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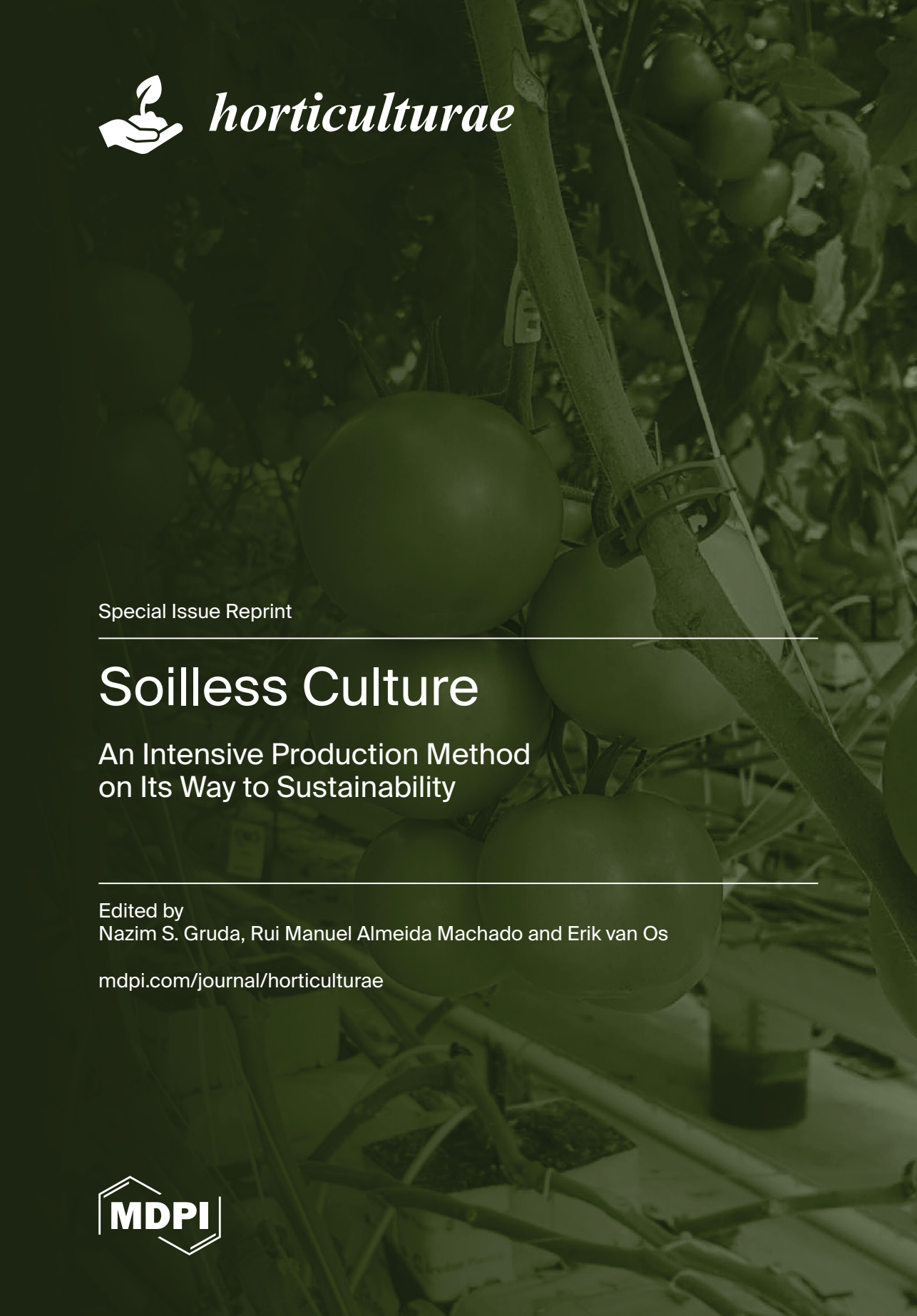

Special Issue Reprint

Soilless Culture

An Intensive Production Method on Its Way to Sustainability

Edited by
Nazim S. Gruda, Rui Manuel Almeida Machado and Erik van Os

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Soilless Culture—An Intensive Production Method on Its Way to Sustainability

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Editors

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Cover image courtesy of Erik van Os

Contents

About the Editors

Nazim S. Gruda

Nazim S. Gruda, University of Bonn, Germany, is a globally renowned authority in horticulture. He is Vice-Chair of the Division of Vegetables, Roots, and Tubers within the International Society for Horticultural Science. With a primary focus on innovative and sustainable horticultural food production systems, his research has significantly contributed to scientific understanding and practical applications. Recently, Burleigh Dodds Science published the book "Advances in Horticultural Soilless Culture" edited by him. According to Stanford University, his influential research places him in the top 2% of global scientists. He is a Member, an Honorary, or a Correspondent Member of three European Academies and a "Distinguished Scientist" of the Academy of Science of China. Additionally, Professor Gruda has actively participated as a consultant for international organizations such as the Food and Agriculture Organization (FAO) and as an expert evaluator for entities including The European Commission.

Rui Manuel Almeida Machado

Rui Manuel Almeida Machado is a Professor in the Department of Crop Science at the School of Sciences and Technology of the University of Évora and a member of the Mediterranean Institute for Agriculture, Environment, and Development (MED). His research area focuses on the fertigation, irrigation, rooting, nutrition, and fertilization of horticultural plants. Current research is focused on creating more sustainable growing media and improving the fertility of poor soils. Throughout his teaching career, he has taught various undergraduate, master's, and postgraduate courses in the scientific field of crop science and agricultural sciences. He has taught curricular units such as herbaceous horticulture, forced crops, plant nutrition, soil fertility and crop fertilization, fertigation, foliar fertilization, and soil-plant relationships.

Erik van Os

Erik van Os was more than 40 years researcher at Wageningen University and Research, dept Greenhouse Horticulture on soilless cultivation systems, approaching the crop from a technical point of view. Research topics were saving water and fertilizers, disinfecting the recirculating nutrient solution, minimizing the emission of fertilizers and pesticides to the environment, choosing substrate and materials and controlling the composition of the nutrient solution. During the last 10 years, the exchange of knowledge to farmers became necessary for high-tech greenhouses in the Netherlands and middle- and low-tech growing systems for farmers with less money to invest. He is currently employed by multiple international organizations, primarily working on the development and implementation of soilless agriculture systems in underdeveloped and developing countries.

Preface

Soilless Culture Systems (SCSs) have been in use commercially for the last 40 years, and now many variations of these systems are available. The primary benefit of SCSs is that they save water and fertilizers, and the surplus water can be reused by the grower or in a cascade system in other crops. This system helps to promote the sustainable use of water and fertilizers. The next step in this process, highlighted in this Reprint, is the use of microorganisms to increase the resilience of plants against pests and diseases, thus reducing the need for chemicals. Additionally, organic growing media/plant substrates will increase further. SCSs are moving towards sustainable agriculture with both high-tech and low-tech methods. It is encouraging to see researchers worldwide embracing this cultivation method to take sustainable steps forward. As editors, we are pleased to have had the opportunity to cooperate and stimulate this development towards sustainable agriculture. We hope that many growers will apply the methods investigated here and that many researchers will continue to develop and improve SCSs.

This reprint comprises 11 original contributions and an editorial written by 45 authors from 10 countries. The numbers differ slightly from what was reported in the editorial, as the editorial was written before the last article was published.

The cover picture illustrates sustainability with a soilless tomato crop. The old leaves on the floor provide a habitat for beneficial predators to eliminate harmful pests.

Nazim S. Gruda, Rui Manuel Almeida Machado, and Erik van Os
Editors

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Editorial

Is Soilless Culture a Sustainable Form of Agriculture?

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1. Introduction

A soilless culture system (SCS) is a technique used for plant production that has recently become increasingly popular [1,2]. For instance, almost all greenhouse areas in the Netherlands use SCSs due to their benefits, including up to 50% savings on water and fertilisers, the ability to steer crop growth vegetatively or generatively, and higher yields with better quality [3–5]. Similar systems with low investments can be used in mid- and low-tech horticulture. SCSs also enable growers to start with a disease-free crop; crop rotation is no longer required [5]. Further, it significantly benefits regions facing water scarcity, unfavourable soil conditions, infertility, soil-borne diseases, salinity, or sodicity [1,2,6]. Especially when water shortage is an issue, circulating surplus nutrient solutions can save water and expensive fertilisers. Alternatively, the surplus can be used in another crop without recirculating [4]. The primary goal is intensification. Thus, an SCS is employed in areas with suitable climate conditions and proximity to major urban centres to ensure and increase productivity.

Diverse crops require varied growing techniques. A growing medium with 30% drainage is utilised for fruit vegetables, accommodating 2–10 plants per square meter. This medium is sterilisable for reuse. NFT or DFT techniques are commonly used for leafy crops like lettuce and kale, sometimes with fixed or movable troughs. The nutrient solution is continuously circulated in this method. Growing media also serve to cultivate herbaceous plants, ornamentals, medicinal and aromatic species, small fruits, and woody crops. Seedlings and transplants are produced using these media in controlled and open agricultural environments [7]. Nevertheless, there is an ongoing discussion regarding the sustainability of SCS and growing media [8,9].

Reducing or replacing the use of peat, improving nutrient and water efficiency, and establishing circular waste flows are crucial steps towards the sustainable cultivation of soilless plants. By utilising renewable and locally available raw materials, appropriate substrate mixtures, biostimulants, and advanced techniques such as artificial intelligence and the Internet of Things, we can develop a new strategy for SCSs. These measures have the potential to pave the way for a promising future for agriculture.

The Special Issue, titled "Soilless Culture—An Intensive Production Method on Its Way to Sustainability", includes recent research on sustainable horticultural plant cultivation using SCSs. It features contributions from diverse experts spanning circular growing media, remote growing, cost-effective methods for increasing profitability, biostimulants, plant nutrition, and water quality. This Special Issue includes 10 original contributions written by 42 authors from 9 countries.

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2. Alternative Peat-Growing Media

The utilisation of peat substrates is the basis of today's growing media in horticulture. Regrettably, peat extraction can damage the environment and ecosystems. Peatlands serve as natural carbon sinks, and using peat as a substrate releases stored carbon, negatively impacting the CO_2 balance and contributing to global warming [1,2]. As a result, the shift from peat to sustainable alternatives in horticultural growth is of utmost importance [1,2,10]. However, this presents a significant challenge due to peat's physical and chemical characteristics. Nevertheless, it is paramount for the horticultural industry to adopt sustainable practices, and reducing or eliminating peat from growing media cannot be disregarded [8]. This Special Issue highlights the significance of transitioning towards sustainable horticultural practices, the difficulties faced, and various strategies to reduce or eliminate peat usage in the growing media industry through a collection of papers.

The first contribution of this Special Issue focuses on replacing peat in horticultural growing media with alternative constituents based on biomass. Using four well-established non-peat materials, namely wood fibre, bark, composted green waste, and coir, has garnered considerable attention in agriculture. Each material presents distinct challenges and possesses specific properties. For instance, wood fibre may lead to nitrogen immobilisation, while composted green waste and bark can exhibit high bulk density and electrical conductivity (EC). However, these materials also offer significant nutrient content, distinguishing them from peat-based alternatives.

It is crucial to emphasise the varying roles of these materials in peat replacement. For instance, it is recommended to use wood fibre as a compound and diluent in growing media; some studies have demonstrated that it can even be utilised alone [1]. Composted bark and coir have the potential to function as complete replacements for peat. Contribution 1 emphasised the sustainability aspects of using no peat materials in the future, underscoring the significance of sustainable agricultural practices in line with the focus of this Special Issue, analysing factors affecting the supply and use of growing media constituents in Germany, including processing infrastructure, competition, and the economic advantage of peat. Although technical solutions for peat-free media exist, this study suggests future evolutions will not lead to the complete substitution of peat in Germany (contribution 1).

Martins et al. (contribution 2) conducted a study on using coir, municipal compost, and biochar to reduce peat content in growing media. They found that lettuce seedlings grown in coir-based media with 17–22% v/v of municipal solid waste compost and biochar grew more vigorously than in other mixtures. The mixes also increased the total phenol content in lettuce leaves, which can improve abiotic stress tolerance. However, further research is needed to evaluate how the seedlings behave after transplantation and to compare these mixes with commercial counterparts for different vegetable transplants [10].

Česonienė et al. (contribution 3) studied a different aspect. They investigated the impact of substituting peat with varying proportions of pine and spruce wood fibres and perlite on the growth of blueberry saplings. The study found that the most favourable outcomes were observed with substrates containing 15–45% v/v of pine wood fibre and 15–30% v/v of spruce wood fibre. Conversely, introducing spruce bark fibres over 30% had a detrimental effect on sapling growth. Analysis of leaf macronutrients revealed challenges, particularly in nitrogen and potassium levels, within substrates containing 30–45% v/v of spruce bark fibres. These findings underscore the potential to reduce peat consumption while supporting the conservation of vital wetland ecosystems (contribution 3).

The utilisation of organic fertilisers in soilless container plant production has increased. However, there is a shortage of techniques to evaluate N release. The release of mineralisable N can be estimated using water-soluble and hydrolysable N and C pools. The Gompertz function is a reliable method for making precise estimations. Nonetheless, this research underscores two primary obstacles confronting agricultural practitioners when utilising organic nitrogen-based fertilisers: firstly, the nitrogen release comprises only 50% of the total nitrogen applied, and secondly, this release transpires swiftly, resulting in

salt-induced damage and rendering complete nitrogen application before planting becomes unfeasible (contribution 4).

3. Cascade Systems

In the realm of cascade systems, which prioritise resource efficiency and sustainability, various studies have demonstrated their effectiveness in optimising nutrient and growing medium utilisation.

As exemplified in a study by Karatsivou et al. (contribution 5), soilless cascade systems are highly proficient in recycling nutrient solutions. This innovative prototype, designed to repurpose drained nutrient solutions from primary tomato crops for secondary crops, yielded a 14% increase in spinach yield. When lettuce and parsley were used as secondary crops, their yield remained unaffected by the system. Furthermore, the environmental advantages were evident as water productivity improved with pure drainage solutions, and there was a remarkable 50% increase in nitrogen and phosphorus use efficiency compared to control treatments.

Similarly, the concept of reusing the substrate of a primary crop for a secondary crop has gained traction in cascade systems. In a study by Machado et al. (contribution 6), various coir-based mixtures were tested for growing spinach. The lettuce plants grown in the reused mixtures exhibited yields similar to those produced in new coir, demonstrating the viability of such practices. These findings underscore the potential of soilless cascade systems to optimise nutrient and growing medium utilisation, thereby reducing the need for supplementary fertilisers or plant substrates, ultimately contributing to environmental sustainability (contributions 5 and 6).

Expanding the scope of cascade cropping systems, contribution 7 explored the feasibility of incorporating biodegradable packaging within the short food supply chain. Specifically, they assessed the impact of replacing conventional petroleum-based bags with PLA film on the shelf life of fresh-cut wild rockets (primary crop) and sea fennel (secondary crop). This study found that using PLA-based film had no significant detrimental effects after storage and demonstrated superior microbiological safety compared to conventional materials. These findings suggest that integrating biodegradable materials like PLA film could further enhance the sustainability of cascade cropping systems, offering an eco-friendly alternative to petroleum-based plastics within a short food supply chain. In this way, cascade systems continue to evolve as a vital component of sustainable agricultural practices.

Greenhouse agriculture faces significant challenges in terms of environmental sustainability, particularly regarding pesticide emissions from crops grown in open or closed systems. To address this issue, contribution 8 tested a greenhouse emission model using stone wool mats for sweet pepper cultivation via drip irrigation. They improved the model's performance by modelling the mats as two equally large tanks. Furthermore, exploring alternative pesticide application methods like spraying or low-volume misting is essential in pursuing environmentally friendly practices, which involve additional processes affecting substance entry into recirculation water.

Examining sustainable agricultural practices, contribution 9 researched the use of sodium hypochlorite as a disinfectant for tomato plants cultivated in recycled nutrient solutions. Surprisingly, this study found that chlorine application did not negatively impact plant growth or gas exchange; instead, it significantly increased the total production of marketable fruits. Tomatoes grown in this manner are safe to consume. However, it is important to note that further research is required to assess the effectiveness of chlorine as a disinfectant in nutrient solutions, especially when combined with crop inoculation against pathogens.

Demonstrating the commitment to environmentally responsible SCS, contribution 10 conducted research that showcased the advantages of integrating mycorrhiza and bacteria alongside 80% mineral fertilisers in capia pepper cultivation. This approach resulted in a remarkable 32.4% increase in yield compared to using 100% mineral fertilisers alone.

Beyond yield, the plants treated with bio-fertilisers exhibited superior fruit parameters, including fruit weight, diameter, volume, electric conductivity of fruit juice, and total soluble solids. These findings provide compelling evidence that incorporating bacteria and mycorrhiza into farming practices can reduce reliance on mineral fertilisers and promote a more sustainable approach to horticulture (Dasgan et al.).

4. Conclusions

SCSs have the potential to enhance water and nutrient utilisation efficiency significantly. However, several aspects of the SCS production process require improvement to improve sustainability.

In pursuit of sustainable agriculture practices, this Special Issue explores alternative biomass-based constituents to replace peat in horticultural growing media. Through the evaluation of four established non-peat materials, namely wood fibre, bark, composted green waste, and coir, the authors of this Special Issue uncover specific advantages and challenges associated with each. Wood fibres emerge as a potential compound or diluent, while composted bark and coir show promise as peat substitutes. Sustainability is a focal point, yet supply and complete peat substitution challenges persist.

Cascade systems represent a substantial leap forward in enhancing sustainability within agriculture. Their multifaceted approach, which encompasses recycling nutrient solutions, minimising pesticide emissions, and integrating eco-friendly practices, holds great promise for the future of farming. These innovative solutions optimise resource utilisation and contribute significantly to promoting environmentally responsible agricultural practices. The results from this Special Issue will contribute to advancing our understanding of cascade systems and their potential to transform agriculture into more sustainable and ecologically mindful efforts. As we face the challenges of a changing world, cascade systems provide a ray of hope for a more sustainable and resilient agricultural future.

In summation, the outcomes of diverse studies within this Special Issue underscore the potential of SCS as a viable form of sustainable agriculture. These investigations accentuate a range of strategies aimed at fortifying the sustainability of SCS, encompassing a reduction in peat use in horticulture, the implementation of efficient nutrient management protocols, the adoption of cascading systems for recycling nutrient solutions, the reuse of GM, and the incorporation of bio fertilisers, among others. Nonetheless, it is imperative to underscore further research in this domain, as it is pivotal for refining and seamlessly integrating these strategies into SCS. This integration should happen by stimulating the yield and quality of horticultural products.

Author Contributions: N.S.G. drafted the manuscript outline and analysed the Special Issue topics. E.A.v.O. contributed to the introduction, while R.M.A.M. contributed to the concluding remarks and future perspectives and formatted the manuscript according to the journal's guidelines. All authors have read and agreed to the published version of the manuscript.

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List of Contributions

1. Hirschler, O.; Thrän, D. Peat Substitution in Horticulture: Interviews with German Growing Media Producers on the Transformation of the Resource Base. Horticulturae, 2023, 9(8), 919. https://doi.org/10.3390/horticulturae9080919
2. Martins, T.C.; Machado, R.M.A.; Alves-Pereira, I.; Ferreira, R.; Gruda, N.S. Coir-Based Growing Media with Municipal Compost and Biochar and Their Impacts on Growth and Some Quality Parameters in Lettuce Seedlings. Horticulturae, 2023, 9(1), 105. https://doi.org/10.3390/horticulturae9010105
3. Česonienė, L.; Krikštolaitis, R.; Daubaras, R.; Mažeika, R. Effects of Mixes of Peat with Different Rates of Spruce, Pine Fibers, or Perlite on the Growth of Blueberry Saplings. Horticulturae, 2023,9(2), 151. https://doi.org/10.3390/horticulturae9020151

4. Lohr, D.; Gruda, N.S.; Meinken, E. Estimating Nitrogen Release from Organic Fertilizers for Soilless Production by Analysis of C and N Pools. Horticulturae, 2023, 9(7), 767. https://doi.org/10.3390/horticulturae9070767
5. Karatsivou, E.; Elvanidi, A.; Faliagka, S.; Naounoulis, I.; Katsoulas, N. Performance Evaluation of a Cascade Cropping System. Horticulturae, 2023, 9(7), 802. https://doi.org/10.3390/horticulturae9070802
6. Machado, R.M.A.; Alves-Pereira, I.; Alves, I.; Ferreira, R.M.; Gruda, N.S. Reusing Coir-Based Substrates for Lettuce Growth: Nutrient Content and Phytonutrients Accumulation. Horticulturae, 2023, 9(10), 1080. https://doi.org/10.3390/horticulturae9101080
7. Gómez, P.A.;Egea-Gilabert, C.; Giménez, A., Benaissa, R.R.,;Amoruso, F.; Signore, A.; Gallegos-Cedilo, V.; Ochoa, J.; Fernández, J.A. Biodegradable Food Packaging of Wild Rocket (*Diplotaxis tenuifolia* L.[DC.]) and Sea Fennel (*Crithmum maritimum* L.) Grown in a Cascade Cropping System for Short Food Supply Chain. Horticulturae, 2023, 9(6), 621. https://doi.org/10.3390/horticulturae9060621
8. Wipfler, E.L.; Boesten, J.J.; van Os, E.A.; Beltman, W.H. Testing the Greenhouse Emission Model (GEM) for Pesticides Applied via Drip Irrigation to Stone Wool Mats Growing Sweet Pepper in a Recirculation System. Horticulturae, 2023, 9(4), 495. https://doi.org/10.3390/horticulturae9040495
9. Lykogianni, M.; Bempelou, E.; Karavidas, I.; Anagnostopoulos, C.; Aliferis, K.A.; Savvas, D. Impact of sodium hypochlorite applied as nutrient solution disinfectant on growth, nutritional status, yield, and consumer safety of tomato (*Solanum lycopersicum* L.) fruit produced in a soilless cultivation. Horticulturae, 2023, 9(3), 352. https://doi.org/10.3390/horticulturae9030352
10. Dasgan, H.Y.; Yilmaz, M.; Dere, S.; Ikiz, B.; Gruda, N.S. Bio-Fertilizers Reduced the Need for Mineral Fertilizers in Soilless-Grown Capia Pepper. Horticulturae, 2023, 9(2), 188. https://doi.org/10.3390/horticulturae9020188

References

1. Gruda, N.S. Increasing Sustainability of Growing Media Constituents and Stand-Alone Substrates in Soilless Culture Systems. *Agronomy* **2019**, *9*, 298. [CrossRef]
2. Gruda, N.S. Advances in Soilless Culture and Growing Media in Today's Horticulture—An Editorial. *Agronomy* **2022**, *12*, 2773. [CrossRef]
3. Putra, A.P.; Yuliando, H. Soilless Culture System to Support Water Use Efficiency and Product Quality: A Review. *Agric. Agric. Sci. Procedia* **2015**, *3*, 283–288. [CrossRef]
4. Santos, M.G.; Moreira, G.S.; Pereira, R.; Carvalho, S.M.P. Assessing the potential use of drainage from open soilless production systems: A case study from an agronomic and ecotoxicological perspective. *Agric. Water Manag.* **2022**, *273*, 107906. [CrossRef]
5. Van der Salm, C.; Voogt, W.; Beerling, E.; Van Ruijven, J.; Van Os, E. Minimising emissions to water bodies from NW European greenhouses; with focus on Dutch vegetable cultivation. *Agric. Water Manag.* **2020**, *242*, 106398. [CrossRef]
6. Savvas, D.; Gruda, N. Application of soilless culture technologies in the modern greenhouse industry—A review. *Eur. J. Hortic. Sci.* **2018**, *83*, 280–293. [CrossRef]
7. Pascual, J.A.; Ceglie, F.; Tuzel, Y.; Koller, M.; Koren, A.; Hitchings, R.; Tittarelli, F. Organic substrate for transplant production in organic nurseries. A review. *Agron. Sustain. Dev.* **2018**, *38*, 35. [CrossRef]
8. D'Amico, A.; De Boni, A.; Palmisano, G.O.; Acciani, C.; Roma, R. Environmental analysis of soilless tomato production in a high-tech greenhouse. *Clean. Environ. Syst.* **2023**, *11*, 100137. [CrossRef]
9. Gonnella, M.; Renna, M. The Evolution of soilless systems towards ecological sustainability in the perspective of a circular economy. Is it really the opposite of organic agriculture? *Agronomy* **2021**, *11*, 950. [CrossRef]
10. Blok, C.; Eveleens, B.; van Winkel, A. Growing media for food and quality of life in the period 2020–2050. *Acta Hort.* **2021**, *1305*, 341–356. [CrossRef]

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Article

Peat Substitution in Horticulture: Interviews with German Growing Media Producers on the Transformation of the Resource Base

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Abstract: Peat is the major constituent of horticultural growing media. Due to its high climate footprint, its extraction and use are controversial and the need to limit its use is widely recognised. The Peat Use Reduction Strategy of the German government aims to phase out its use and replace it with renewable materials. Despite large potential, stakeholders consider the availability of peat substitutes in sufficient quantity and quality as a critical issue. The goal of this research is to systematically investigate the challenges and opportunities for substituting peat in the resource base of the growing media industry. Based on deep-dive interviews with German growing media producers, the factors determining the supply and use of the main growing media constituents—peat, green compost, wood fibres, composted bark and coir products—were analysed. The results show the critical role of the processing infrastructure on transportation distances and the quality and quantity of the market supply. Additionally, competition with other sectors affects the availability of materials for the growing media industry. Moreover, peat is still economically advantageous compared with its substitutes. Even if this advantage declines due to consumer awareness and the end of domestic extraction, the end of peat use would probably imply new policy measures.

Keywords: peat; peat substitute; compost; wood fibres; growing media; industrial production; interviews

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1. Introduction

The present paper focuses on the replacement of peat in horticultural growing media with alternative constituents based on biomass.

The process of extracting and using horticultural peat from peatland soils is a significant source of greenhouse gas (GHG) emissions that accounts for about 12.6 Mt CO2-eq for the European Union (Convention) and 2.2 Mt CO2-eq for Germany (Data 2019, [1]). Peat extraction only represents a small share of the total GHG emissions from peatlands but generates, through the fast destruction of the soil carbon stock, by far the highest climate impact per hectare compared with any other human activity on peatlands. The climate footprint of peat is also by far the highest among all other horticultural growing media constituents [2,3]. Moreover, peat extraction potentially represents a threat to ecosystems in regions where the extraction takes place on living mires and without renaturing measures afterwards [4]. In Europe, Germany ranks first for the extraction of peat for horticultural purposes, second for peat imports, first for the use of peat in growing media production, first for exports of peat and peat products and first for the use of peat in horticulture [5]. As presented in Figure 1, Schmatzler [6] forecasted an almost complete end to peat extraction in 2040 in Lower Saxony, where German peat extraction is concentrated [7]. The reasons behind this trend are the limitation of land availability through competition with agriculture

and restrictions on the issuance of new extracting permits. Despite important uncertainties concerning data on German peat extraction [5], a long-term trend of decrease in the amount of domestically extracted peat can be observed, compensated by increasing imports from other EU states, especially the Baltic states.

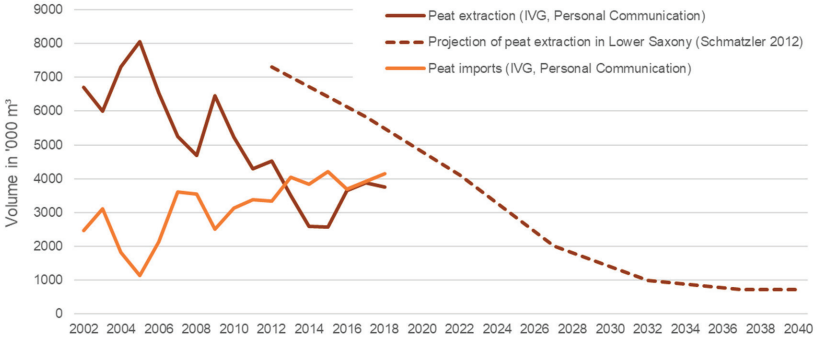

Figure 1. Evolution of peat extraction, peat imports and projection of peat extraction in Germany.

In a context of rising awareness regarding climate change [8] and the importance of protecting peatland carbon stocks [9–11], the extraction and use of peat are increasingly controversial. In the last decade, the horticultural sector increasingly acknowledged its role in limiting its climate footprint, especially by reducing peat use in growing media [12–18]. Against this background, in 2016, the German government set a goal to end peat extraction and reduce peat use in the Climate Action Plan 2050 [19]. The targets of the Peat Use Reduction Strategy developed by the Ministry of Food and Agriculture are to end peat use in hobby gardening by 2026 and reduce it as much as possible for professional horticulture by 2030 [20]. This strategy includes discussions with stakeholders, research funding, extension on alternatives and public awareness programs. Governments in other European countries have also committed to reduce the use of peat, namely the United Kingdom, Switzerland, Norway, the Netherlands and Austria [21–25]. An overview of the political initiatives to reduce peat use in Europe is presented in Hirschler et al. [26] and Gruda et al. [27]. Furthermore, discussions on a European strategy to reduce peat use in horticulture have started, as proposed by Germany at the EU Agriculture and Fishery Council in May 2022 [28]. Furthermore, the inclusion of the emissions of peat extraction in the mitigation targets of the EU member states through the LULUCF Regulation (EU) 2018/841 [29] is expected to further increase their relevance for climate policy. However, outside of Germany, there are no initiatives on a national or EU level to address the extraction of peat for horticulture in the currently extracting countries. Therefore, even if domestic peat extraction is expected to stop in Germany, the supply constituted by peat imports is expected to continue being available in the foreseeable future.

Peat is by far the most used constituent for the production of growing media in Germany and in Europe [30,31]. Growing media is defined in the fertilising products regulation (EU) 2019/1009 [32] as "a product other than soil in situ, the function of which is for plants or mushrooms to grow in". They are mixtures of organic constituents, sometimes complemented by mineral constituents, in which the plant roots develop and constitute the supporting medium for fertilisation and water supply. According to the Industrieverband Garten e.V. (IVG), the German industry produced around 8.1 million cubic meters of growing media in 2022 [33]. In the last decade, this volume has remained relatively stable between 8 and 10 million cubic meters. Blok et al. [34] forecasted an important increase in the need of growing media worldwide between 2017 and 2050. It is unclear to what extent this demand will be able to be met and, since this trend mostly concerns Asia, how this development will apply to the German industry. Growing media are used by hobby gardeners as potting soil; this represents 66% of German production for the domestic market. The rest is mostly used in a wide range of professional horticultural systems such

as in the production of flowers, nursery stock, vegetable seedlings and mushrooms. A smaller proportion of growing media is also used in the landscaping sector. Around 28% of German production is exported, mostly for professional horticulture.

The other main growing media constituents apart from peat, also referred as peat substitutes or alternative constituents, are green compost, wood fibres, composted bark and coir products. As presented in Figure 2, the share of alternative constituents used in growing media in Germany has regularly increased, especially for the hobby sector.

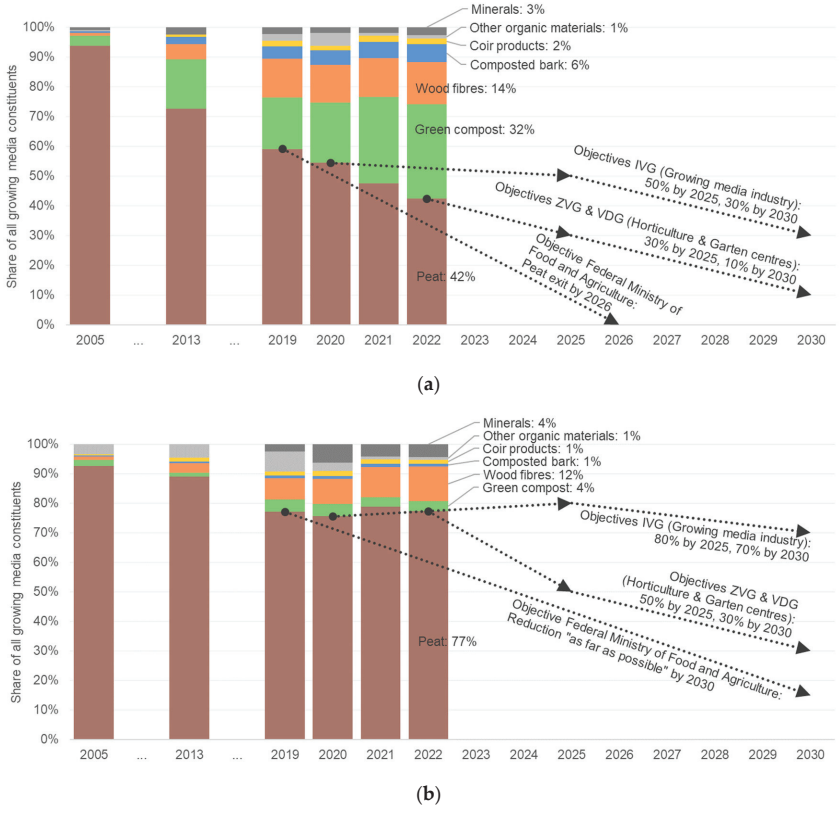

Figure 2. Evolution of the composition of growing media in Germany in (**a**) the hobby sector and (**b**) the professional sector and peat use reduction objectives set by stakeholders. For the years 2005 and 2013: total growing media production in Germany (source: [31,35]). For the years 2019–2022: growing media production in Germany for the domestic market (source: [33]). Objectives apply for growing media used in Germany. The curve starts at the year of decision.

The properties of growing media constituents and their use in horticulture are largely documented in the literature [15,17,36–43]. Green compost is produced from green waste though a composting process. After shredding, mixing and sieving, the material is left loose on a surface and turned over several times. For a good quality product, the process needs to attain a certain temperature and last several months. The properties of green compost are generally limited by its high pH, nutrient content and bulk density. Neumaier and Meinken [38] set a general limit of 40% of green compost in growing media. Wood fibres are produced from coniferous wood chips through a thermomechanical process carried out with a machine (in general an extruder or refiner). Wood fibre use in growing media has increased significantly in the last years, especially in the professional sector. The use of wood fibres is said to be mostly limited by nitrogen immobilisation, which

complicates fertilisation. A rate of 40% wood fibres in growing media is set as a limit by Neumaier and Meinken, but other sources and projects showed successful experiences with 50% rates and even at 100% as stand-alone substrate [44–47]. The composting of bark is conceptually comparable with the composting of green waste. The use of composted bark is generally limited by its water holding capacity and, in some cases, heavy metal concentrations. According to Neumaier and Meinken, it should not be used at a rate of more than 50% in growing media. Uncomposted bark can also be used in growing media but does not represent important quantities in Germany [33]. Coir products for growing media are by-products from the processing of coconut fibres, themselves by-products of coconut production. Coir pith, also named "cocopeat", and coir fibres are both part of coir products. Before arriving in Europe, coir products are washed to decrease salinity. Coco pith is supplied compressed in blocks and needs to be processed in order to obtain a loose material usable for mixing in growing media. In some cases, coco pith is further processed by buffering to improve fertilisation. Coir products can be limited by their salt content, but good quality products have been shown to be used as stand-alone growing media [38,48]. Other growing media constituents are investigated but do not represent significant amounts in Germany yet, notably fresh *Sphagnum* moss [49], fibres from *Miscanthus*, flax, reed, hemp and various plants [50–53], biochar [54], biogas digestate [55,56] or rice hulls.

Although the necessity to reduce peat use seems to be accepted by the vast majority of stakeholders in Germany, the extent and speed of this transformation are subject to intense debate. As a result, the IVG and representatives of the horticultural sector have expressed doubts about the governmental goals and set their own targets on peat reduction [57,58], as presented in Figure 2. According to discussions between stakeholders, the reduction of peat in growing media composition seems to face two main categories of problems. The first category of problems relates to the challenge of using growing media in horticulture with different properties due to the change in its composition. In addition to the extensive research on the subject, plant production in peat-reduced and peat-free growing media has been the subject of successful investigations in practice in Germany [59,60]. The second challenge concerns the supply of alternative constituents in case of an increase in their demand as a consequence of peat replacement and is often designated as the problem of the "availability" ("Verfügbarkeit" in German) of peat substitutes, further referred to as the "availability problem". This latter problem is presented as critical by industry stakeholders. Investigations on the potential amounts of bio-based constituents show largely sufficient resources for the replacement of peat in Germany and in Europe [26]. The results of the approach based on the potential amounts imply that the growing media industry will encounter difficulties in accessing the existing amounts of raw materials and peat substitutes, limiting the effective available supply. Factors such as competition with the energy sector for the access to biomass or the quality of compost are mentioned in the many discussions surrounding the reduction of peat use between stakeholders and based on specific situations.

In this context, the goal of the present paper is to systematically investigate and list the factors enabling and limiting the use of growing media constituents and the transformation of the resource base of the industry for the reduction of peat use.

2. Materials and Methods

2.1. General Method

The research was conducted specifically on the German industry. Given the limited scientific literature on the subject and that the orientation of the research question focused on the concrete situation in the industry, the investigations were based on the experience of growing media producers. For this, in-depth expert interviews were carried out and analysed following qualitative methods.

The method is presented as it was carried out, based on the following steps: (1) sampling, (2) questionnaires and interviews and (3) qualitative analysis.

2.2. Sampling

The sample was prepared based on a list of growing media producers in Germany. This list was created using an internet search completed by the list of the members of the German industry group IVG and personal contacts. As a result, 83 companies were identified.

The goal of the sample selection was to include a variety of companies regarding size (number of employees, production), geographical region, own peat extraction activities (with/without), outlet market (hobby/professional/landscaping sector) and public argumentation of the company. These elements were assumed to be potentially linked to the situation and the readiness of the companies regarding peat substitution. The information on these criteria was collected or estimated, when possible, using the companies' websites. Four companies refused to take part of the interviews. In order to complete the sample, other companies were selected. In accordance with the goals set, 9 growing media producers were surveyed.

2.3. Questionnaires and Interviews

The interviewees were either heads of the companies or members of the management teams. In some cases, two people represented one company. Before the interview, the interviewees were asked to fill out a questionnaire in order to collect data on the situation of the company and to produce the first elements of discussion for the interview. The questionnaire is available in Table A1.

The questionnaire and interviews were conducted in German and took place between November 2022 and January 2023. The interviews were carried out via videoconference and mostly lasted between 1 and 1.5 h. They were led in a semi-directed form inspired by the concept of the "problem-centered interview" proposed by Witzel [61].

The structure of the interview aimed to (1) obtain an overall understanding of the supply and production chain for growing media and growing media constituents in the company, (2) understand and gather the factors determining and potentially limiting the use of growing media constituents and (3) obtain the perspective of the company regarding the reduction of peat use in Germany. The list of themes and questions can be found in Table A2.

We focused on the main organic constituents used in growing media in Germany: (1) peat, (2) green compost, (3) wood fibres, (4) composted bark and (5) coir products. In case one of these five main constituents was not mentioned actively in an interview, the interviewer explicitly asked about it. When mentioned by the interviewee, information on other materials was included in the analysis.

2.4. Qualitative Analysis

The interviews were entirely recorded, transcribed and qualitatively analysed using MAXQDA following the method of the thematic analysis described by Braun and Clarke [62]. The approach mixed deductive and inductive processes, and the coding system was adapted along the analysis in order to gain relevance.

On this basis, factors determining the supply and the use of growing media constituents were systematically listed and explained. For each element, the number of interviewees mentioning it and the number of times mentioned are used as indicators of the relevance of the element in the current situation. These mentions are often pointing problems. Some elements, although potentially important, could have not been mentioned because they were not considered problematic in the current situation. Therefore, the relevance of an element in the current situation should not be confounded with its importance. Moreover, due to the limited sample, those mentioned should not be considered statistically representative.

The number of interviewees mentioning a specific idea are presented in the results in the following form: (N = X), where X is the number of interviewees mentioning the idea. When it applies, the number of times an idea is mentioned, Y, is added to the number of interviewees, X, using the following form: (N = X, n = Y).

2.5. Interest and Position of the Interviewees

In a potential conflictual political situation due to different interests, it seems important to acknowledge the position of the researcher and the perception of the role of the research team and of the subject by the interviewees. The research team of the first author is mandated by the Ministry of Food and Agriculture to carry out investigations on the possibilities and the consequences of the Peat Use Reduction Strategy in order to support decision-making processes. Therefore, the perspective and goals of the present research focus on the transformation towards peat reduction and not, for example, on questioning its relevance. Moreover, the debate surrounding the preliminary research of the research team published in the Thünen Working Paper 190 [26] on the potential amounts gave an insight into the sensitivity of research on the subject. However, all companies that were asked to take part in the investigation showed an interest for the research and the interviewees answered the questions very openly. The four companies refusing to take part in the interviews attested it was due to time constraints and not linked to any defiance toward the research team or the subject. The conclusions of the potential analysis were mentioned as background at the beginning of each interview and accepted by all interviewees. The relevance of the research was not questioned.

3. Results

3.1. Description of the Sample

3.1.1. Company Characteristics

Based on answers of the questionnaire and completed by information from the interviews, the characteristics of the sample were analysed. The results are presented with the intention to preserve the anonymity of the participating companies. As presented in Figure 3, the fact that the company extracts peat or not and the major outlet market (plant production vs. hobby and landscaping) were directly linked to each other and were also strongly linked to the geographical position, the size and the average peat rate in growing media.

As presented in Table 1, the approach prioritising diversity leads to an overrepresentation of the perspective of bigger companies. Since the peat reduction goals focus on amounts, the sample can be considered a good balance between a diversity of situations and the significance regarding the amounts of growing media produced.

Table 1. Comparison of structural parameters between the sample and the whole German industry.

	Sample	**German Industry**
Total companies	9	83
Total growing media production (m^3/a) *	3.15×10^6	8.1×10^6
Average growing media production per company * (m^3/a)	350,550 m^3	97,590 m^3
Number of companies above 20 employees *	56% (5/9)	29% (24 **/83)
Number of companies with majority of production in North-Western Germany *	56% (5/9)	48% (40/83)

Source: own analysis. * Activity of the company within Germany. ** Source: [63].

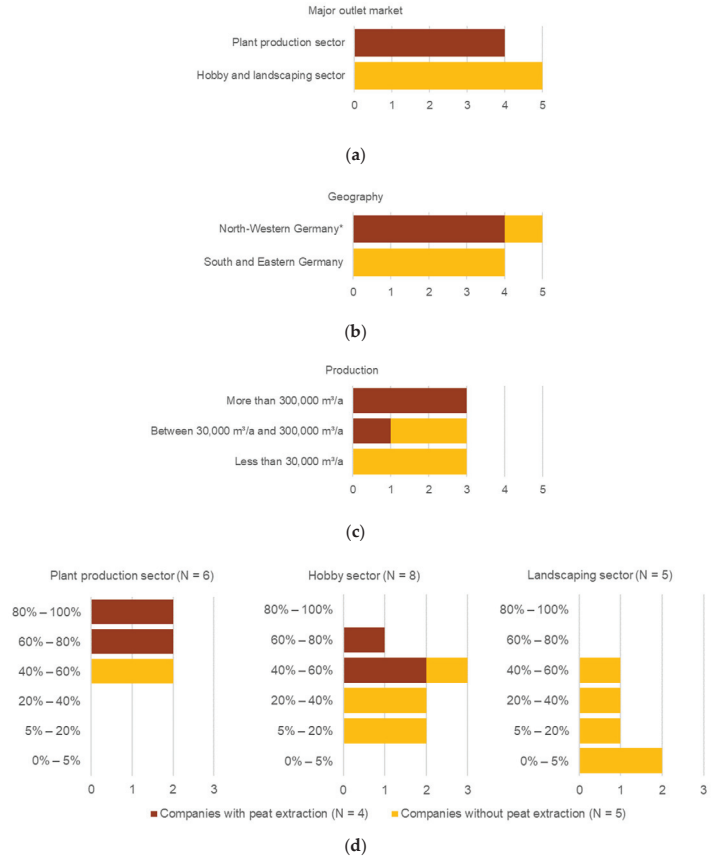

Figure 3. Number of surveyed companies (N = 9) depending on (**a**) major outlet market of growing media, (**b**) geographical location of the majority of the production sites (* Lower Saxony, Schleswig-Holstein, Bremen, Hamburg, Nord Rhine-Westphalia), (**c**) yearly volume of growing media production and (**d**) peat rate and outlet market of produced growing media.

3.1.2. Supply and Processing of Growing Media Constituents

The numbers of companies surveyed using and processing the different growing media constituents considered are presented in Figure 4. All companies surveyed used peat and compost. Most of them (N = 8) used wood fibres and composted bark. Coir products were used by N = 4 companies, from which N = 3 produced growing media mostly for plant production. Some companies had their own facilities to process wood fibres (N = 1), compost bark (N = 6) and compost green waste (N = 3). Green waste was either brought by private gardeners and collected directly in a facility of the company or obtained from waste disposal facilities. All wood chips and bark used by the growing media producers came from sawmill companies. Coir products were bought from distributors importing them from Asia, mostly India and Sri Lanka. The transport to Europe was carried out by sea, mostly to the Netherlands, and then by truck to the growing media producer.

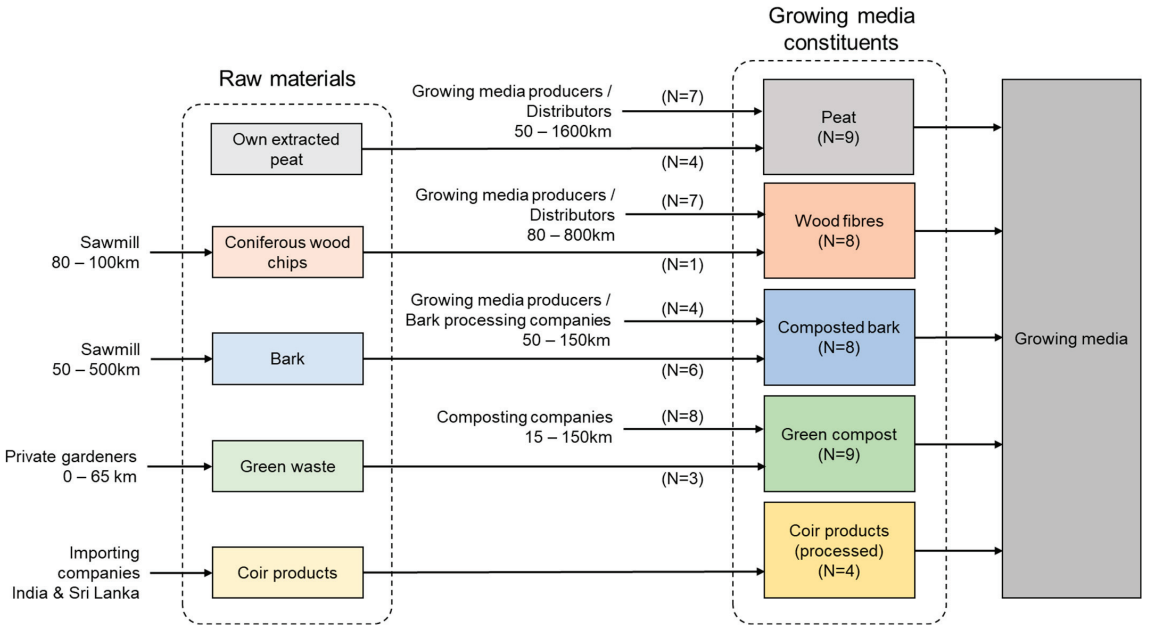

Figure 4. Supply chains of raw materials and growing media constituents in the companies surveyed (N = 9).

The size of the company and the fact that the company has no peat extraction activities seem to be positive linked to the presence of processing capacities for peat substitutes. Among the companies surveyed, a majority concretely declared planning investments to process raw materials for the production of peat substitutes (N = 5).

3.1.3. Position on the Peat Use Reduction Strategy

All companies surveyed declared that they were aiming to reduce peat use in their growing media production. When asked about their position regarding the goals of the Ministry of Food and Agriculture on the reduction of peat use, more interviewees said that it was very favourable (N = 2) or favourable (N = 2) than unfavourable (N = 3). For companies that expressed reservations, the reason was that these goals were considered too ambitious and not realistic given the actual conditions and measures. There was no apparent link between the position regarding peat reduction and the considered variables (peat extraction, growing media production, major outlet sector or geography).

3.2. Challenges and Requirements of the Growing Media Sector

In the following section, the factors determining the choices of the interviewed growing media producers in the supply of growing media constituents are presented and explained based on the analysis of the interviews.

3.2.1. Products Requirements for Final Use

The requirements for growing media and growing media constituents are strongly influenced by their specific utilisation. This has implications for the choices of growing media producers in supplying the materials they work with. In some cases where the production is carried out for another company, the growing media producer has limited to no latitude on the constituents used (N = 3, n = 4).

Because of their influence on nutrient and water supply as well as root development, the properties of growing media are critical for plant health and development. The suitabil-

ity for plant growth is by far the most mentioned quality criteria for growing media and its constituents (N = 9, n = 52). For this purpose, peat is considered to bring particularly favourable, uniform and constant properties (N = 8, n = 21). As a consequence, the reduction of the peat share in growing media is associated with quality challenges (N = 9, n = 33). Among the four main peat substitutes, the potential of coir products was underlined due to its properties and its ability to replace peat by up to 100% (N = 2, n = 2). For the plant production sector, in which the economic viability requires a successful and uniform plant growth and quality, the suitability of growing media for plant growth is critical to avoid outages in production. Within plant production, the importance of these requirements was said to be higher for the production of vegetable seedlings than for ornamental plants (floriculture and nursery stock) (N = 3, n = 6). The importance of quality was said to be lower for the hobby sector than for the plant production sector (N = 6, n = 11) and lower for the landscaping sector than for the hobby sector (N = 1, n = 3). The differences in the quality requirements between the different sectors were confirmed by the different peat rates in the sample (Figure 3) and in the whole German growing media production, as well as the differentiated reduction goals set by the stakeholders (Figure 2). Higher quality requirements for growing media imply relying on more specific materials and thus augment the challenge of supply.

In order to compare growing media constituents, we chose to differentiate comments on the suitability for plant growth between two perspectives: (1) the general suitability that applies to the maximal potential quality for this constituent and (2) the actual quality available, which refers to the quality of materials currently available on the market. Problems regarding the general suitability can only be overcome by research and development, whereas the actual quality can be increased up to the general suitability by improved practices. The actual quality of green compost available was often identified as a problem (N = 5, n = 12). These issues were due to the presence of biowaste and impurities in the green waste, as well as the seasonal variability of the composition of green waste. In order to achieve the requirements for growing media, green compost needs to be based on a limited amount of lawn cuttings, bringing high salt content and a minimal amount of woody material bringing structure. Growing media producers generally use external certification systems to assure the quality of growing media constituents bought from other companies. Although information on the certification was not systematically collected, almost all interviewees (N = 8) said they used certification for green compost.

The interviewees reported the increasing attention of customers regarding environmental and social standards, leading to an increasing demand for peat-free and peat-reduced products (N = 7, n = 13). Unlike for the plant production and landscaping sectors, where growing media only constitute a part of the product supplied to the end customer, sustainability standards play a significant role in the demand for hobby growing media. The attention to sustainability was also mentioned as a moral driver for the growing media producers themselves, playing a role in their choices additionally to their economic logic (N = 7, n = 16).

As another quality criterion for users, the interviewees also mentioned the weight of the product sold in bags for hobby gardeners (N = 3, n = 4). The biological safety for human health was mentioned once as a potential issue (N = 1, n = 1). The presence of viable weed seeds or plant pathogens was not mentioned. The interviewees also mentioned criteria linked to habits with peat products but not influencing its performance, such as the appearance (N = 2, n = 4).

3.2.2. Price of Materials

The plant production sector is generally exposed to a strong competition, including on an international level, and depends on small margins compared with the production costs. For this reason, the growers require a low price for growing media. However, the professional sector also uses growing media with more expensive components, such as coir products or sod peat, than the hobby sector and the landscaping sector. Therefore, the

readiness to pay more for a growing media with specific quality standards seems to be higher in the professional sector than in the hobby sector. Some information on prices was gathered during interviews but this was not systematically collected.

The price of raw materials first depends on their production costs. The production costs of the raw materials used for peat substitutes were not mentioned, which could be explained by the fact that these materials are generally by-products or wastes from other activities. However, the interviewees underlined the importance of the competing uses of these raw materials by other sectors as a critical factor influencing their price (N = 7, n = 30). This competition is mostly linked to the energy use of wood chips (for example in form of pellets), bark and the woody part of green waste (N = 7, n = 27). The subsidies for the energy use of biomass were said to exacerbate this competition (N = 2, n = 3). Other competing sectors mentioned were the use of wood chips and bark for construction material (N = 3, n = 4) and green compost for agriculture (N = 2, n = 2).

In addition to the price of raw materials, processing costs play a role in the price of the finished growing media constituent. These costs can be direct, such as the energy input needed for the production of wood fibres (N = 3, n = 3), or indirect, through space and time requirements, which constitutes a logistical challenge, for example in the case of the composting of green waste and bark or the loosening of coir products (N = 6, n = 13).

3.2.3. Transportation

The qualitative and quantitative importance of commentaries related to transportation issues (N = 9, n = 81) suggests that this aspect is critical in the assessment of the availability of materials. This confirms that transportation costs are a "substantial part" of the overall costs of materials, as was explicitly stated by an interviewee. The importance of transportation costs is confirmed by the difference in the use of peat between North-Western Germany, where it is extracted, and Southern Germany, where it needs to be transported. The transportation distances are determined by the regional market availability of the material considered. In addition to their effect on costs, long transportation distances also affect the reliability of the supply by increasing the risk of incidents as well as the transport time and thus the necessary notice to obtain a material (N = 5, n = 10). The interviewees also underlined the sustainability issue of long transportation distances and the importance of a local or regional supply (N = 6, n = 9). This aspect was also underlined to defend local peat over imports from the Baltics.

The transport of material for growing media is generally carried out by truck for all products, except in specific cases where companies have access to a port terminal and import peat via shipments. In addition to transportation distances, transportation costs are also directly influenced by the transportability of the material (N = 6, n = 16), which can be defined by the cost per volume unit per kilometre. The transportability of the material seems to be determined by the density of the material, which can limit the amount of material per load and increase the fixed costs per cubic meter transported. Especially for green compost, the high bulk density was presented as a limiting factor for its transportation (N = 2, n = 4). For material for which the density does not limit the volume to be transported, the possibility to compress it can lead to a decrease in the fixed costs per load, as it is the case for peat, wood fibres or coir products. The transport of some materials, such as peat or coir products, over long distances (>1000 km), when others are only locally sourced, such as green compost or transported bark (<150 km), can be explained by their properties and thus their value as growing media constituents, in addition to their transportability. The possibility to optimise logistics by avoiding return trips can be a factor to reduce costs, which is enabled by exchanges with partners or suppliers.

3.2.4. Reliability of the Supply

The demand for growing media is seasonal (N = 4, n = 7), with most of it occurring between January and May. Especially for plant growers who need to optimise their cultivation system and have limited storage capacity, a reliable supply of the growing media is

crucial. As a consequence, in order to assure the reliability of their delivery and production (time, quality and amounts), growing media producers are dependent on two strategies: a reliable supply in the short term and the storage of materials in advance.

When relying on a short-term supply, growing media producers can be exposed to supply shortages when the market offer is limited (N = 4, n = 11), which was mentioned for wood fibres, bark, green compost and certain qualities of peat. The competition with other sectors can lead to limited amounts being available on the market and induce shortages. In opposition to transactions on the free market, a long-term relationship with suppliers, formalised through contracts, cooperation or shareholding, is a facilitating factor for the organisational challenges and risk management (N = 9, n = 28). A long-term relationship with the supplier also enables optimisation of the products to the producer's needs and brings security in the quality of the product. Long-term contracts for the supply guarantee price stability but imply higher prices. The relationship with the supplier is also facilitated by the local nature of the relationship. The supply can also be facilitated when assured by companies within the same consortium or group (N = 5, n = 7). Some interviewees also mentioned the number of vehicles available for transport (their own or from freight companies) as potentially limiting during the production season (N = 3, n = 3).

3.2.5. Internal Storage and Processing Capacities

The possibility of storing material in advance is another way to gain flexibility and to avoid production shortages due to the difficulties linked to problems in logistics, market availability or high material prices (N = 4, n = 8). The storability of the material can be a problem or imply a specific form of storage (packed, under roof). In particular, the sensibility of wood fibres to biological activity represents an inconvenience for open-air storage (N = 2, n = 4). Some packaging, such as big bales, can also provide more efficiency in storage and a better storability.

The ability to process raw materials themselves also constitutes a strong advantage for growing media producers (N = 8, n = 32). This enables a reduction in transportation distances and gives more control over the quality of the product through process monitoring, which is particularly interesting for compost to limit quality problems and transportation. The control over the process gives more flexibility in terms of the quality of the raw materials used, as opposed to buying a finished product. Raw materials are also generally more available on the market than finished products. For example, wood chips can be locally sourced, whereas wood fibres generally have to be transported over long distances (Figure 4). Moreover, processing capacities limit costs by internalising the added value. In some cases, space is scarce for the companies planning to increase processing capacity or increasingly relying on external supply. The relevance of space availability and management was summarised by an interviewee by the following statement: "Almost every problem [. . .] can be solved with space".

The development of processing and storage facilities implies investments, for which the decision is based on the costs, the volume of material needed, the future market situation of materials and the time needed for the equipment to be usable (N = 5, n = 7). The regulatory processes needed to obtain authorisations were mentioned as a burden for the construction of composting facilities and machinery equipment for the production of wood fibres (N = 3, n = 5).

In the current situation, growing media producers are dependent on the activity of the sawmill industry for the supply of wood chips and bark and on the disposal of green waste from gardens and green spaces for green compost (N = 4, n = 15). Only through peat extraction can growing media producers have control of the production process. This possibility, in addition to the storage of peat on the extraction site in form of piles, constitutes a considerable advantage for the flexibility and the reliability of the supply (N = 1, n = 3).

3.2.6. Political and Long-Term Situation

The interviewees underlined the importance of the future evolution of resources (N = 7, n = 16) and the evolution of the political context (N = 7, n = 21) on their long-term strategy for a reliable supply. In particular, the interviewees identified the development of policy in Germany and potentially in Europe in the future as a threat for sourcing and using peat and an important driver of change (N = 7, n = 18). This was summarised by an interviewee in the following: "without this political pressure, the peat industry would never move". The environmental and social concerns are the drivers behind this political factor, as it is for the evolution in customer demand. Some interviewees see political threats to the future use of coir products due to environmental critics, as it is the case in Switzerland (N = 2, n = 3). Additionally, growing media producers mentioned the risk, in the long term, of relying on only peat and the security brought by an enlargement of the resource base (N = 4, n = 5). Questions on the evolution of raw material resources due to economic evolutions, especially for wood chips and bark due to the decreasing activities of the sawmill industry or the decreasing amounts of coniferous trees in German forests were mentioned (N = 5, n = 6). Even if potential amounts are unlikely to limit the supply, these evolutions could trigger economic competition for these resources.

3.3. Assessment of Materials

All the relevant factors for the use of growing media constituents are not only linked to the nature and the situation specific to the constituents but often also depends on the internal resources of the companies, for example space. In order to assess each constituent, the critical factors specific to growing media constituents were selected in order to create the matrix available in Figure 5.

Criteria	Comments	Wood fibres	Composted bark	Green compost	Coir products	Peat
General suitability for plant growth	(N=8, n=33) more important for plant production	Negative (N=3, n=5) limited share possible, N-immobilisation / Positive (N=1, n=1) good structure	Positive (N=2, n=2) stability, pH / Negative (N=1, n=1) limited share	Negative (N=4, n=6) limited share possible, Salt content, high pH	Positive (N=2, n=2) fibre structure necessary to replace peat / Negative (N=2, n=2) structure	Positive (N=8, n=21) structure, pH, water capacity, considered as reference / Negative (N=1, n=1) quality limitation of black peat
Actual quality available	(N=7, n=19) including aspects regarding uniformity, constancy of quality		Positive(N=1, n=1) constancy / Negative (N=1, n=1) small production units	Negative (N=5, n=12) lack of qualitative production, share of biowaste, impurities	Positive (N=1, n=1) constancy / Negative (N=1, n=1) lack of transparency	Negative (N=3, n=3) quality depends on weather conditions, quality problems Baltic peat
Market demand	(N=7, n=13) concerns more directly the hobby sector (direct commercialisation of growing media)				Negative (N=1, n=1) label cocopeat-free	Negative (N=7, n=13) increasing demand label peat-free and peat-reduced
Competition with other sectors	(N=7, n=30) influence on price of raw material, market availability	Negative (N=6, n=17) wood chips: Energy, pellets, wood product industry	Negative (N=5, n=7) energy use of bark (although low energy value)	Negative (N=5, n=7) energy use of green waste, agriculture use of compost		Negative (N=2, n=2) energy use of peat (Finland)
Transportation distances and reliability	(N=9, n=58) including regional market availability and transport reliability. Direct influence on transportation costs	Positive (N=5, n=7) local/nationally produced product / Negative (N=2, n=3) lack of regional production wood fibres	Positive(N=1, n=1) local / Negative (N=1, n=1) lack of regional production	Positive (N=5, n=8) local product / Negative (N=1, n=1) lack of regional production	Negative (N=6, n=13) high costs and long transportation time from Asia, insecurity	Negative (N=5, n=10) imported peat / Positive (N=4, n=4) local peat in Northern Germany, established supply chain
Transportability	(N=6, n=16) direct influence on transportation costs, bulk density also a criteria for users	Positive (N=2, n=3) wood fibres: light and compressible / Negative (N=2, n=2) wood chips : heavy	Negative (N=1, n=1)	Negative (N=2, n=4) heavy	Positive (N=2, n=2) compressible	Positive (N=3, n=3) light and compressible
Market availability	(N=4, n=12) limit of market offer in current situation	Negative (N=2, n=4) shortages	Negative (N=1, n=1) shortages	Negative (N=2, n=2) not enough for demand	Positive (N=2, n=2) always available on the market	Negative (N=1, n=1) shortage specific quality
Resources necessary for processing	(N=7, n=29) including costs, space, time, work, knowledge. Influence of time and space on logistics	Negative (N=3, n=3) energy input / Positive (N=1, n=1) short processing time	Negative (N=3, n=4) time and space	Negative (N=6, n=14) necessary space, time and know-how	Negative (N=2, n=5) water for loosening, time and space	Positive (N=1, n=1) easy to process
Costs and conditions For processing equipment	(N=6, n=12) influence the readiness to invest for new processing facilities	Negative (N=3, n=3) investment for machine, regulatory process for permit		Negative (N=4, n=4) long regulatory process for permit for composting, investment in composting hall		
Storability	(N=2, n=10) can be influenced by storage form: big bales or roofed area	Negative (N=2, n=4) needs to stay dry	Negative (N=1, n=1) needs to stay dry	Negative (N=1, n=1) needs to stay dry		Positive (N=2, n=6) in big bales, stability, storage on extraction site
Long-term evolution of resources	(N=9, n=41) evolution of resources and of the political context	Negative (N=4, n=4) uncertainty on evolution of the activity of saw mill industry, wood use in other sectors, forest composition (less coniferous) / Positive (N=1, n=1) large amounts	Negative (N=1, n=2) uncertainty on evolution of the activity of saw mill industry		Negative (N=2, n=3) risk of political action to restrain imports (example Switzerland)	Negative (N=9, n=26) political action and expected coercive measures to restrain use, end of extraction rights in Germany / Positive (N=1, n=1) large resources in Scandinavia

Number of participants mentioning the idea: N (Positive) – N (Negative)

-9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9

Figure 5. Assessment of growing media constituents based on the interview data and using the critical criteria identified.

At the end of the interviews, the interviewees were systematically asked to rank constituents depending on the difficulties associated with the increase in their use. The results are presented in Figure 6. Wood fibre was generally assessed more critical than other peat substitutes, but the answers do not permit us to establish a general ranking for the constituents. For composted bark and green compost, the fact that the company processes the product seems to be linked to a less critical assessment of the situation. This

can be interpreted as a confirmation of the advantages provided by processing. The opinion on coir products was notably split between interviewees, with some mentioning its great potential for peat substitution and the good market availability (N = 2) and others not considering it viable based on the long transportation distances and ecological and social considerations (N = 3).

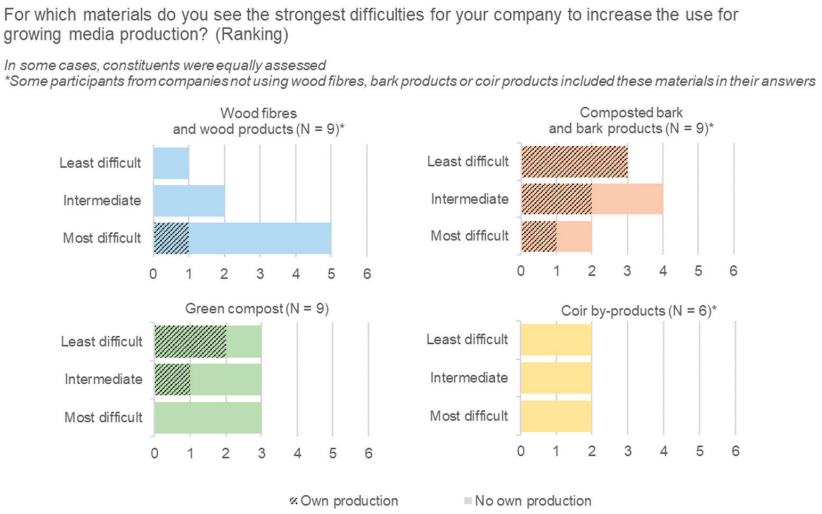

Figure 6. Ranking of growing media constituents based on the difficulty of increasing their use.

The interviewees mentioned the development or the use in limited amounts of other constituents. Among them, fresh *Sphagnum* moss from paludiculture or semi-natural mires (N = 3) and products based on *Miscanthus* (N = 3), hemp fibres (N = 3) and digestate from biomass fermentation (N = 2) were considered as having the potential to play a significant role in replacing peat in the future. In the current situation, the use of new constituents was generally said to be limited either by their price or by their quality, limiting the development of their production in significant amounts for growing media. The development of new alternative constituents was mentioned as an important and, for some participants, necessary element for the success of the future transformation towards the reduction of peat use (N = 5, n = 8).

3.4. Assessment of the Transformation

3.4.1. The "Availability Problem" as a Future Challenge

During the discussions, the interviewees expressed their general assessment of the situation of the availability of growing media constituents. Although a lot was said about the challenges, a large majority of the interviewees (N = 8) explicitly presented their current situation regarding the supply of peat substitutes for their company as not problematic. The other interviewee did not mention being in a problematic situation.

However, the majority of interviewees (N = 6, n = 31) expressed general concerns about the availability of materials in the future perspective of an increased demand for alternative constituents, often referring to the situation of the market in Germany in general. In a lot of cases, the problem was presented in terms of available amounts being limited for the whole industry, leading to an increased competition within the growing media sector. In particular, concerns were expressed that after the expected end of peat use in the hobby sector, the availability of alternative resources will be limited for the transformation of the plant production sector, which is expected to occur later. The concerns on the transformation also included a temporal aspect, with a repeated mention that solutions

would need time to develop and could not happen "overnight" ("von heute auf morgen") (N = 6, n = 13).

When asked about the difficulty of the transformation of the companies towards peat-free growing media production in 2030 as presented in Figure 7, the vast majority estimated that a complete replacement of peat was possible (N = 8); however, for some of them, only under strong changes to the current conditions (N = 3). The answers were polarised depending on the presence of peat extraction. The higher difficulty mentioned by companies with peat extraction facilities can be interpreted as the specialisation of their infrastructure and supply on peat and their focus on the professional sector.

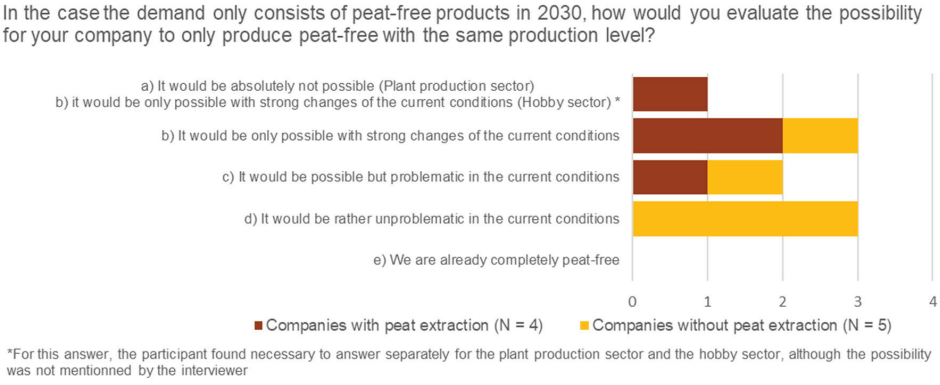

Figure 7. Assessment of the difficulty for the company to only produce peat-free products by 2030.

3.4.2. Current Limiting Factors of the Transformation

In the following paragraphs, we propose a causal chain explaining the limitation of the transformation of the resource base towards peat substitutes for the production of growing media. This causal chain is illustrated in Figure 8. This interpretation enables us to redefine the "availability problem" as the limit of the amounts of material on the market of sufficient quality, at a reasonable price and within a transportation radius allowing the growing media producers to competitively produce growing media. The amounts and quality of constituents available on the market as well as the transportation distances necessary to source these amounts are directly linked to the development of the processing infrastructure and the attention of the suppliers to the requirements of the growing media production. Additionally, the price and the market availability of the material is affected by competition with other sectors, itself exacerbated by state subsidies for the energy use of biomass. In the current situation, these aspects limit the economic interest in using more peat substitutes, which itself limits the interest of suppliers or growing media producers for producing these constituents and developing the corresponding infrastructure. In addition, regulatory burdens can slow down, disincentivise or prevent the development of new processing facilities and additional storage space.

The relative advantageous situation of peat represents a disincentive for the use of its substitutes (N = 7, n = 13). This advantage does not necessarily imply a lower price for peat compared with the other constituents but a higher ratio between quality and price.

3.4.3. Current Drivers of the Transformation

The results show that the current drivers behind the increased use of peat substitutes are apparently not due to the positive evolution of the quality and availability of growing media constituents but by the necessity to reduce peat use. Three drivers behind this current trend are identified: (1) the increasing demand for peat-reduced and peat-free products (N = 7, n = 13) triggered by the growing awareness of consumers, (2) a degradation of the economic situation of peat due to the end of peat extraction in Germany (N = 5,

n = 10) bringing numerous new disadvantages for its supply: longer transportation distance, dependence on new stakeholders abroad, loss of storage areas on extraction sites, bringing higher costs and more insecurity concerning the supply and the quality of peat (N = 5, n = 10) and (3) the threat of future coercive policy measures on peat at a German or European level (N = 7, n = 18).

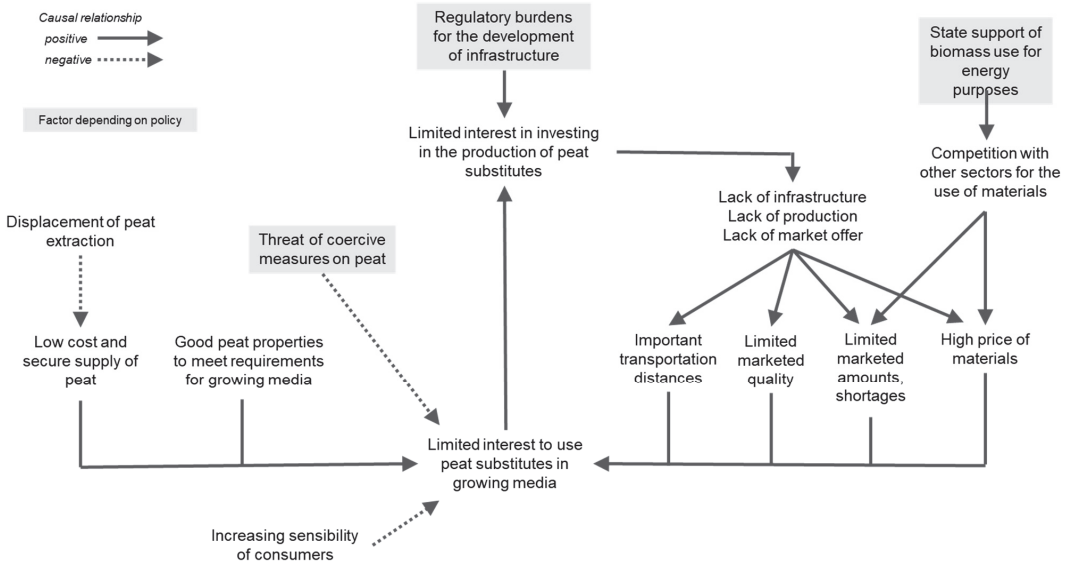

Figure 8. Causal chain explaining the limitation of the transformation of the resource base towards peat substitutes for the production of growing media.

3.4.4. Transformation in a Competitive Framework

Generally, the competitive framework within the growing media sector, especially internationally, seems to play an important role in the assessment of the difficulties related to the reduction in peat use (N = 5, n = 13). The interviewees explicitly stated, while supporting the idea of reducing peat use and even coercive measures, the need for such strategy to apply to all stakeholders, including importers of growing media (N = 5, n = 9). Thus, the price of growing media seems to be more critical due to the competition within the sector, nationally and internationally, than due to the willingness of customers to pay. This would also mean that for a company, increases in prices are not necessarily critical if they also affect the competition. For example, an interviewee noted that the strong price increase for growing media and other resources in 2022 concerning the whole market did not lead to negative changes in the demand for growing media for the company (in this case mostly for the professional horticultural sector).

3.5. Specific Situation of 2022

As for numerous sectors of the European economy, the year 2022 was a particular year for the German growing media industry due to the consequences of the war in Ukraine. A differentiation between aspects and their importance specifically linked to this situation and those applying more generally and in the long term was not completely possible. The interviewees mentioned several aspects specifically linked to the year 2022. First, they mentioned a general increase in material prices due to the energy crisis (N = 6, n = 9) leading to an increased competition for biomass, the abrupt stop of peat imports from Russia and Belarus (N = 1, n = 1) and competition for compost with the agricultural use due to high fertiliser prices (N = 1, n = 1). Additionally, an increase in transportation costs were mentioned due to high fuel prices and logistical problems (N = 2, n = 3). For the

interviewees, the situation had already improved compared with earlier in the year, but the time needed for a way out of the situation was uncertain. In general, the economic situation, although bringing difficulties, was not said to be particularly critical. There was one mention of good economic results during the COVID-19 period in the previous years (N = 2, n = 2), which were accompanied by a strong increase in growing media production in Germany. This could partially explain the capacity of the industry to overcome the crisis in 2022.

4. Discussion

4.1. Validity of Preliminarily Calculated Potentials

In this paragraph, we discuss, in the light of the present results, the validity of the potential amounts presented in Hirschler et al. [26], based on physical amounts of resources for the production of peat substitutes. The results show that quality problems for green compost represent a limitation for its use as a growing media constituent. The issues linked to the presence of biowaste and impurities can be considered avoidable by improving waste management in the future. In order to compensate for the seasonal variability of the composition of green waste, larger processing facilities could enable mixing of different charges of green waste to obtain a more suitable homogeneous quality. Therefore, the entire green waste supply can be considered as potentially usable for the growing media industry with a development of the supply chain. The potential for green compost presented in Hirschler et al. can be still considered valid. No further limitations due to the quality of raw materials were found for the other constituents—wood fibres, composted bark and coir products.

The results show further challenges linked to the economic situation that could be included in further work on the potential to identify possible limitations of a complete transformation and the conditions necessary to overcome them. In particular, the limitations due to transportation distances could be included by considering the geographic repartition of potential and the infrastructure for processing growing media and its constituents. Since the majority of the growing media production takes place in Lower Saxony, such research would assess the possibility to regionally source renewable materials or if substituting peat while maintaining production implies a decentralisation of the industry.

4.2. Future Evolution of Transformation Factors

In this paragraph, we discuss the evolution of drivers and limiting factors and their influence on the future reduction of peat use.

The first driver concerns the increasing price and insecurity of the peat supply associated with the end of domestic extraction. A total displacement of peat extraction is expected to occur after 2040. Since the peat industry in the Baltic states plans to extend peat extraction in order to supply the growing Asian market, it is to be assumed that peat resources in Europe will not be limited in the next decades. As a consequence, the displacement process is very likely to lead to a partial reduction in peat use, as the examples of other Western European countries relying on imports show [5]. A limitation could occur though political action and would be only feasible within the establishment of a European strategy. The evolution of market demand, another driver of the reduction in peat use, depends on the sensibility of consumers regarding peat-free products and the readiness of retailers to increase their offerings. According to the interviews carried out for the HOT project in Germany, a third of hobby gardeners consider peat-free as a criterion for their choice of potting soil [64]. It is questionable that this sensibility will apply to the entire consumer market in the future. The influence of these drivers is expected to be more important for the hobby market than for the professional market, with higher economic pressure and quality requirements, which can already be observed by the different evolutions of the peat rate between 2019 and 2022 (Figure 2).

Parallelly, the transformation will only be possible with the development of the infrastructure for the production, the processing and the storage of peat substitutes. The

rapidity of this transformation depends on the economic pressure on peat, the evolution of the competition with other sectors and the regulatory framework for the construction of facilities for the processing of peat substitutes. Due to the increasing need for biomass in the future in other economic sectors (for example energy and construction), in order to attain climate goals, it is unlikely that the market for bio-based raw material will, without intervention, advantageously develop for the growing media industry.

Finally, other aspects considered secondary in our analysis participate in enabling or accelerating the transformation and could gain importance in the future. Further education and more information on constituents and peat-free growing media could be needed, especially for consumers and professional users. Parallelly, the development of growing media analysis could also increase the capacity of professional growers to adapt to changing properties. Additionally, the development of technologies and market availability of other constituents could bring new possibilities. However, our research shows that even if new constituents can be developed, for example with processing technology, the limit to their use in growing media is generally because of their costs in comparison with the price of peat and ease of availability. This is typically the case for *Sphagnum* moss produced from paludiculture, which presents very good properties as a growing media constituent but whose production costs are high and whose development depends on a large-scale rewetting of peatlands [65].

4.3. Implication for the Peat Use Reduction Strategy

As stated in the previous paragraph, the consequences of the current drivers—displacement of peat supply and evolution of the sensibility of consumers—are unlikely to lead to a complete end to peat use in the timeframe set in the Peat Use Reduction Strategy. The threat of coercive measures beyond the voluntary policy is itself one of the drivers behind peat use reduction. However, this threat cannot be expected to have a sustainable effect on the industry if not followed by concrete measures.

Therefore, in the absence of measures affecting the economic situation of peat and/or its substitutes, the achievement of a peat exit can be considered unlikely or would depend on the emergence of other factors in the future.

Figure 9 illustrates, based on Figure 8, the positive evolution of the use of peat substitutes and the associated policy measures that could enable the transformation. Coercive measures could lower the attractiveness of peat, for example based on market-based instruments such as a carbon pricing on peat or a regulatory limitation or ban. Given the importance of international competition, it would be critical for such measures to affect all of the products sold in Germany including imports. A common EU policy would prevent international distortion of concurrence at a European level. Supporting measures for the use of biomass in the growing media sector could represent direct incentives and accompany the development of the supply chain of peat substitutes. This could also imply revising the distribution of subsidies between sectors in order to limit competition with the growing media sector. Such measures would need to be prepared in accordance with other policies on the use of biomass at the scale of the economy. Further investigations to reduce the regulatory burdens for the extension of the production, processing and storing infrastructure of biomass could facilitate its establishment. This especially concerns authorisation processes for composting plants and could also apply for the development of products from paludiculture such as fresh *Sphagnum* moss.

4.4. Conclusions and Further Research

Although the contribution of the reduction in peat use for the limitation of greenhouse gases emissions is widely accepted, this article brings a new perspective on the economic logic behind the use of materials in the growing media industry and the implication for the transformation toward a peat-free horticulture. This study confirms the role of environmental factors on the design of growing media, which has strongly gained importance in the last decade [12–18]. The origins of these considerations are explained from the

perspective of economic stakeholders: evolution of consumer demand and political risks as well as a focus on the local products to limit supply chain costs and insecurity. Additionally, critical factors determining the use of constituents are underlined, especially transportation distances and the competition with other sectors for the use of materials. The difficulties identified in the supply of peat substitutes are strongly linked to a lack of infrastructure for the storage and the processing of alternative materials. The results identify the economic advantage of peat over peat substitutes as a central challenge for the transformation of the growing media sector. Even if solutions for the technical implementations of the use of peat-reduced and peat-free growing media in horticulture exist and are largely documented, the factors identified in this study suggest that future evolutions will not lead to a complete substitution of peat. For this reason, political interventions making peat more expensive, for example through carbon pricing, or less available and/or supporting the use of peat substitutes and the development of the infrastructure are needed to achieve the targets of the Peat Use Reduction Strategy. Further research on the prices of materials and products would be needed to evaluate more precisely the influence of future trends and potential political measures on the relative economic advantages of constituents and on the reduction of peat use in horticulture.

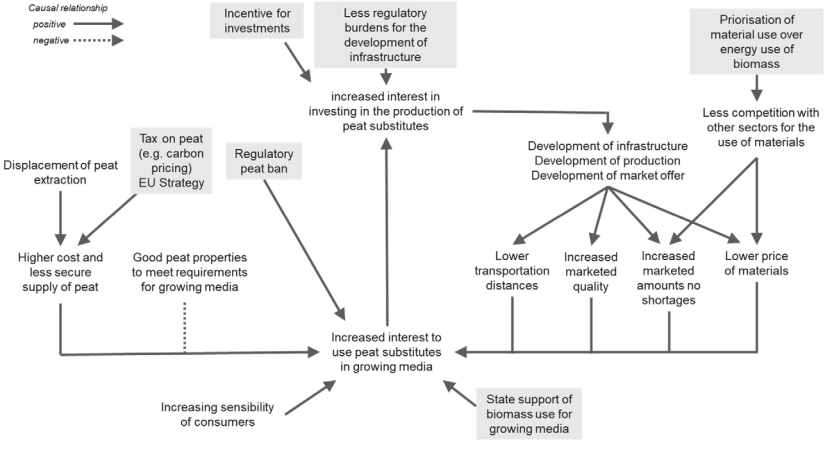

Figure 9. Causal chain explaining the potential transformation of the resource base towards peat substitutes for the production of growing media and associated policy measures.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Structure of the questionnaire.

	Question	Type of Question/Choices
1	Name:	Open field
2	Company:	Open field
3	E-mail:	Open field
4	Is your company member of the Industrieverband Garten e.V. (IVG)?	Yes/No
5	How many employees are working in your company in the growing media sector in Germany?	Multiple choice: (a) 1–5 (b) 6–10 (c) 11–20 (d) 21–50 (e) 51–100 (f) 101–200 (g) more than 200
6	Is the growing media production the main activity of you company in Germany?	Yes/No
7	How much growing media do you produce annually in Germany? • For the hobby sector: • For the landscaping sector: • For the plant production sector:	For each: Open field
8	What is the average peat rate in your growing media? • For the hobby sector: • For the landscaping sector: • For the plant production sector:	For each, multiple choice: (a) 0–5% (b) 5–20% (c) 20–40% (d) 40–60% (e) 60–80% (f) 80–100%
9	Where are your production facilities located in Germany?	For each site: Open field
10	Does your company have own peat extraction sites?	Yes/No
10b	(If yes) where are they located?	Multiple answer: (a) In Germany (b) Outside Germany
11	Does your company have the goal to increase the use of peat alternatives in growing media?	Yes/No
12	Please indicate in case your company has quantified or timely defined goals on peat reduction or increase of use of peat alternatives	Open field
13	The strategy of the Ministry of Food and Agriculture aims to end the use of peat in Germany in the hobby sector by 2026 and to reduce it to the greatest extent in the professional sector. What is the position of you company regarding these goals?	Multiple choice: (a) Very favourable (b) Rather favourable (c) Neutral (d) Rather unfavourable (e) Very unfavourable
14	You can further explain the position of your company here:	Open field
15	Do you have additional commentaries?	Open field

Table A2. Structure of the interviews.

Step	Content/Questions
Introduction	Presentation of the interviewer Presentation of the project Presentation of the background including preliminary research on potentials Presentation of the research question Do you have any commentaries or question?
Clarifying questions	Questions related to the questionnaire, especially definition for growing media in the statistics
Supply and processing chain	Growing media constituents used Own processing or not Supply chain of materials Distance from suppliers Type of suppliers Relationship to supplier / contracts
Challenges linked to the constituents	Challenges linked to the availability and the use of constituents Reasons to reduce peat use
Closed question 1	"For which materials do you see the strongest difficulties for your company to increase the use for growing media production?" Ranking: (a) wood fibres and wood products (b) composted bark and bark products (c) green compost (d) coir products
Closed question 2	"In the case the demand only consists of peat-free products in 2030, how would you evaluate the possibility for your company to only produce peat-free at the same production level?" Multiple choice: (a) It would be absolutely not possible (b) It would be only possible with strong changes of the current conditions (c) It would be possible but problematic in the current conditions (d) It would be rather unproblematic in the current conditions (e) We are already completely peat-free
Closing discussion	What would be the solutions to the challenges mentioned regarding the reduction of peat use? Who would be responsible for implementing these solutions? Are there things that we did not mention in the interview and that would be worth mentioning?

References

1. Secretariat of the United Nations Framework Convention on Climate Change. Greenhouse Gas Inventory Data—Peat Extraction. Flexible Queries Annex I Countries. Available online: https://di.unfccc.int/flex_annex1 (accessed on 6 May 2022).
2. Peano, L.; Loerincik, Y.; Margni, M.; Rossi, V. Comparative Life Cycle Assessment of Horticultural Growing Media Based on peat and Other Growing Media Constituents: Final Report. 2012. Available online: https://warumtorf.kundenprojekt.info/fileadmin/user_upload/comparative-life-cycle-assessment-of-horticultural-growing-media-based-on-peat-and-other-growing-media-constituents.pdf (accessed on 30 May 2022).
3. Stucki, M.; Wettstein, S.; Mathis, A.; Amrein, S. *Erweiterung der Studie «Torf und Torfersatzprodukte im Vergleich»: Eigenschaften, Verfügbarkeit, Ökologische Nachhaltigkeit und Soziale Auswirkungen, Wädenswil*; ZHAW Zürcher Hochschule für Angewandte Wissenschaften: Winterthur, Switzerland, 2019. [CrossRef]
4. Lindholm, T.; Heikkilä, R. Destruction of mires in Finland. In *The Finnish Environment [Online]*; Finnish environment Institute: Helsinki, Finland, 2006; pp. 179–192. Available online: https://www.researchgate.net/publication/261070498_Destruction_of_mires_in_Finland (accessed on 26 July 2023).
5. Hirschler, O.; Osterburg, B. Peat extraction, trade and use in Europe: A material flow analysis. *Mires Peat* **2022**, *28*, 1–27. [CrossRef]
6. Schmatzler, E. Die Torfindustrie in Niedersachsen—Ergebnisse einer Umfrage zur Zukunft der Torfgewinnung in Niedersachsen. *TELMA-Berichte der Deutschen Gesellschaft für Moor-und Torfkunde* **2012**, *42*, 27–42.

7. Caspers, G.; Schmatzler, E. Vorkommen und Verwendung von Torf in Deutschland. *TELMA Beiheft zu den Berichten der Deutschen Gesellschaft für Moor-und Torfkunde* **2009**, *39*, 75–98.
8. Secretariat of the United Nations Framework Convention on Climate Change. *Adoption of the Paris Agreement. I: Proposal by the President (Draft Decision)*; United Nations Office: Geneva, Switzerland, 2015.
9. Parish, F.; Sirin, A.; Charman, D.; Joosten, H.; Minayeva, T.; Silvius, M.; Stringer, L. *Assessment on Peatlands, Biodiversity and Climate Change: Main Report*; Global Environment Centre: Kuala Lumpur, Malaysia; Wetland International: Wageningen, The Netherland, 2008; ISBN 978-983-43751-0-2.
10. Leifeld, J.; Menichetti, L. The underappreciated potential of peatlands in global climate change mitigation strategies. *Nat. Commun.* **2018**, *9*, 1071. [CrossRef]
11. Joosten, H.; Tapio-Biström, M.-L.; Tol, S. *Peatlands—Guidance for Climate Change Mitigation through Conservation, Rehabilitation and Sustainable Use*, 2nd ed.; Food and Agriculture Organization of the United Nations: Rome, Italy, 2012; ISBN 978-92-5-107302-5.
12. Prasad, M. Review of the Use of Peat Moss in Horticulture: Final Report of the Chairman of the Working Group. 2021. Available online: https://assets.gov.ie/213283/b110a6f6-bebf-4496-9c76-92f3cf0faf35.pdf (accessed on 26 July 2023).
13. Gruda, N. *Advances in Horticultural Soilless Culture*; Burleigh Dodds Science Publishing Limited: Cambridge UK, 2020; ISBN 9781003048206.
14. Gruda, N. Sustainable peat alternative growing media. *Acta Hortic.* **2012**, *927*, 973–979. [CrossRef]
15. Gruda, N.S. Current and Future Perspective of Growing Media in Europe. *Acta Hortic.* **2012**, *960*, 37–43. [CrossRef]
16. Schmilewski, G. Producing growing media responsibly to help sustain horticulture. *Acta Hortic.* **2014**, *1034*, 299–306. [CrossRef]
17. Gruda, N.S. Advances in Soilless Culture and Growing Media in Today's Horticulture—An Editorial. *Agronomy* **2022**, *12*, 2773. [CrossRef]
18. Savvas, D.; Gruda, N. Application of soilless culture technologies in the modern greenhouse industry—A review. *Europ.J.Hortic.Sci.* **2018**, *83*, 280–293. [CrossRef]
19. Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety. Climate Action Plan 2050: Principles and Goals of the German Government's Climate Policy. 2016. Available online: https://www.bmuv.de/fileadmin/Daten_BMU/Pools/Broschueren/klimaschutzplan_2050_en_bf.pdf (accessed on 15 June 2023).
20. Federal Ministry of Food and Agriculture. Going Peat-Free, Protecting the Climate: The Peat Use Reduction Strategy of the Federal Ministry of Food and Agriculture. 2022. Available online: https://www.bmel.de/SharedDocs/Downloads/EN/Publications/peat-use-reduction-strategy.pdf?__blob=publicationFile&v=6 (accessed on 9 June 2023).
21. UK Government. England Peat Action Plan. 2021. Available online: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1010786/england-peat-action-plan.pdf (accessed on 26 July 2023).
22. Federal Council of Switzerland. Bericht des Bundesrates in Erfüllung des Postulats 10.3377 Diener Lenz «Torfausstiegskonzept». 2012. Available online: https://www.newsd.admin.ch/newsd/message/attachments/29089.pdf (accessed on 26 July 2023).
23. Norwegian Ministry of Climate and Environment. Norway's Climate Action Plan for 2021–2030: Meld. St. 13 (2020–2021) Report to the Storting (White Paper). 2022. Available online: https://www.regjeringen.no/contentassets/a78ecf5ad2344fa5ae4a394412ef8975/en-gb/pdfs/stm202020210013000engpdfs.pdf (accessed on 9 June 2023).
24. Ministry of Agriculture, Nature and Food Quality; Ministry of Economic Affairs and Climate Policy; Netherlands Association of Potting Soil and Substrate Manufacturers; Netherlands Agricultural and Horticultural Association; Greenhouse Horticulture Netherlands; Plantum; Tuinbranche Nederland; Dutch Association of Biowaste Processors; Dutch Association of Wholesalers in Floricultural Products; Dutch Flower Auctions Association; et al. Covenant on the Environmental Impact of Potting Soil and Substrates. 2022. Available online: https://turfvrij.nl/wp-content/uploads/2023/02/Convenant-english.pdf (accessed on 9 June 2023).
25. Bundesministerium für Landwirtschaft, Regionen und Tourismus. Moorstrategie Österreich 2030+. 2022. Available online: https://info.bml.gv.at/dam/jcr:b1db9395-5df4-4863-8d3b-f0d97b83cc67/Moorstrategie%20%C3%96sterreich%202030+.pdf (accessed on 9 June 2023).
26. Hirschler, O.; Osterburg, B.; Weimar, H.; Glasenapp, S.; Ohmes, M.-F. *Peat Replacement in Horticultural Growing Media: Availability of Bio-Based Alternative Materials: Thünen Working Paper 190*; Thünen Working Paper; Johann Heinrich von Thünen Institute: Braunschweig, Germany, 2022. [CrossRef]
27. Gruda, N.S.; Hirschler, O.; Stuart, J. Peat reduction in horticulture—An overview of Europe. *Acta Hortic.* **2023**, *in print*.
28. Council of the European Union. Agriculture and Fisheries Council. 24 May 2022. Available online: https://www.consilium.europa.eu/en/meetings/agrifish/2022/05/24/ (accessed on 9 June 2023).
29. *Regulation (EU) 2018/841 of the European Parliament and of the Council of 30 May 2018 on the Inclusion of Greenhouse gas Emissions and Removals from Land Use, Land Use Change and Forestry in the 2030 Climate and Energy Framework, and Amending Regulation (EU) No 525/2013 and Decision No 529/2013/EU (Text with EEA Relevance)*; O.J. (L 156) 1; European Union: Maastricht, The Netherlands, 2018.
30. Growing Media Europe. The Role of Peat in Growing Media. Available online: https://www.growing-media.eu/peat (accessed on 9 June 2023).
31. Schmilewski, G. Growing media constituents used in the EU in 2013. *Acta Horticulturae* **2017**, *1168*, 85–92. [CrossRef]
32. *Regulation (EU) 2019/1009 of the European Parliament and of the Concil of 5 June 2019 Laying Down Rules on the Making Available on the Market of EU Fertilising Products and Amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and Repealing Regulation (EC) No 2003/2003 (Text with EEA Relevance)*; O.J. (L 170) 1; European Union: Maastricht, The Netherlands, 2019.

33. Industrieverband Garten e.V. 2020–2023 Data on (1) Use of Constituents for Growing Media for the Domestic Market, (2) Production of Growing Media and (3) Peat Extraction and Imports; Presentation and Documents. Personal communication.
34. Blok, C.; Eveleens, B.; van Winkel, A. Growing media for food and quality of life in the period 2020–2050. *Acta Hortic.* **2021**, *1305*, 341–356. [CrossRef]
35. Schmilewski, G. Growing medium constituents used in the EU. *Acta Hortic.* **2009**, *819*, 33–46. [CrossRef]
36. Martins, T.; Machado, R.; Alves-Pereira, I.; Ferreira, R.; Gruda, N. Coir-Based Growing Media with Municipal Compost and Biochar and Their Impacts on Growth and Some Quality Parameters in Lettuce Seedlings. *Horticulturae* **2023**, *9*, 105. [CrossRef]
37. Barrett, G.E.; Alexander, P.D.; Robinson, J.S.; Bragg, N.C. Achieving environmentally sustainable growing media for soilless plant cultivation systems—A review. *Sci. Hortic.* **2016**, *212*, 220–234. [CrossRef]
38. Neumaier, D.; Meinken, E. Peat substitutes in growing media—Options and limitations. *Acta Hortic.* **2015**, *1099*, 159–166. [CrossRef]
39. Gruda, N. Increasing Sustainability of Growing Media Constituents and Stand-Alone Substrates in Soilless Culture Systems. *Agronomy* **2019**, *9*, 298. [CrossRef]
40. Schmilewski, G. Kultursubstrate und Blumenerden—Eigenschaften; Ausgangsstoffe, Verwendung, Industrieverband Garten e.V., Düsseldorf, Germany. 2018. Available online: https://substratbuch.ivg.org/static/flipbook/flipbook.html (accessed on 26 July 2023).
41. Carlile, W.R.; Cattivello, C.; Zaccheo, P. Organic Growing Media: Constituents and Properties. *Vadose Zone J.* **2015**, *14*, vzj2014.09.0125. [CrossRef]
42. Laun, N.; Weinheimer, S.; Lutz, F.; Emmel, M.; Gruda, N. Peat reduced substrates for vegetable seedlings. *Acta Hortic.* **2021**, *1321*, 23–30. [CrossRef]
43. Gruda, N.S.; Fernández, J.A. Optimising Soilless Culture Systems and Alternative Growing Media to Current Used Materials. *Horticulturae* **2022**, *8*, 292. [CrossRef]
44. Muro, J.; Irigoyen, I.; Samitier, P.; Mazuela, P.; Salas, M.C.; Soler, J. Wood Fiber as Growing Medium in Hydroponic Crop. *Acta Hortic.* **2005**, *697*, 179–185. [CrossRef]
45. Jackson, B.E.; Wright, R.D. Pine tree substrate: An alternative and renewable substrate for horticultural crop production. *Acta Hortic.* **2009**, *819*, 265–272. [CrossRef]
46. Woznicki, T.; Kusnierek, K.; Sønsteby, A. Performance of wood fibre as a substrate in hydroponic strawberry production under different fertigation strategies. *Acta Hortic.* **2021**, *1309*, 289–296. [CrossRef]
47. Beretta, D.; Ripamonti, M. Evaluation of wood fiber as component of substrates for container nursery crops. *Acta Hortic.* **2021**, *1305*, 77–82. [CrossRef]
48. Machado, R.M.A.; Alves-Pereira, I.; Ferreira, R.; Gruda, N.S. Coir, an Alternative to Peat—Effects on Plant Growth, Phytochemical Accumulation, and Antioxidant Power of Spinach. *Horticulturae* **2021**, *7*, 127. [CrossRef]
49. Gaudig, G.; Krebs, M.; Prager, A.; Wichmann, S.; Barney, M.; Caporn, S.J.M.; Emmel, M.; Fritz, C.; Graf, M.; Grobe, A.; et al. Sphagnum farming from species selection to the production of growing media: A review. *Mires Peat* **2018**, *20*, 1–30. [CrossRef]
50. Vandecasteele, B.; Muylle, H.; de Windt, I.; van Acker, J.; Ameloot, N.; Moreaux, K.; Coucke, P.; Debode, J. Plant fibers for renewable growing media: Potential of defibration, acidification or inoculation with biocontrol fungi to reduce the N drawdown and plant pathogens. *J. Clean. Prod.* **2018**, *203*, 1143–1154. [CrossRef]
51. Nguyen, T.H. Miscanthus as Primary Feedstock for Growing Media in Soilless Cultivation. Ph.D. Dissertation, University of Bonn, Bonn, Germany, 2022.
52. Dittrich, C.; Pecenka, R.; Løes, A.-K.; Cáceres, R.; Conroy, J.; Rayns, F.; Schmutz, U.; Kir, A.; Kruggel-Emden, H. Extrusion of Different Plants into Fibre for Peat Replacement in Growing Media: Adjustment of Parameters to Achieve Satisfactory Physical Fibre-Properties. *Agronomy* **2021**, *11*, 1185. [CrossRef]
53. Both, A.K.; Helle, M.A.; Madireddy, G.; Cheung, C.L. Green Chemical Approach to Fabricate Hemp Fiber Composites for Making Sustainable Hydroponic Growth Media. *ACS Agric. Sci. Technol.* **2021**, *1*, 499–506. [CrossRef]
54. Prasad, M.; Chrysargyris, A.; McDaniel, N.; Kavanagh, A.; Gruda, N.S.; Tzortzakis, N. Plant Nutrient Availability and pH of Biochars and Their Fractions, with the Possible Use as a Component in a Growing Media. *Agronomy* **2020**, *10*, 10. [CrossRef]
55. Crippa, L.; Zaccheo, P.; Orfeo, D. Utilization of the solid fraction of digestate from anaerobic digestion as container media substrate. *Acta Hortic.* **2013**, 367–373. [CrossRef]
56. Restrepo, A.P.; Medina, E.; Pérez-Espinosa, A.; Agulló, E.; Bustamante, M.A.; Mininni, C.; Bernal, M.P.; Moral, R. Substitution of Peat in Horticultural Seedlings: Suitability of Digestate-Derived Compost from Cattle Manure and Maize Silage Codigestion. *Commun. Soil Sci. Plant Anal.* **2013**, *44*, 668–677. [CrossRef]
57. Industrieverband Garten e.V. Selbstverpflichtung der Substratindustrie zur Torfminderung, Düsseldorf, Germany. 2020. Available online: https://www.ivg.org/fileadmin/downloads/Torfminderung/IVG_Selbstverpflichtung_Torfminderung_Kurzversion.pdf (accessed on 15 June 2023).
58. Zentralverband Gartenbau e.V.; Verband Deutscher Garten-Center e.V. Empfehlung zur Torfminderung, Berlin, Germany. 2022. Available online: https://www.derdeutschegartenbau.de/wp-content/uploads/2022/08/2022_03_zvg_vdg_torfminderung.pdf (accessed on 14 June 2023).
59. Fachagentur Nachwachsende Rohstoffe e.V. TerZ—Torfersatz im Zierpflanzenbau. Available online: https://torfersatz.fnr.de/torfersatz/forschung-zu-torfersatz-im-gartenbau/torfersatz-im-zierpflanzenbau (accessed on 26 July 2023).

60. Fachagentur Nachwachsende Rohstoffe e.V. ToSBa—Torfersatz in Baumschulen: Modell- und Demonstrationsvorhaben zur Praxiseinführung von torfreduzierten Substraten in Baumschulen. Available online: https://torfersatz.fnr.de/torfersatz/forschung-zu-torfersatz-im-gartenbau/torfersatz-in-baumschulen (accessed on 14 June 2023).
61. Witzel, A. Das problemzentrierte Interview. In *Qualitative Forschung in der Psychologie: Grundfragen, Verfahrensweisen, Anwendungsfelder*; Jüttemann, G., Ed.; Beltz: Weinheim, Germany, 1985; pp. 227–255, ISBN 978-3-407-54680-7.
62. Braun, V.; Clarke, V. Thematic analysis. In *APA Handbook of Research Methods in Psychology, Vol 2: Research Designs: Quantitative, Qualitative, Neuropsychological, and Biological*; Cooper, H., Camic, P.M., Long, D.L., Panter, A.T., Rindskopf, D., Sher, K.J., Eds.; American Psychological Association: Washington, DC, USA, 2012; pp. 57–71, ISBN 1-4338-1005-0.
63. Statistisches Bundesamt. GENESIS-Online, Tabelle 42271-0002: Beschäftigte und Umsatz der Betriebe im Verarbeitenden Gewerbe: Deutschland, Jahre, Wirtschaftszweige (WZ2008 2-/3-/4-Steller). Kategorie "Torfgewinnung". Available online: https://www-genesis.destatis.de/genesis/online (accessed on 14 June 2023).
64. Herbes, C.; Lohr, D. Torffreier Hobbygartenbau bis 2026—Herausforderungen und Lösungsansätze (HOT): Konsument*innen-Wahrnehmungen und pflanzenbauliche Realität. Presentation at FNR Workshop Torminderung – eine Branche im Wandel, Online. 21 March 2023; Available online: https://veranstaltungen.fnr.de/torfminderung-eine-branche-im-wandel/rueckblick/rueckblick-21-maerz-2023 (accessed on 26 July 2023).
65. Wichmann, S.; Krebs, M.; Kumar, S.; Gaudig, G. Paludiculture on former bog grassland: Profitability of Sphagnum farming in North West Germany. *Mires Peat* **2020**, *26*, 1–18. [CrossRef]

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Coir-Based Growing Media with Municipal Compost and Biochar and Their Impacts on Growth and Some Quality Parameters in Lettuce Seedlings

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Abstract: The purpose of this study was to develop substrates with little or no peat by combining coir-based growing media with municipal compost and/or acacia biochar, two locally produced renewable resources, and to assess their effects on lettuce seedling emergence and growth, as well as their content in photosynthetic pigments and total phenols. Two experiments were carried out, the first with six mixes using compost and biochar blended with perlite, pine bark, and blonde peat to adjust some physicochemical characteristics. The mixes of coir: compost: pine bark: blonde peat (73:12:5:10, *v*/*v*) and coir: compost: biochar: blonde peat (73:12:10:5, *v*/*v*) had physicochemical characteristics closer to or within the normal range of the substrates. The presence of 12% compost and 10% biochar in the mixtures had no adverse effect on lettuce seed germination and cumulative seed emergence, which ranged from 90 to 99%. The seedling growth in those mixes was vigorous and higher than in other mixtures. Coir-based growing media with municipal solid waste compost and compost plus biochar can reduce the use of peat to a percentage of 5–10% *v*/*v* and the use of 17–22% *v*/*v* of locally produced renewable resources. In addition, mixtures affected the total phenol content in the lettuce leaves. Future research is needed to assess the behavior of seedlings after their transplantation.

Keywords: *Lactuca sativa* L.; sustainable substrate; peat alternatives; pH; electrical conductivity; seedling emergence; total phenols

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1. Introduction

Transplanting seedlings is the most-common method for vegetable crop establishment in Portugal and it is increasing worldwide. It promotes plant growth, early maturation, yield, harvest-time plant uniformity, and efficient land use. Furthermore, it may require less irrigation water and herbicides than crops established by sowing [1].

Peat or peat-rich substrates are the most-common growing medium for vegetable transplants. However, peat is a non-renewable resource and its extraction has detrimental effects on the environment and ecosystems. In addition, peatlands are natural carbon sinks [2,3]. Hence, when peat is used as a substrate, stored carbon is released, negatively affecting CO_2 balance [3]. Therefore, peat use in horticulture is restricted or regulated, mainly in some European countries [4,5]. As a result, using less peat is the primary goal of soilless-grown plants today [2,5,6].

An alternative strategy could be to use selectively collected municipal solid organic waste (MSW) and biochar, two organic resources, in substrates [7–9]. The use of MSW contributes to reducing organic waste accumulation in landfills and carbon footprint while

also boosting nutrient recycling [10–12]. Perhaps the most beneficial effect of compost inclusion in a growth medium is its nutritional contribution. Matured compost may act as a slow-release fertilizer [13]. Additionally, it might have a biostimulant effect and suppress plant diseases caused by various pathogens and pests [1,14]. Organic compost tends to have peat-like porosity and aeration properties [15].

Biochar can be a beneficial component in substrates as it can increase plant growth, reduce dependence on non-renewable substrate components, and contribute to carbon sequestration [7]. In addition, biochar can replace peat [16,17], perlite, and vermiculite without compromising seedling quality [17].

Raw materials used as growing media constituents should be free from phytotoxic compounds [18] and should demonstrate good chemical properties, such as a suitable pH [19,20] and the content of certain elements and/or salt content [5,21–23]. However, municipal organic compost and biochar have some chemical characteristics, such as pH and electrical conductivity (EC), that may negatively affect plant growth, limiting their use as stand-alone substrates. For example, most biochar often has a high pH [20,24]. The pH of biochar varies depending on the feedstock type, temperature during its production [25,26], and, as recently investigated, particle size. The fractions from the same biochar can have different pH levels [5,20]. In addition, MSW compost usually has high electrical conductivity and pH values [27].

Finally, it should be noted that plants are more sensitive to salt stress at earlier plant-growth stages (germination, seedling, establishment) than plants at later stages [28]. Therefore, organic composts may also have human pathogens. To reduce them in the future, the compost must be certified as to the raw materials used and the maximum temperature and time of exposure to these during the thermophilic phase. The use of green compost can reduce the risk of human pathogens' presence [4]. A mixture of MSW and coir could overcome its limitations. Coir has a low pH and density, good physical stability, aeration, and water-holding capacity [9,29–31]. The effects of biochar incorporation on plant growth in container substrates depend on biochar properties, plant type, percentage of biochar applied, and other container substrate components mixed with biochar [32]. For example, tomato plant heights and bell pepper (*Capsicum annuum* L.) dry weights increased with the addition of 1, 3, and 5% (*w*/*w*) to a soilless mixture of coconut fiber and tuff (volcanic ash) [33]. On the other hand, the mix of coir with MSW and biochar in a ratio of 3:1 (75% coir by volume in the mixture) decreased the EC and pH, but not to adequate levels [34].

Municipal compost and biochar are two locally produced renewable resources whose use lessens Portugal's dependency on peat and coir imports, keeps organic waste out of landfills, and reduces the carbon footprint [35] and greenhouse gas (GHG) emissions [12].

This research aimed to reduce the use of peat in substrates by investigating the suitability of coir-based substrates in combination with selectively harvested municipal solid organic waste and/or acacia biochar for successful horticultural plant cultivation. Further, we investigated the effect of mixes on lettuce seedling emergence, growth, and the content of photosynthetic pigments and total phenols.

2. Materials and Methods

Two experiments were conducted at the Center of Studies and Experimentation of Mitra in Évora, Portugal (38°57′ N. 8°32′ W. elevation 200 m).

We designed the first experiment to create mixes with the characteristics of an ideal substrate. First, we mixed a coir-based medium with municipal compost or biochar to achieve adequate substrate characteristics. Next, we added perlite, blonde peat, and pine bark to blend the mixes.

Further, we evaluated how adding fertilizer to mixes affected their physicochemical properties. Finally, we investigated the emergence and growth of lettuce seedlings and the levels of photosynthetic pigments and total phenols in the leaves.

The second experiment was realized in sequence with the first. The goal was to enhance the mixes' physicochemical properties, particularly pH and EC. Moreover, we

aimed to evaluate these effects on seedling growth, photosynthetic pigment content, and total phenol content in a lettuce.

2.1. Components of Mixes

The following components were used to make the mixtures: coir, municipal solid organic compost, acacia wood biochar, perlite, pine bark, and blonde peat.

A 100% coir pith was used. The coir had a pH of 5.5 to 6.0, an electrical conductivity (EC) greater than 1.5 dS m^{-1}, granulometry 0–10 mm, total porosity = 95% *v*/*v*, air = 25% *v*/*v*, and CEC = 60–120 meq/100 g. The physiochemical characteristics of compost (Nutrimais, Lipor Company, Baguim do Monte, Portugal) and acacia wood biochar (Ibero Massa, Oliveira de Azeméis, Portugal) are presented in an earlier study [35]. Biochar was pyrolyzed at a temperature of 400 to 500 °C. The raw materials used in the "Nutrimais" manufacturing process include horticultural products, food scraps carefully selected from restaurants, canteens, and similar establishments, forest exploitation residues (e.g., branches and foliage), and green residues (e.g., flowers, grasses, and pruning). According to the manufacturer, the compost used in this study is free of pathogens. The compost had wood fragments larger than 1 cm, which were ground down to a particle size of between 3 and 4 mm to allow for the introduction of the mixtures into the wells of the plastic trays.

Perlite (Knauf, Dortmund, Germany) has particles from 2 to 6 mm (coarse perlite), is pH-neutral, and is chemically inert. The pH and the electrical conductivity (1:5 H_2O) of the blonde peat (Greenterra Ltd., Riga, Latvia) were, respectively, 5.5 to 6.5 and 1 dS m^{-1}. The pH and the EC of the components in the mixes were measured in the aqueous extract (1:5 substrate: distilled water, *w*/*v*) according to the methodology presented by Machado et al. (2021). The nitrate (NO_3-N) levels in aqueous extracts (1:5 substrate:water, *v*/*v*) were also determined using an ion-specific electrode and meter (Crison Instruments, Barcelona, Spain).

2.2. Seedling-Growth Experiments

2.2.1. Growth Conditions and Mixes

The experiments were conducted between 27 March and 11 May 2022. The average daily temperature inside the greenhouse at the shoot seedling level ranged from 17 to 26 °C. These values were within the range of temperatures suitable for the germination of lettuce seeds (15 to 25 °C) [36].

Solar radiation ranged from 127.3 to 348.8 $W \cdot m^{-2} \cdot d^{-1}$. Seeds of lettuce (*Lactuca sativa* L. cv. Grand Rapids) with a mean germination rate of 95%, evaluated through a germination test, were used in both experiments.

Experiment 1 was carried out with twelve treatments: six mixes unfertilized and fertilized (Table 1).

Table 1. Constitution and proportion of the different components in the mixes (experiment one).

	Mixes (%, *v*/*v*)					
Mixes [1] (Treatments)	**C**	**B**	**MSW**	**P**	**Pi**	**BP**
C + B + P	84	14	-	2	-	-
C + B + Pi	70	20	-	-	10	-
C + B + Pi + BP	65	20	-	-	5	10
C + MSW + P	84	-	14	2	-	-
C + MSW + Pi	70	-	20	-	10	-
C + MSW + Pi + BP	65	-	20	-	5	10

[1]—C—Coir, B—Biochar, MSW—Municipal solid organic waste, P—Perlite, Pi—Pine bark, BP—Blonde peat.

The mixes were fertilized with a 1 g controlled-release fertilizer (6N-5.3P-10K + 1.2% Mg + 0.02% B + 0.05% Cu, 0.2% Fe, 0.06% Mn 0.02%, Mn and 0.015% Zn) l^{-1} of growing media. Each replicate's treatments (mixes) occupied two rows of plastic trays (26 wells). Each well had a volume of 25 cm^3.

The second experiment was carried out with five mixes, whose constitution is presented in Table 2. At each mix, we added 1 g of controlled-release fertilizer (6N-5.3P-10K + 1.2% Mg + 0.02% B + 0.05% Cu, 0.2% Fe, 0.06% Mn, 0.02% Mn, and 0.015% Zn) per L of growing medium.

Table 2. Constitution and proportion of the different components in the mixes (experiment two).

	Mixes (%, *v*/*v*)					
Mixes [1] (Treatments)	**C**	**MSW**	**B**	**P**	**Pi**	**BP**
C + MSW + P	85	13	-	2	-	-
C + MSW + BP	80	12	-	-	-	8
C + MSW + Pi	80	12	-	-	8	-
C + MSW + Pi + BP	73	12	-	-	5	10
C + MSW + B + BP	73	12	10	-	-	5

[1]—C—Coir, B—Biochar, MSW—Municipal solid organic waste, P—Perlite, Pi—Pine bark, BP—Blonde peat.

Both experiments were arranged in a complete randomized block design with five replicates. Each replicate's treatments (mixes) occupied two rows of plastic trays (26 wells). Each well had a volume of 25 cm^3. The seeds were manually sown in plastic trays; one seed was placed in each compartment at 1.5 cm depth and covered. Nursery trays were watered by micro sprinklers three to six times per day in order to keep the substrate well-moistened.

Fresh tap water was used for irrigation; it had an electrical conductivity (EC) of 0.3 d*S* m^{-1}, a pH of 7, and 0.10 to 0.30 mmol L^{-1} NO_3, 0.12 to 0.20 mmol L^{-1} Ca, 0.15 to 0.22 mmol L^{-1} Mg, and 2.1 mmol L^{-1} Cl and 0.7 mmol L^{-1} Na.

2.2.2. Measurements

In both experiments, the same methodology was used to evaluate the initial physicochemical properties of the mixes and their effects on the emergence and growth of seedlings. Thus, the measurements made will be presented together. The initial physicochemical characteristics of the mixtures measured were pH, EC, mass wetness, moisture content, total porosity, and bulk density. The pH and the EC were measured in the aqueous extract (1:5 substrate: water, *w*/*v)* according to the methodology presented by [9]. Moisture content, total porosity, and bulk density were determined following the methodology described in [37]. The number of seedlings that emerged in each mix of all replicates was recorded throughout the experimental period. In each treatment, six seedlings were randomly collected from each replication. In these, the weight of the root system and the shoot, the number of leaves, and the leaf area were measured. The root system of the seedlings was separated from the substrate by washing it in running water with a net underneath to avoid root loss. We measured the leaf area using a leaf area meter (LI-COR Model LI–3000A).

Leaf samples of 0.5000 g from three lettuce plants were collected from all repetitions in each treatment. The seedlings were macerated in a mortar and homogenized in 4 mL of methanol/water (90:10, *v*/*v*; MW90 extract) or methanol/water (80:20, *v*/*v*; MW80 extract) for 1 min. Aliquots of the methanolic extracts MW90 or MW80 were obtained after centrifugation at 4 °C and 6440× *g* for 5 min was preserved at −20 °C for later use [37].

The following equations were used to determine the concentration (mg/100 g FW) of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Cc) of MW90 extract, where A denotes absorbance, following [38]:

1. $\text{Chl a} = 16.82A_{665.2} - 9.28A_{652.4}$
2. $\text{Chl b} = 36.92A_{652.4} - 16.54A_{665.2}$
3. $\text{Cc} = (1000A_{470} - 1.91\text{Chl-a} - 95.15\text{Chl-b})/225$

The total phenolic compound (TPC) content in the MW80 extract was determined according to that described by [39] by reacting an appropriate volume of sample or standard with 1/10 diluted Folin–Ciocalteau reagent and 7.5% sodium carbonate. After stirring the reaction mixture in the vortex, we waited for 90 min at room temperature in the dark. The absorbance

of the chromophore then formed and was read at 760 nm. Finally, the TPC concentration, expressed as milligrams of gallic acid equivalent (GAE) per 100 g of fresh weight (FW), was calculated using a calibration curve (GAE, n = 6 concentrations from 0 to 200 mg/L).

2.2.3. Data Analysis

Data were analyzed using analysis of variance using SPSS 25 software (Chicago, IL, USA) licensed to the University of Évora. The means were separated at the 5% level using Duncan's new multiple-range test.

3. Results and Discussion

3.1. Physicochemical Characteristics of the Components

Table 3 shows some of the characteristics of the components used in the mixes. The pH and EC were relatively high in the municipal solid organic compost, as was the nitrate content. Further, also noteworthy was the high pH of biochar (8.76), while the EC was low (0.22 dS m^{-1}) (Table 3).

Table 3. Physicochemical characteristics of the components.

Components	pH	EC (dS m^{-1})	Bulk Density (g cm^{-3})	Nitrate (NO_3^-) (ppm)
Coir	5.66	1.5	0.12	-
MSW [1]	7.91	8.62	0.23	91.1
Biochar [2]	8.76	0.22	0.36	4.45
Pine bark	4.84	0.13	0.18	12.1
Perlite	7.06	0.04	0.14	-
Blonde peat	5.5	0.11	0.12	-

[1]—MSW—Municipal solid organic waste. [2]—The granulometry of the biochar was also determined through the use of sieves, as described by [40]. The biochar granulometry, expressed as a percentage by weight, was: ≥ 2 mm (28.11%); ≥1 mm < 2 mm (30.05%); ≥ 0.5 mm < 1 mm (15.60%); < 0.5 mm (26.24%).

3.2. Experiment 1

3.2.1. Initial Physicochemical Characteristics of the Mixes

The physicochemical properties of the mixes were unaffected by the interactions of treatments with the fertilizer supply (Table 4). Despite the initial pH of biochar being higher than that of MSW (Table 1), the pH of mixtures containing biochar ranged from 7.14 to 7.77, while that of mixtures including compost ranged from 7.81 to 8.09. This was probably due to the high cation-exchange capacity of composts, which increased the buffering capacity of the growing medium [41]. On the other hand, fresh biochar typically has a low CEC, as the high temperatures during pyrolysis reduce the concentration of functional groups (e.g., –OH, –COOH, –CH, and –C=O) [42].

Table 4. Physicochemical characteristics of the mixes of experiment 1.

Mixes [1]	pH	EC (d*S* m^{-1})	Bulk Density (g cm^{-3})	Mass Wetness [3] (g Water/g Substrate)	Total Porosity (%)	Moisture Content (%, *w*/*w*)
C + B + P	7.51 c [2]	1.60 c	0.18 bc	5.39 ab	97.95 a	81.06 ab
C + B + Pi	7.77 b	1.14 d	0.18 c	4.96 bc	98.75 a	79.56 bc
C + B + Pi + BP	7.14 d	0.98 d	0.18 c	5.03 bc	99.22 a	78.17 c
C + MSW + P	7.81 b	2.80 b	0.18 c	5.71 a	98.90 a	82.88 a
C + MSW + Pi	8.09 a	3.42 a	0.21 a	4.92 bc	99.28 a	81.73 ab
C + MSW + Pi + BP	7.95 ab	3.25 a	0.19 b	4.69 c	98.66 a	82.11 ab
Significance	***	***	***	***	NS	***

[1]—C—Coir, B—Biochar, MSW—Municipal solid organic waste, P—Perlite, Pi—Pine bark, BP—Blonde peat. [2]—Means followed by different letters within a column are significantly different. *** significant at $p < 0.001$ level. NS—not significant. Mean separation was performed using Duncan's multiple-range test. Means are based on four replicates. [3]—Mass wetness—the water content of a sample on a dry mass basis; this is calculated as (wet weight—dry weight)/dry weight.

Regardless of the differences, the pH values of the different blends were higher than the maximum value of the adequate range for plant growth in substrates (6.5) [43–45]. As a result, the ratios of the components in the mixes of experiment 2 (Table 2) were altered to decrease the pH.

The EC of mixes with biochar (ranging from 0.98 to 1.60 dS m^{-1}) was much lower than that of mixtures with MSW (Table 4). Regarding the mixes with biochar, compost led to increases in EC of 1.2 to 2.3 dS m^{-1}. EC values in mixes with compost ranged from 2.80 to 3.42 dS m^{-1} (Table 3), which may influence seed germination and seedling growth. Lettuce is moderately sensitive to salinity, having a salinity threshold of 2 dS m^{-1} in soil. Nevertheless, plants in their early stages (germination, seedling) are more susceptible to salt stress than plants in their later stages [28]. Although the EC value of substrates varies depending on the method used to determine it [46,47], the highest EC level in the range appropriate for growing plants in substrates is generally higher than in soil. According to Martinez and Roca [44], the appropriate range of the EC for substrates ranges from 0.75 to 3.5 dS m^{-1}, but they did not discriminate the method used to determine EC. Salinity levels ranging from 2 to 3.49 dS m^{-1} in saturated media extract are satisfactory for most plants, but the growth of some sensitive plants may be reduced [46]. The EC in mixes with MSW was lower in the mixture (coir + MSW + perlite) (2.8 dS m^{-1}) than in the other mixes. As a result, the change in the proportions of the components in this mixture in experiment 2was reduced (Table 2).

Bulk density ranged from 0.18 to 0.21 g cm^{-3}. These values were adequate for substrates [45,48,49]. However, the bulk density of an ideal substrate for vegetable seedlings should not exceed 0.4 g cm^{-3} [43].

Although perlite is generally added to the substrate to increase the proportion of large pores and reduce the water-holding capacity, the mass wetness was higher in mixes with perlite. This may be due to the low proportion of perlite added (2%) (Table 1). All mixtures had a total porosity above 85% (from 97.9 to 99.3%), which is regarded as suitable for substrates [46] (Table 4). Moisture content ranged from 78.2 to 82.8, with the coir + biochar + perlite + biochar mix having a lower moisture content.

3.2.2. Seed Emergence

The addition of fertilizer and the interaction between treatments did not affect the seedling emergence. At 5 DAS (days after sowing), the percentage of seed emergence was affected by the mixture (Figure 1). The seed emergence was higher in mixes with biochar at 5 DAS, ranging from 97 to 100%. Low rates of biochar can have a stimulatory effect on germination [42]. The seed emergence precocity was lower in the coir + MSW + blonde peat + pine bark (65:20:5:10. v/v) and coir + MSW + pine bark (70:20:10, v/v) mixes as compared to other mixtures. This may be due to the high percentage of MSW in the mixture (20%, v/v) (Table 1). Reference [50] reported that the percentage of MSW in mixes with peat affected seed emergence. However, at 16 DAS, the cumulative seedling emergence ranged from 98 to 100% and was not significantly affected by the mixes ($p < 0.05$). This indicates that the presence of MSW and biochar in percentages ranging from 14 to 20% v/v did not affect the germination since the average cumulative seedling emergence and seedling survival were higher than the average germination rate of the seeds determined (95%).

3.2.3. Photosynthetic Pigments and Total Phenols

The interaction between treatments significantly affected leaf photosynthetic pigments and total phenol content (Table 5). However, adding fertilizer appears to increase the content of chl a, chl b, total chl, and carotenoids (Cc) in all substrates. Nutrient availability is essential for photosynthetic pigment biosynthesis [51]

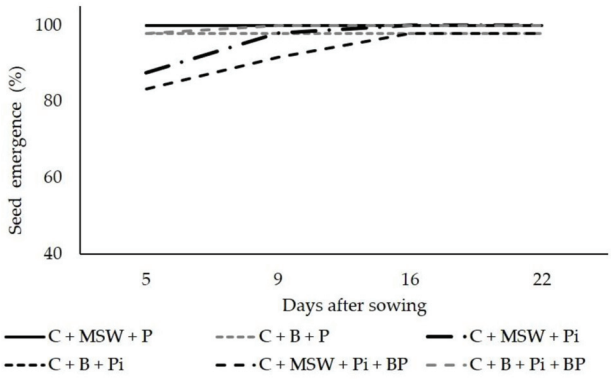

Figure 1. Influence of mixes on cumulative seedling emergence (C—Coir, B—Biochar, MSW—Municipal solid organic waste, P—Perlite, Pi—Pine bark, BP—Blonde peat).

Table 5. Effect of fertilization and mixes on leaf photosynthetic pigments and in total phenol content.

Mixes [1]	Chl a	Chl b	Chl Total	Cc	TPC
	(mg 100 g^{-1} FW)				(mg GAE 100 g^{-1} FW)
Unfertilized					
C + B + P	7.90 def [2]	9.69 d	17.59 e	5.22 d	68.16 fg
C + B + Pi	8.49 cde	8.82 d	17.32 e	6.98 c	149.37 a
C + B + Pi + BP	7.64 def	9.50 d	17.14	7.82 c	122.70 b
C + MSW + P	6.37 fg	9.57 d	15.94 ef	7.13 c	104.05 cd
C + MSW + Pi	7.40 efg	8.34 d	15.74 ef	5.50 d	67.58 fg
C + MSW + Pi + BP	5.83 g	7.98 d	13.81 f	4.84 d	115.88 bc
Fertilized					
C + B + P	14.73 a	19.18 a	33.91 a	10.40 b	68.27 fg
C + B + Pi	12.80 b	17.9 ab	30.71 b	12.88 a	62.67 fg
C + B + Pi + BP	9.28 cd	12.06 c	21.34 d	12.80 a	107.01 bcd
C + MSW + P	9.3 cd	13.49 c	22.80 d	9.18 b	77.26 ef
C + MSW + Pi	9.64 c	16.13 b	25.77 c	12.72 a	54.43 g
C + MSW + Pi + BP	10.16 c	16.29 b	26.44 c	13.40 a	93.42 de
Significance					
Fertilizer	***	***	***	***	***
Mixes	***	***	***	***	***
Interaction	***	***	***	***	***

[1]—C—Coir, B—Biochar, MSW—Municipal solid organic waste, P—Perlite, Pi—Pine bark, BP—Blonde peat. FW—Fresh weight. [2]—Means followed by different letters within a column are significantly different. *** significant at $p < 0.001$ level. NS—not significant. Mean separation was performed using Duncan's multiple-range test.

Chl a, Chl b, and total Chl contents were higher in the fertilized coir + biochar + perlite mix (Table 5). Chl a, Chl b, and total Chl contents ranged from 5.8 to 14.7, 7.98 to 19.2 and 13.8 to 33.9 mg/g of leaf fresh weight, respectively. These values were in the same range or slightly higher than those reported by [51] for lettuce seedlings. Chl b, as reported by [52], also had higher contents than Chl a.

Regarding the total content of phenols, it appears that adding fertilizer contributed to its decrease, except in the coir + biochar + perlite mix. This indicates that the seedlings may have been subjected to significant abiotic stress in the unfertilized mixtures, probably due to nutrient deficiency. Nutrient deficiency in lettuces increases total phenol content [53].

The fertilized mixes with four components, with blonde peat (10%, v/v), had the highest levels of total phenols (Table 5). The TPC in the different treatments ranged from 54.43 to 149.37 (mg GAE 100^{-1} FW). These values are lower than those mentioned in lettuce seedlings by [23], which range between 400 and 600 mg GAE 100^{-1} FW. However, as is

known, TFC is affected by many factors, including genotype, growing conditions, and others [54].

3.2.4. Seedling Growth

Growth parameters, except for dry matter %, were not significantly affected by the interaction of treatments (Table 6).

Table 6. Effect of the mix on seedling growth, experiment 1.

Mixes	Shoot Fresh Weight	Shoot Dry Weight	Seedling Total Dry Weight	Seedling Dry Weight	Leaf Area	Leaves
		(g/Plant)		(%)	(cm^2)	(N°)
Unfertilized						
C + B + P [1]	0.89 def [2]	0.09 ef	0.14 e	8.21 a	21.6 def	6.00 fg
C + B + Pi	0.67 f	0.06 f	0.09 f	6.24 b	16.75 f	5.92 fg
C + B + T + Pi	0.72 ef	0.07 f	0.09 f	6.40 b	19.02 ef	5.58 g
C + MSW + P	1.08 cde	0.10 de	0.14 de	6.03 b	28.37 cde	6.67 def
C + MSW + Pi	1.73 b	0.14 c	0.20 c	5.94 b	46.33 b	7.08 cde
C + MSW + Pi + BP	2.05 b	0.18 b	0.25 b	6.44 b	54.02 ab	7.75 bc
Fertilized						
C + B + P	1.19 cd	0.12 cde	0.18 cd	6.44 b	31.08 cd	7.58 bcd
C + B + Pi	1.21 cd	0.12 cde	0.17 cde	6.84 b	31.28 cd	7.33 cde
C + B + Pi + BP	1.22 cd	0.13 cd	0.18 cde	6.50 b	31.88 cd	6.50 efg
C + MSW + P	1.35 c	0.13 cd	0.19 c	6.48 b	35.62 c	7.33 cde
C + MSW + Pi	1.84 b	0.17 b	0.23 b	6.13 b	49.90 b	8.50 b
C + MSW + Pi + BP	2.57 a	0.22 a	0.29 a	5.97 b	66.11 a	9.50 a
Significance						
Fertilizer	***	***	***	NS	***	***
Mixe	***	***	***	*	***	***
Interaction	NS	NS	NS	*	NS	NS

[1]—C—Coir, B—Biochar, MSW—Municipal solid organic waste, P—Perlite, Pi—Pine bark, BP—Blonde peat. [2]—Means followed by different letters within a column are significantly different. * and *** significant at $p < 0.05$ and 0.001 levels, respectively. NS—not significant. Mean separation was performed using Duncan's multiple-range test.

Lettuce seedling growth was significantly affected by fertilizer addition and growing media mixtures (Table 6). In unfertilized mixes, seedlings grown in biochar grew less than those grown in mixes with MSW. This indicates that MSW contributed to seedling nutrition and that mixes with biochar have a lower ability to feed them. The EC of mixes with biochar had low EC values (0.98 to 1.60 dS m^{-1}). Biochar had a lower EC and nitrate level than MSW (Table 3). Biochar has a low content of extractable macronutrients, except for K [55]. Chrysargyris et al. [24] also reported that applying fertilizer to mixtures containing biochar can increase the growth of lettuce seedlings, but it depends on the percentage of biochar in the mix.

On the other hand, the biochar particle size of the fractions from the same biochar could also influence pH and the nutrient availability of Ca and Mg. This could lead to nutrient imbalances during the cultivation of plants [5,20]. Thus, future research is required to determine whether the lower growth of seedlings on substrates containing biochar is due to a nutritional deficit.

Additional fertilizer to the mixes with biochar increased seedling growth (Table 6). Fertilizer addition to mixes with MSW also increases the shoot and total dry weight. The total dry weight of the seedlings in mixtures containing the same proportion of MSW and biochar (Table 1), without or with fertilizer, was significantly higher in the mixtures containing MSW.

Seedling growth in coir + MSW + pine bark + blonde peat (65:20:5:10, v/v) and coir + MSW + pine bark (70:20:10, v/v) mixes was higher than that in the other mixes (Table 6). Compared to the Coir + MSW + pine bark mix, the mix with 10% blond peat (Coir + MSW +

pine bark + blonde peat) boosted shoot dry and total dry weight by nearly 30%. The seedlings grown in these mixes, despite having a pH > 7.9 and an EC > 3.2 (Table 3), presented a higher growth than those grown in the other mixes. They did not present any visual symptoms of nutrient deficiencies or excess salts and the roots were healthy (Figure 2). This may be due to the presence of humic acids, which account for 13% [34] of the dry weight of the compost, which was higher in these mixes (20%). Humic acids may contribute to the availability of nutrients, especially micronutrients, by chelating and co-transporting micronutrients to plants [56] and increasing H^+ exudation [57].

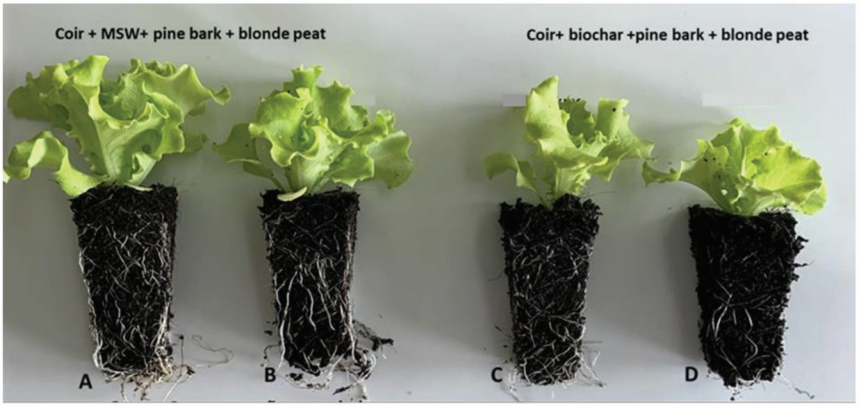

Figure 2. Seedlings grown in coir + MSW + pine bark + blonde peat (65:20:5:10, *v*/*v*) (**A**—fertilized, **B**—unfertilized) and in coir + biochar + pine bark + blonde peat (65:20:5:10, *v*/*v*) (**C**—fertilized, **D**—unfertilized).

Although plants are more sensitive in the initial phase, lettuce seedlings from the mixes with high EC (> 3.2) did not present any visual symptoms of excess salts. According to [58], the initial EC of the mixes with compost assessed in the saturated extract should not exceed 2.5 d*S* m^{-1} for tomato seedlings, which are more tolerant to salt stress than lettuce. However, the response to salinity depends on environmental conditions and the moisture content of the substrate. In the present study, the effects of salt stress may have been reduced due to mild temperatures and frequent irrigation that decrease the substrate's osmotic potential. On the other hand, humic acids may also reduce the salt stress effects since they may increase osmoprotection and ion homeostasis [59,60].

3.3. Experiment 2

3.3.1. Initial Physicochemical Characteristics of the Mixes

The average pH values were affected by the mix. The mixes with blonde peat had a lower pH than other mixes (Table 7). In the mixes' coir + organic compost + blonde peat and coir + organic compost + pine bark + blonde peat, the pH (6.56) was within the range considered suitable for substrates. In the mixture with biochar (10%. *v*/*v*), the average pH value (7.16) was slightly higher than the maximum value of the adequate range. Except for the coir + MSW + perlite mix, the goal of lowering the initial pH in the mixes with MSW was met. The mixes also affected EC, ranging from 2.44 to 2.79 dS/m (Table 7). Despite the differences in EC of the mixes, they are within an adequate range for substrates, as previously mentioned. The addition of blonde peat to the mixtures contributed to the decrease in EC (Table 7).

Bulk density ranged from 0.12 to 0.14 g/cm^3 (Table 7). As previously mentioned, these values were within an adequate range for substrates. The total porosity was not significantly affected by the mixtures and was above 85%, as required for substrates. Mass wetness was consistently higher than 6.32 g of water per g substrate in all growing media, and their values increased relative to the previous experiment.

Table 7. Physicochemical characteristics of the mixes of experiment 2.

Mixes [1]	pH	EC (dS m^{-1})	Bulk Density (g/cm^3)	Mass Wetness [3] (g Water/g Substrate)	Total Porosity (%)	Moisture (% *w*/*w*)
C + MSW + P	7.25 a [2]	2.79 a	0.12 c	7.38 a	98.58 a	75.03 b
C + MSW + BP	6.56 b	2.56 bc	0.12 c	7.18 a	98.67 a	75.12 b
C + MSW + Pi	7.17 a	2.73 b	0.13 b	6.82 b	98.52 a	77.64 a
C + MSW + Pi + BP	6.56 b	2.44 c	0.13 b	6.67 b	98.62 a	74.74 b
C + MSW + B + BP	7.16 a	2.53 bc	0.14 a	6.32 c	98.45 a	76.23 ab
Significance	***	***	***	***	NS	*

[1]—C—Coir, B—Biochar, MSW- Municipal solid organic waste, P—Perlite, Pi—Pine bark, BP- Blonde peat. [2]—Means followed by different letters within a column are significantly different. * and *** significant at $p < 0.05$ and 0.001 levels, respectively. NS—not significant. Mean separation was performed using Duncan's multiple-range test. Means are based on four replicates. [3]—Mass wetness—the water content of a sample on a dry mass basis; this is calculated as (wet weight—dry weight)/dry weight.

3.3.2. Seed Emergence

Seedling emergence in coir + compost + blond peat was lower than in the other mixes but still very high (91%) (Figure 3). At 9 DAS, the emergence was rapid in the remaining substrates, ranging from 91 to 100%. In these mixes at 23 DAS, cumulative seedling emergence ranged from 97 to 100%. The presence of MSW (12–13%) and MSW (12%) + biochar (10%) in the mixture, as in the previous experiment, had no significant effect on seed germination and cumulative seed emergence.

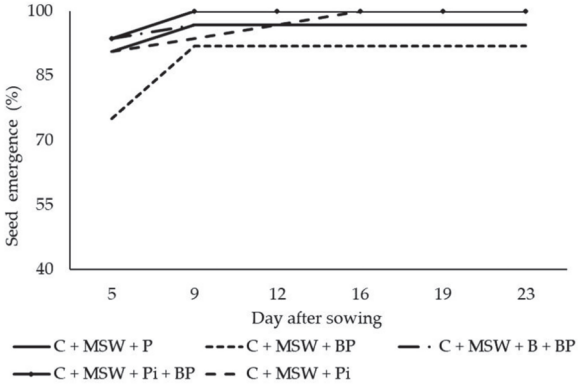

Figure 3. Influence of mixes on cumulative seedling emergence (C—Coir, B—Biochar, MSW—Municipal solid organic waste, P—Perlite, Pi—Pine bark, BP—Blonde peat).

3.3.3. Photosynthetic Pigments and Total Phenols

The mixes affected the average content of photosynthetic pigments in the leaves. For example, seedlings grown in the coir + MSW + blonde peat (80:12:8. *v*/*v*) mix had higher levels of chl a, chl b, total chl, and carotenoids in their leaves than plants grown in the other mixes (Table 8).

Chl a, chl b, and total Chl content ranged from 10.1 to 13.2, 13.5 to 15.4, and 24.8 to 29.7 mg/g of leaf fresh-weight, respectively. These values were slightly higher than those reported by [52] for lettuce seedlings.

The average TPC in the different mixes ranged from 45.79 to 70.04 mg GAE 100^{-1} FW (Table 8). These values were lower than those reported by [24] for lettuce seedlings.

The TPC of seedlings grown in the mixes coir + MSW + biochar + blonde peat (45.79 mg GAE 100 g^{-1} FW) and Coir + MSW + pine bark (49.05 mg GAE 100 g^{-1} FW) was lower than that grown in the other mixes. In lettuce seedlings grown on substrates with blonde peat and biochar, the total phenol content decreased with the addition of biochar, regardless of fertilization [24].

The TPC in the seedlings of the other mixes was higher than in previous mixes. Seedlings with high total phenol content may have a more remarkable ability to resist abiotic stress after transplantation. Thus, future research is required to assess how the seedlings from different mixes behave following transplantation.

Table 8. Effect of mix on leaf photosynthetic pigments and in total phenol content.

Mixes [1]	Chl a	Chl b	Chl Total	Cc	TPC
	(mg.100 g^{-1} FW)				(mg GAE 100 g^{-1} FW)
C + MSW + P	11.92 ab [2]	15.54 ab	27.46 ab	13.37 b	69.63 a
C + MSW + BP	13.15 a	16.50 a	29.65 a	15.28 a	62.20 a
C + MSW + Pi	11.79 ab	15.45 ab	27.23 ab	11.60 c	49.05 b
C + MSW + Pi + BP	10.10 b	13.85 b	24.84 b	9.80 d	70.04 a
C + MSW + B + BP	12.55 ab	13.53 b	26.08 ab	10.42 d	45.79 b
Significance	**	**	**	**	*

[1]—C—Coir, B—Biochar, MSW—Municipal solid organic waste, P—Perlite, Pi—Pine bark, BP—Blonde peat, FW—fresh weight. [2]—Means followed by different letters within a column are significantly different. * and ** significant at $p < 0.05$ and 0.01 levels, respectively. NS—not significant. Mean separation was performed using Duncan's multiple-range test.

3.3.4. Seedling Growth

The mixes significantly affected seedling growth, which was higher in the mixtures with four components than in the mixtures with three (Table 9). Seedling shoot (0.20 g/plant) and total dry weight (0.27 g/plant) were higher in seedlings grown in the coir + MSW + pine bark + blonde peat (73:12:5:10, v/v) mix than the other mixes. It should be noted, nonetheless, that seedling shoot fresh weight, leaf area, number of leaves, and total dry weight, grown in the mix with biochar coir + MSW + biochar + blond peat (73:12:10:5; v/v), did not differ significantly from those grown in the coir + MSW + pine bark + blonde peat mix (Table 9). However, the shoot dry weight of the seedlings grown in coir + MSW + biochar + blond peat was lower than that grown in the mix coir + MSW + pine bark + blonde peat due to a higher allocation of biomass in the root system. This may indicate that seedlings in a mixture with biochar were subject to higher growth-constraining resources than those grown in the coir + MSW + pine bark + blonde peat. When nutrients are scarce, roots may allocate more biomass [61]. The two previous mixes had the highest seedling dry-matter accumulation, and their physicochemical characteristics were within an adequate range for substrate characteristics or slightly higher in the case of pH.

Table 9. Effect of the mix on seedling growth, experiment 2.

	Fresh Weight	Dry Weight				
Mixes[1]	Shoot	Shoot	Seedling	Seedling Dry Weight	Leaf Area	Leaves
		(g/Plant)		(%)	(cm^2)	(N°)
C + MSW + P	1.96 b [2]	0.13 b	0.18 b	4.65 a	67.86 b	6.25 a
C + MSW + BP	2.12 b	0.15 b	0.20 b	4.75 a	68.83 b	6.15 a
C + MSW + Pi	2.38 ab	0.15 b	0.20 b	4.44 a	76.27 ab	6.25 a
C + MSW + Pi + BP	2.92 a	0.20 a	0.27 a	4.80 a	92.99 a	6.69 a
C + MSW + B + BP	2.49 ab	0.16 b	0.22 ab	4.61 a	81.04 ab	6.73 a
Significance	*	*	*	NS	*	NS

[1]—C—Coir, B—Biochar, MSW- Municipal solid organic waste, P—Perlite, Pi—Pine bark, BP—Blonde peat. [2]—Means followed by different letters within a column are significantly different * significant at $p < 0.05$ level. NS—not significant. Mean separation was performed using Duncan's multiple-range test.

The growth parameters do not correlate with the content of leaf photosynthetic pigments or total phenol. However, seedling total dry weight increased linearly with total shoot Chl (seedling total dry weight (g) = 0.466 × (total shoot Chl) + 0.0832, r^2 = 0.824,

$p < 0.01$) that was higher in mixes with four components. Seedling survival after transplanting is related to dry-weight accumulation. These results indicate that the mixes may use between 17 and 22% v/v of locally produced renewable resources and are suitable for lettuce seedling growth.

The percentage of compost could be further increased when green raw materials are used. For instance, ref. [62] suggests the use of up to 50% compost as a component in a growing medium. Green waste compost is made from greenhouse vegetables, nursery shrubs, branches, plant trimmings, leaves, and grass from gardens, public green spaces, and other landscapes. The woody material is chopped, mixed with the remaining green residues, and gathered in clamps [4,63].

In the remaining mixes, the shoot fresh weight, shoot total dry weight, leaf area, and number of leaves did not differ significantly ($p < 0.05$) from those mixes grown in coir + MSW + biochar + blond peat (73:12:10:5; v/v). Despite the differences in seedling growth in different mixes, all seedlings from different treatments had well-developed shoot and root systems. The seedlings presented vigorous growth without any visual symptoms of deficiencies or toxicities. These results agree with those from [64]. Lettuce seedling growth in a fine-wood fiber substrate showed a good development in root mass and a lower leaf/root dry weight ratio, even by reducing the pot size. The pot size decreased, to some degree, the quality of lettuce seedling parameters. However, no differences in lettuce yield were found after transplanting to the field [64]. According to the authors, culture methods, such as, for instance, irrigation and good root development of seedlings in wood fiber substrates, have been responsible for these results. Thus, an adapted irrigation strategy to the substrate used plays a crucial role [65,66].

As previously mentioned, in addition to affecting biomass accumulation, in our study, the mixes also affected leaf TPC, which may influence seedling tolerance to abiotic stress after transplanting. Therefore, future research will be needed to assess the behavior of the seedlings after transplantation and compare their growth to that of seedlings grown on commercial substrates.

4. Conclusions

The findings of this study show that coir-based growing media with municipal solid waste compost and compost plus biochar can reduce the use of peat to a percentage of 5–10% v/v and the use of 17–22% v/v of locally produced renewable resources. The initial EC and pH of the mixes coir + MSW + pine bark + blonde peat (73:12:5:10 v/v) and coir + MSW + biochar + blonde peat (73:12:10:5, v/v) were within or were slightly higher than the maximal values of the range considered adequate for substrates. The presence of MSW (12%) and MSW (12%) + biochar (10%) in the mixtures had no adverse effects on seed germination and cumulative seed emergence. The seedling growth in those mixes was vigorous and higher than that of those grown in other mixtures. However, further research must compare lettuce seedling growth in these and commercial mixes and their use to grow other vegetable transplants. In addition, coir-based mixes affect total phenol content. As total phenol content increases tolerance to abiotic stress, future studies are needed to evaluate the behavior of the seedlings of the mixes after transplantation.

Author Contributions: R.M.A.M. conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools, or data; and wrote the paper. T.C.M. performed the experiments and analyzed the data. I.A.-P. and R.F. performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools, or data; and wrote the paper. N.S.G. reviewed, corrected, and edited the paper. All authors have read and agreed to the published version of the manuscript.

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References

1. Pascual, J.A.; Ceglie, F.; Tuzel, Y.; Koller, M.; Koren, A.; Hitchings, R.; Tittarelli, F. Organic substrate for transplant production in organic nurseries. A review. *Agron. Sustain. Dev.* **2018**, *38*, 35. [CrossRef]
2. Gruda, N.S. Increasing sustainability of growing media constituents and stand-alone substrates in soilless culture systems. *Agronomy* **2019**, *9*, 298. [CrossRef]
3. Gruda, N.; Bisbis, M.B.; Tanny, J. Impacts of protected vegetable cultivation on climate change and adaptation strategies for cleaner production—A review. *J. Clean. Prod.* **2019**, *225*, 324–339. [CrossRef]
4. Gruda, N.; Bragg, N. Developments in alternative organic materials as growing media in soilless culture systems. In *Advances in Horticultural Soilless Culture*; Gruda, N., Ed.; Burleigh Dodds Science Publishing Limited: Cambridge, UK, 2021; ISBN 9781786764355. [CrossRef]
5. Gruda, N.S. Advances in Soilless Culture and Growing Media in Today's Horticulture—An Editorial. *Agronomy* **2022**, *12*, 2773. [CrossRef]
6. Atzori, G.; Pane, C.; Zaccardelli, M.; Cacini, S.; Massa, D. The Role of Peat-Free Organic Substrates in the Sustainable Management of Soilless Cultivations. *Agronomy* **2021**, *11*, 1236. [CrossRef]
7. Dumroese, R.K.; Heiskanen, J.; Englund, K.; Tervahauta, A. Pelleted biochar: Chemical and physical properties show potential use as a substrate in container nurseries. *Biomass Bioenergy* **2011**, *35*, 2018–2027. [CrossRef]
8. Steiner, C.; Harttung, T. Biochar as a growing media additive and peat substitute. *Solid Earth* **2014**, *5*, 995–999. [CrossRef]
9. Machado, R.M.A.; Alves-Pereira, I.; Ferreira, R.; Gruda, N.S. Coir, an Alternative to Peat—Effects on plant growth, phytochemical accumulation, and antioxidant power of spinach. *Horticulturae* **2021**, *7*, 127. [CrossRef]
10. Veeken, A.; Adani, F.; Fangueiro, D.; Jensen, L.S. *The Value of Recycling Organic Matter to Soils: Classification as Organic Fertiliser or Organic Soil Improver*; EIP-AGRI Focus Group—Nutrient Recycling: Brussels, Belgium, 2017; pp. 8–10. Available online: https://ec.europa.eu/eip/agriculture/sites/agri-eip/files/fg19_minipaper_5_value_of_organic_matter_en.pdf (accessed on 14 November 2022).
11. De Corato, U. Agricultural waste recycling in horticultural intensive farming systems by on-farm composting and compost-based tea application improves soil quality and plant health: A review under the perspective of a circular economy. *Sci. Total Environ.* **2020**, *738*, 139840. [CrossRef] [PubMed]
12. Raza, S.T.; Wu, J.; Rene, E.R.; Ali, Z.; Chen, Z. Reuse of agricultural wastes, manure, and biochar as an organic amendment: A review on its implications for vermicomposting technology. *J. Clean. Prod.* **2022**, *360*, 132200. [CrossRef]
13. Lazcano, C.; Arnold, J.; Tato, A.; Zaller, J.G.; Domínguez, J. Compost and vermicompost as nursery pot components: Effects on tomato plant growth and morphology. *Span. J. Agric. Res.* **2009**, *7*, 944–951. [CrossRef]
14. Vallance, J.; Déniel, F.; Floch, G.L.; Guérin-Dubrana, L.; Blancard, D.; Rey, P. Pathogenic and beneficial microorganisms in soilless cultures. In *Sustainable Agriculture*; Lichtfouse, E., Hamelin, M., Navarrete, M., Debaeke, P., Eds.; Springer: Dordrecht, The Netherland, 2011; Volume 2, pp. 711–726.
15. Fascella, G. Growing substrates alternative to peat for ornamental plants. In *Soilless Culture—Use of Substrates for the Production of Quality Horticultural Crops*; Asaduzzaman, M., Ed.; Intech Publication: Rijeka, Croatia, 2015; pp. 47–67. [CrossRef]
16. Vaughn, S.F.; Kenar, J.A.; Thompson, A.R.; Peterson, S.C. Comparison of biochars derived from wood pellets and pelletized wheat straw as replacements for peat in potting substrates. *Ind. Crops Prod.* **2013**, *51*, 437–443. [CrossRef]
17. Matt, C.P.; Keyes, C.R.; Dumroese, R.K. Biochar effects on the nursery propagation of 4 northen Rocky Mountain native plant species. *Nativ. Plants J.* **2018**, *19*, 14–26. [CrossRef]
18. Gruda, N.; Rau, B.J.; Wright, R.D. Laboratory Bioassay and Greenhouse Evaluation of a Pine tree substrate used as a container Substrate. *Eur. J. Hortic. Sci.* **2009**, *74*, 73–78.
19. Jackson, B.E.; Wright, R.D.; Gruda, N. Container medium pH in a pine tree substrate amended with peat moss and dolomitic limestone affects plant growth. *Hortscience* **2009**, *44*, 1983–1987. [CrossRef]
20. Prasad, M.; Chrysargyris, A.; McDaniel, N.; Kavanagh, A.; Gruda, N.S.; Tzortzakis, N. Plant nutrient availability and pH of biochar and their fractions, with the possible use as a component in a growing media. *Agronomy* **2020**, *10*, 10. [CrossRef]
21. Gruda, N.; Schnitzler, W.H. Wood fiber substrates as a peat alternative for vegetable production. *Eur. J. Wood Prod.* **2006**, *64*, 347–350. (In German) [CrossRef]
22. Gruda, N. Sustainable peat alternative growing media. *Acta Hortic.* **2012**, *927*, 973–980. [CrossRef]
23. Giménez, A.; Fernández, J.; Pascual, J.; Ros, M.; Saez-Tovar, J.; Martinez-Sabater, E.; Gruda, N.; Egea-Gilabert, C. Promising composts as growing media for the production of baby leaf lettuce in a floating system. *Agronomy* **2020**, *10*, 1540. [CrossRef]
24. Chrysargyris, A.; Prasad, M.; Kavanagh, A.; Tzortzakis, N. Biochar type. ratio. and nutrient levels in growing media affects seedling production and plant performance. *Agronomy* **2020**, *10*, 1421. [CrossRef]
25. Lehmann, J.; Rilling, M.C.; Thies, J.; Masiello, C.A.; Hockaday, W.C.; Crowley, D. Biochar effects soil biota—A review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836. [CrossRef]
26. Ding, Y.; Liu, Y.; Liu, S.; Li, Z.; Tan, X.; Huang, X.; Zeng, G.; Zhou, L.; Zheng, B. Biochar to improve soil fertility. A review. *Agron. Sustain. Dev.* **2016**, *36*, 36. [CrossRef]
27. Hargreaves, J.C.; Adl, M.S.; Warman, P.R. A review of the use of composted municipal solid waste in agriculture. *Agric. Ecosyst. Environ.* **2008**, *123*, 1–14. [CrossRef]

28. Machado, R.; Serralheiro, R. Soil salinity: Effect on vegetable crop growth. management practices to prevent and mitigate soil salinization. *Horticulturae* **2017**, *3*, 30. [CrossRef]
29. Michel, J.C. The physical properties of peat: A key factor for modern growing media. *Mires Peat* **2010**, *6*, 1–90.
30. Gruda, N.; Caron, J.; Prasad, M.; Maher, M. Growing media. In *Encyclopedia of Soil Science*; CRC Press: Boca Raton, FL, USA, 2016; pp. 1053–1058.
31. Savvas, D.; Gruda, N. Application of soilless culture technologies in the modern greenhouse industry—A review. *Eur. J. Hortic. Sci.* **2018**, *83*, 280–293. [CrossRef]
32. Huang, L.; Gu, M. Effects of biochar on container substrate properties and growth of plants—A review. *Horticulturae* **2019**, *5*, 14. [CrossRef]
33. Graber, E.R.; Harel, Y.H.; Kolton, M.; Cytryn, E.; Silber, A.; David, D.R.; Tsechansky, L.; Borenshtein, M.; Elad, Y. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. *Plant Soil* **2010**, *337*, 481–496. [CrossRef]
34. Machado, R.M.A.; Bui, H.H. Physicochemical characteristics of substrates of the mixture of coir with municipal solid waste compost and with biochar. *Acta Hortic.* **2021**, *1320*, 399–404. [CrossRef]
35. Machado, R.M.A.; Alves-Pereira, I.; Morais, C.; Alemão, A.; Ferreira, R. Effects of Coir-Based Growing Medium with Municipal Solid Waste Compost or Biochar on Plant Growth, Mineral Nutrition, and Accumulation of Phytochemicals in Spinach. *Plants* **2022**, *11*, 14. [CrossRef]
36. Almeida, D. *Manual de Culturas Hortícolas*; Editorial Presença: Lisboa, Portugal, 2006; Volume 1, pp. 196–300. ISBN 9789722335515.
37. Fonteno, W.C.; Harden, C.T. *Procedures for Determining Physical Properties of Horticultural Substrates Using the NCSU Porometer, Horticultural Substrates Laboratory*; North Carolina State University: Raleigh, NC, USA, 2003; Available online: https://www.ncsu.edu/project/hortsublab/pdf/porometermanual.pdf (accessed on 3 November 2022).
38. Lichtenthaler, H.K.; Buschmann, C. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Curr. Protoc. Food Anal. Chem.* **2001**, *1*, F4.3.1–F4.3.8. [CrossRef]
39. Bouayed, J.; Hoffmann, L.; Bohn, T. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties. Bioaccessibility and potential uptake. *Food Chem.* **2011**, *128*, 14–21. [CrossRef]
40. Bilderback, T.E.; Warren, S.L.; Owen, J.S.; Albano, J.P. Healthy substrates need physicals too! *HortTechnology* **2005**, *15*, 747–751. [CrossRef]
41. Sonneveld, C.; Voogt, W. Plant nutrition in future greenhouse production. In *Plant Nutrition of Greenhouse Crops*; Springer: Dordrecht, The Netherland, 2009; pp. 393–403.
42. Joseph, S.; Cowie, A.L.; Van Zwieten, L.; Bolan, N.; Budai, A.; Buss, W.; Cayuela, M.L.; Graber, E.R.; Ippolito, J.A.; Kuzyakov, Y.; et al. How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. *GCB Bioenergy* **2021**, *13*, 1731–1764. [CrossRef]
43. Abad, M.; Noguera, P.; Bures, S. National inventory of organic wastesfor use as growing media for ornamental potted plant production: Case study in Spain. *Bioresour. Technol.* **2001**, *77*, 197–200. [CrossRef] [PubMed]
44. Bailey, D.A.; Nelson, P.V.; Fonteno, W.C. *Substrates pH and Water Quality*; North Carolina State University: Raleigh, NC, USA, 2000.
45. Martínez, P.F.; Roca, D. Sustratos para el cultivo sin suelo. Materiales, propiedades y manejo. In *Sustratos, Manejo del Clima, Automatización y Control en Sistemas de Cultivo sin Suelo*; Flórez, R.V.J., Ed.; Editorial Universidad Nacional de Colombia: Bogotá, Colombia, 2011; pp. 37–77.
46. Bunt, B.R. *Media and Mixes for Container-Grown Plants: A Manual on the Preparation and Use of Growing Media for Pot Plants*, 2nd ed.; Springer Science & Business Media: London, UK, 2012; pp. 86–93.
47. Camberato, D.M.; Lopez, R.G.; Mickelbart, M.V. *pH and Electrical Conductivity Measurements in Soilless Substrates*; HO-237-W; Purdue University: West Lafayette, IN, USA, 2009; pp. 1–6.
48. Gruda, N.; Schnitzler, W.H. Suitability of wood fiber substrates for production of vegetable transplants. I. Physical properties of wood fiber substrates. *Sci. Hortic.* **2004**, *100*, 309–322. [CrossRef]
49. Reis, M.M.F. *Material Vegetal e Viveiros: Manual de Horticultura em Modo de Produção Biológico*; Escola Superior Agrária Ponte de Lima/Instituto Politécnico de Viana do Castelo: Refóios do Lima, Portugal, 2007; pp. 19–52.
50. Tzortzakis, N.; Gouma, S.; Paterakis, C.; Manios, T. Deployment of municipal solid wastes as a substitute growing medium component in marigold and basil seedlings production. *Sci. World J.* **2012**, *2012*, 285874. [CrossRef] [PubMed]
51. Hosseinzadeh, S.R.; Amiri, H.; Ismaili, A. Effect of vermicompost fertilizer on photosynthetic characteristics of chickpea (*Cicer arietinum* L.) under drought stress. *Photosynthetica* **2016**, *54*, 87–92. [CrossRef]
52. Shin, Y.K.; Bhandari, S.R.; Jo, J.S.; Song, J.W.; Lee, J.G. Effect of drought stress on chlorophyll fluorescence parameters, phytochemical contents, and antioxidant activities in lettuce seedlings. *Horticulturae* **2021**, *7*, 238. [CrossRef]
53. Luna, M.C.; Martínez-Sánchez, A.; Selma, M.V.; Tudela, J.A.; Baixauli, C.; Gil, M.I. Influence of nutrient solutions in an open-field soilless system on the quality characteristics and shelf life of fresh-cut red and green lettuces (*Lactuca sativa* L.) in different seasons. *J. Sci. Food Agric.* **2012**, *93*, 415–421. [CrossRef]
54. Howard, L.R.; Pandjaitan, N.; Morelock, T.; Gill, M.I. Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season. *J. Agric. Food Chem.* **2002**, *50*, 5891–5896. [CrossRef]

55. Blok, C.; Van der Salm, C.; Hofland-Ziljstra, J.; Streminska, M.; Eveleens, B.; Regelink, I.; Fryda, L.; Visser, R. Biochar for horticultural rooting media improvement: Evaluation of biochar from gasification and slow pyrolysis. *Agronomy* **2017**, *7*, 6. [CrossRef]
56. Yang, F.; Tang, C.; Antonietti, M. Natural and artificial humic substances to manage minerals, ions, water, and soil microorganisms. *Chem. Soc. Rev.* **2021**, *50*, 6221–6239. [CrossRef]
57. Zandonadi, D.B.; Canellas, L.P.; Façanha, A.R. Indolacetic and humic acids induce lateral root development through a concerted plasmalemma and tonoplast H^+ -pumps activation. *Planta* **2007**, *225*, 1583–1595. [CrossRef] [PubMed]
58. Diaz-Pérez, M.; Camacho-Ferre, F. Effect of composts in substrates on the growth of tomato transplants. *HortTechnology* **2010**, *20*, 361–367. [CrossRef]
59. Sani, M.N.H.; Yong, J.W. Harnessing Synergistic Biostimulatory Processes: A Plausible Approach for Enhanced Crop Growth and Resilience in Organic Farming. *Biology* **2021**, *11*, 41. [CrossRef] [PubMed]
60. Jiménez-Arias, D.; García-Machado, F.J.; Morales-Sierra, S.; García-García, A.L.; Herrera, A.J.; Valdés, F.; Luis, J.C.; Borges, A.A. A beginner's guide to osmoprotection by biostimulants. *Plants* **2021**, *10*, 363. [CrossRef] [PubMed]
61. Mašková, T.; Herben, T. Root: Shoot ratio in developing seedlings: How seedlings change their allocation in response to seed mass and ambient nutrient supply. *Ecol. Evol.* **2018**, *8*, 7143–7150. [CrossRef] [PubMed]
62. Prasad, M.; Maher, M.J. The use of composted green waste (CGW) as a growing medium component. *Acta Hortic.* **2001**, *549*, 107–114. [CrossRef]
63. Gruda, N. Current and Future Perspective of Growing Media in Europe. *Acta Hortic.* **2012**, *960*, 37–43. [CrossRef]
64. Gruda, N.; Schnitzler, W.H. Alternative raising systems by head lettuce. *Rep. Agric./Ber. Über Landwirtsch.* **2006**, *84*, 469–484. (In German)
65. Gruda, N.; Schnitzler, W.H. The effect of water supply on bio-morphological and plant-physiological parameters of tomato transplants cultivated in wood fiber substrate. *J. Appl. Bot.* **2000**, *74*, 233–239. (In German)
66. Gruda, N.; Schnitzler, W.H. The effect of water supply of seedlings, cultivated in different substrates and raising systems on the bio-morphological and plant-physiological parameters of lettuce. *J. Appl. Bot.* **2000**, *74*, 240–247. (In German)

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Effects of Mixes of Peat with Different Rates of Spruce, Pine Fibers, or Perlite on the Growth of Blueberry Saplings

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Abstract: Investigations of substrates for growing plant saplings is the basis for the search for new components. Currently, large numbers of saplings are grown for blueberry plantations. Studies on the use of various organic and inorganic components in substrates is relevant in order to reduce the amount of excavated peat. The goal of this study was to analyze the effects of mixes of peat with different rates of spruce, pine fibers and perlite on the growth of blueberry saplings. To define the suitability of substrates, plant vigor assessments of the cultivar 'Duke', including plant height and leaf weight, as well as the chlorophyll fluorescence, content of extractable macronutrients and organic carbon in leaves, were investigated. The best effect on the growth of blueberry saplings, the optimal content of macronutrients in the leaves, was shown for substrates in which a part of the peat was replaced by 15–45% *v*/*v* of pine wood fiber and by 15–30% *v*/*v* of spruce wood fiber. Pine bark fiber in the mix should not exceed 30% *v*/*v*. The addition of spruce bark fibers in the different rates had a negative effect on the vegetative growth of the saplings. The quantity of peat in the substrates can also be significantly reduced by adding 15–45% *v*/*v* of perlite. These results confirm that pine and spruce fibers or perlite in substrates for blueberry sapling growing could reduce the demand for peat and should significantly contribute to the preservation of unique wetland ecosystems.

Keywords: blueberry; fiber; peat; substrate; sapling

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1. Introduction

Substrates (media) for the cultivation of berry plants are an important component of a sustainable food production chain. The use of suitable growing substrates in modern industrial horticulture meets the needs of plants and ensures their productivity. Peat currently represents 77–80% of the growing substrates used annually in the horticultural industry in Europe [1]. Peat is an extremely important component in substrates; however, its extraction threatens sensitive ecosystems, causes carbon sinks, and increases greenhouse-gas emissions [2–4]. Different studies on bogs have confirmed that these ecosystems can substantially contribute to reducing atmospheric greenhouse gases [5,6].

Therefore, substrates in which peat can be replaced by alternative components of organic or mineral origin are relevant to preserving unique wetland ecosystems. The suitability of various growing substrates in horticulture has been studied, i.e., certain quality parameters have been evaluated, including the degree of decomposition, the content of extractable nutrients, pH, bulk density, electrical conductivity and porosity. Various scientific sources indicated the possibility of using tree or coconut fibers, compost, tree bark, perlite, and other components that can be mixed to create appropriate growing substrates [7,8]. When studying substrate compositions, the vegetative growth of plants should be assessed because the substrate should provide plants with an appropriate amount of water and nutrients [9].

Recently, there has been a rapid increase in interest in blueberries and cranberries in Europe and around the world. Consequently, plantations of species from the Ericaceae Juss. family have increased the demand for large quantities of planting material. Wild species of the genus *Vaccinium* L. grow in areas such as high moors and bogs, where the soil may be peaty and the pH ranges from 2.6 to 6.0 [10]. As Hoover et al. [11] reported, blueberries tolerate a wide range of soils. Notwithstanding, it was found that standard substrates containing higher amounts of fertilizers, especially nitrogen fertilizers, are not suitable for these plants [12]. When searching for substrates for blueberry, it is important to consider the characteristics of its roots. Blueberry roots do not have root hairs, and the thin roots that are responsible for water and nutrient absorption are inhabited by mycorrhiza [13].

In the production of substrates, renewable resources, such as wood chips and tree bark, can be used. Such substrates contribute to the utilization of logging waste [14,15]. Lignin, which is found in plant cell walls, degrades more slowly compared to cellulose or hemicellulose and the degradation process of the substrate also slows down [16]. Meanwhile, the bark of trees is rich in organic compounds (lignin, terpenes, fats, resins, sterols, glycosides, tannins, saccharides, acids, and others), which can change the quality of the substrates and affect the germination of plant seeds or the growth of saplings. It was determined that the quantitative and qualitative chemical composition of the bark of different tree species varies, and it is important to determine these variations. The bark's compounds can affect the chemical characteristics of the substrates differently, for example, approximately 8% mannose has been found in spruce bark and approximately 9% arabinose in pine bark [7,17]. Other studies showed that the phloem and the outer bark are richer in chemical compounds than the wood and also differ significantly among wood species [18]. Kemppainen et al. [19] investigated Norway spruce bark and detected a significant amount of tannins, 10.0%. Other researchers reported that fibrous materials are strong contenders in the replacement of peat in growing media, with a focus on the physical properties [16]. As Vandecasteele et al. [20] indicated, plant fibers have the potential for peat replacement and can provide protection against plant diseases.

Perlite is a non-renewable resource and is used throughout the world in horticultural applications. The physical and chemical properties of perlite as an component of substrates and the effect of this material on human health have been analyzed, and different studies confirmed that perlite can improve porosity and oxygenation to plant roots [21].

Based on these previous studies, we hypothesized that the mix of spruce and pine fibers or perlite additions with peat could ensure the growth and quality of blueberry saplings. In this experiment, the effects of mixes of peat with different rates of spruce or pine fibers and perlite on vegetative growth, the content of extractable macronutrients and chlorophyll fluorescence in the leaves of blueberry saplings were studied.

2. Materials and Methods

2.1. Plant Material and Substrate Composition

Five hundred saplings of the highbush blueberry cultivar 'Duke' were purchased from a commercial nursery PLANTIN (Poland). Plants were propagated in in-vitro cultures in the laboratory of this nursery and were replanted to multi-pots after acclimatization. For this experiment, saplings with 1–2 lateral branches reaching a height of 8–12 cm were used. The saplings were transplanted into 2.0 L plastic containers filled with the appropriate substrates. In each substrate variant, thirty saplings were planted.

The substrates were composed of Scots pine *Pinus sylvestris* L. and Norway spruce *Picea abies* (L.) H.Karst. wood or bark fibers and high moor peat, which were used in various proportions. The experiment included 15 treatments: five mixes (peat + fiber of pine wood, peat + fiber of spruce wood, peat + fiber of pine bark, peat + fiber of spruce bark, and peat + perlite), each with three rates (Figure 1, Table 1).

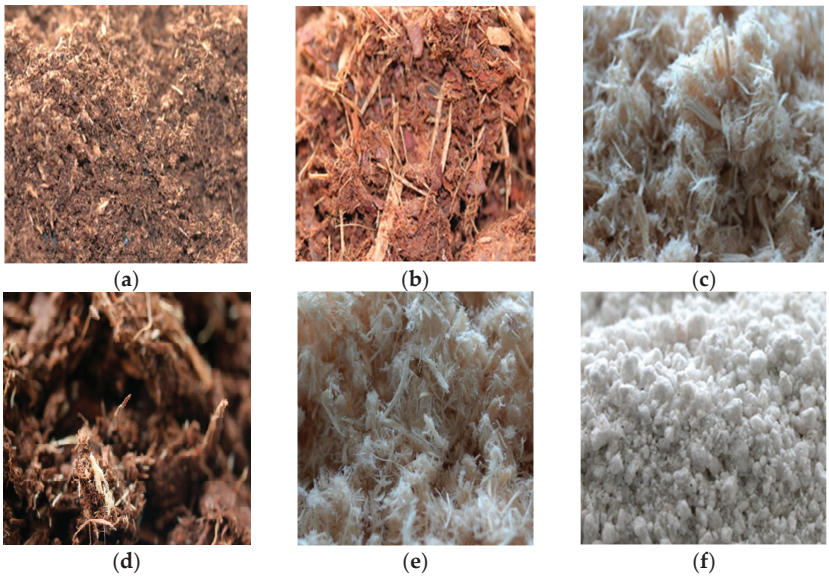

Figure 1. Components of the studied substrates: (**a**)—high moor peat, (**b**)—fiber of pine bark, (**c**)—fiber of pine wood, (**d**)—fiber of spruce bark, (**e**)—fiber of spruce wood, and (**f**)—perlite.

Table 1. Composition of the substrates.

Substrate	Substrate Variant	Perlite (%, *v*/*v*)	Peat (%, *v*/*v*)	Fiber (%, *v*/*v*)
Peat + fiber of pine wood	1	0	85	15
	2	0	70	30
	3	0	55	45
Peat + fiber of spruce wood	1	0	85	15
	2	0	70	30
	3	0	55	45
Peat + fiber of pine bark	1	0	85	15
	2	0	70	30
	3	0	55	45
Peat + fiber of spruce bark	1	0	85	15
	2	0	70	30
	3	0	55	45
Peat + perlite	1	15	85	0
	2	30	70	0
	3	45	55	0

The blueberry saplings were grown under natural light conditions in the greenhouse. The greenhouse temperature and relative humidity were maintained at 25 °C (day) and 15 °C (night) and 60%, respectively.

2.2. Content of Extractable Macronutrients and Organic Carbon and Peat Decomposition in Substrates

Before the planting of the rooted saplings, the content of extractable macronutrients was evaluated in the substrates. Additionally, the content of organic carbon and the degree of peat decomposition were assessed.

The degree of peat decomposition was determined according to LST 1957:2022 [22] and the pH was determined according to LST EN 13037 with the potentiometric method [23].

The pH values of the prepared substrate mixes ranged from 4.5 to 5.4. As Trehane [10] reported, blueberries require a soil pH between 4.0 and 5.2. The content of organic carbon ranged from 27.54% to 41.66%, and the decomposition of peat was 29.1–38.8% (Table 2).

Table 2. Chemical composition of the substrates.

Substrate	Substrate Variant	* pH	** N-NO_3 +N-NH_4, mg L^{-1}	** P, mg L^{-1}	*** K, mg L^{-1}	** Ca, mg L^{-1}	Organic carbon, %	Degree of Peat Decomposition, %
Peat + fiber of pine wood	1	4.9	2.4	0.45	4.0	15.2	39.27	33.1
	2	4.5	1.2	0.4	5.2	11.3	37.83	32.3
	3	4.9	0.6	0.48	6,2	11.0	38.17	29.1
Peat + fiber of spruce wood	1	4.5	3.9	0.48	3.0	9.1	37.76	34.7
	2	4.7	1.3	0.52	4,0	9.3	40.39	34.4
	3	4.9	0.7	0.39	6,0	10.0	39.89	29.3
Peat + fiber of pine bark	1	4.5	1.0	0.61	8.3	12.1	37.30	35.4
	2	4.9	0.7	0.51	13.2	13.3	41.66	36.5
	3	4.6	0.8	0.64	19.5	19.3	38.64	34.2
Peat + fiber of spruce bark	1	4.5	1.5	0.23	9.2	9.0	39.50	34.5
	2	4.7	0.9	0.31	21.6	21.5	38.71	32.8
	3	4.9	0.7	0.4	38.1	38.3	38.86	34.7
Peat + perlite	1	4.9	3.3	0.15	2.0	22.2	37.30	35.4
	2	5.1	2.0	0.41	2.1	2.1	29.89	37.4
	3	5.4	1.7	0.16	2.0	2.4	27.54	38.8

* Using potentiometric method with an error ±0.2; ** using spectrometric analysis method with an error ±10%; *** using flame photometry method with an error ±20%.

The detection of elements available for plants (K, P, Ca, and Mg) was accomplished according to LST EN 13652 [24]. In the water extracts, the phosphorus (P) concentration was detected using the spectrometric method with ammonium molybdenum complexes in Shimadzu UV 1800; the concentration of potassium (K) was measured using the flame photometric method with a Flame Photometer Sherwood M410; and the calcium (Ca) and magnesium (Mg) concentrations were determined using the atomic absorption spectrometric method using Atomic Absorption Spectrometer (Perkin Elmer AAnalyst 200, Waltham, MA, USA) (Table 2).

Mineral nitrogen (N) was extracted in a 1:5 (wt./vol) substrate suspension of 1 M KCl solution. The suspension was shaken for 60 min at 20 ± 2 °C. After shaking, the suspension was filtrated and analyzed using a flow injection analysis (FIA) system with FIASTAR 5000 analyzer. After the addition of an acidic sulfanilamide solution, the nitrates in the substrate extract were converted to nitrites in the cadmium column. They, then, reacted with N-(1-naphthyl) ethylenediamine dihydrochloride to form a purple azo dye whose absorbance can be measured at 540 nm and 720 nm. The substrate extract was injected into a flowing carrier solution, where ammonium was mixed with sodium hydroxide to form gaseous ammonia, which passed through a gas-permeable membrane into the indicator stream. Acidic indicators changed color in this stream when they reacted with ammonium gas. Photometric measurements were performed at 540 nm and 720 nm. The calculation of mineral nitrogen involved adding the combined amounts of nitrate and nitrite nitrogen to the ammonia nitrogen.

The organic carbon content was determined using dry combustion, where the sample was heated to 900 °C in a stream of air, and the carbon dioxide formed was measured using infrared spectroscopy. The amount of organic carbon in the substrate was determined according to the standard ISO 10694:1995 with an organic carbon analyzer multi-EA 4000 Analytik Jena [25].

2.3. Growth of Blueberry Saplings and Content of Extractable Macronutrients in the Leaves

Saplings' height and fresh leaf weight per plant were determined for all variants of substrates by evaluating all thirty saplings. Leaves were collected from each plant separately

and the average weight was determined at the end of the first growth flush of vegetative shoots, i.e., at 90–95 days after transplanting, in the last decade of July. Plant height was measured with a measuring tape. To determine the nutrient concentration, samples of fully expanded leaves from the current season shoots were prepared. From five to ten leaves were collected per plant and mixed before being sent to the laboratory. A total of 200 g of raw material per substrate was prepared. The leaves of the blueberry saplings were air dried, then ground and analyzed using the appropriate methods: nitrogen (N) with the Kjeldahl method, potassium (K) with the flame photometric method, phosphorus (P) with the photometric method with molybdovanadate, calcium (Ca) with the oxalic acid method, and magnesium (Mg) with atomic absorption spectrophotometry at 285·2 nm [26–29]. The amounts of organic carbon in the blueberry leaves were determined according to the standard ISO 10694:1995 with the organic carbon analyzer multi-EA 4000 Analytik Jena [25]. All analyses were performed in triplicate.

2.4. Determination of Chlorophyll Fluorescence

For the investigation of the maximum photochemical efficiency of Photosystem II (Fv/Fm), the leaves were fully dark, adapted, prior to the measurement. Dark-adapted leaf areas were achieved using lightweight leaf clips for 20 min. The chlorophyll fluorescence was measured with a chlorophyll fluorimeter (Pocket PEA Chlorophyll Fluorimeters, Hansatech Instruments Ltd., Norfolk, UK) with a Fv/Fm test duration of 1 s. A total of 5 measurements per plant were taken from leaves located in different directions, at an average height of 0.3–0.4 m, using leaf clips. Ten replications for each substrate variant were performed. The maximum photochemical efficiency of photosystem II was quantified (Fv/Fm) using the following relationship proposed by Maxwell and Johnson [30].

According to Murchie and Lawson [31], the Fv/Fm of non-stressed plant material should be in the range of 0.81–0.83. A much smaller relative interval ($0.79 \leq Fv/Fm \leq 0.84$) was indicated by Maxwell and Johnson [30].

2.5. Statistical Analysis

In the evaluation of the average height and leaf weight of saplings, and chlorophyll fluorescence, a non-parametric Kruskal–Wallis test comparing the ranks of the samples was used. For all hypotheses, statistically significant differences were evaluated at a significance level of $p = 0.05$. The same level of significance was used in testing for differences between means by employing a one-way ANOVA with a multiple (pairwise) comparison procedure using Duncan's test. The statistical calculations were carried out using the IBM SPSS Statistics 27 software application.

3. Results and Discussion

3.1. Effect of Different Substrate Mixess on the Growth of Blueberry Saplings

As presented in Table 3 and Figure S1, the height of blueberry saplings grown in mixes of peat with different amounts of pine wood fiber were not significantly different.

Table 3. Effect of mixes of peat with different amounts of wood and bark fibers and perlite on the height of blueberry saplings.

Substrates	Height, cm		
	1 *	2 *	3 *
Peat + fiber of pine wood	44.26 ± 10.45 *ab*	48.09 ± 7.69 *a*	47.41 ± 8.50 *a*
Peat + fiber of spruce wood	52.59 ± 8.28 *a*	44.02 ± 8.29 *b*	40.57 ± 5.52 *b*
Peat + fiber of pine bark	44.31 ± 6.95 *a*	31.48 ± 9.41 *b*	27.74 ± 5.32 *c*
Peat + fiber of spruce bark	38.51 ± 4.59 *a*	26.97 ± 7.98 *b*	19.90 ± 3.28 *c*
Peat + perlite	44.95 ± 9.54 *ab*	46.51 ± 7.62 *ab*	50.41 ± 8.41 *a*

* Substrate variants: 1—15% *v*/*v*; 2—30% *v*/*v*; 3—45% *v*/*v*. Values followed by different lowercase letters, within the line, indicate statistically significant differences by Duncan's test, $p = 0.05$.

The same trend was found for the average weight of leaves per plant (Table 4, Figure S2). Among the substrates with the spruce wood fiber, significantly higher blueberry saplings (52.59 ± 8.28 cm) were detected for the substrate with the lowest amount of fibers (15% *v*/*v*), while the larger fiber content (30% *v*/*v*) led to the significantly lower height in plants (44.02 ± 8.29 cm). Accordingly, the minimum leaf weight was determined for plants growing in the substrate with 45% *v*/*v* of spruce wood fiber (9.31 ± 2.89 g). In substrates with pine bark fiber, blueberry saplings reached the maximum height when fibers made up 15% *v*/*v* of the total capacity. It was determined that the height of the blueberry saplings decreased significantly as the amount of spruce bark fiber increased. When assessing the influence of perlite on plant growth, it was established that the plants reached a height ranging from 44.95 ± 9.54 cm (15% *v*/*v* of perlite) to 50.41 ± 8.41 cm (45% *v*/*v* of perlite). No differences in leaf weight were found among substrates with this mineral addition.

Table 4. Effect of mixes of peat with different amounts of wood and bark fibers and perlite on the leaf weight of blueberry saplings.

Substrates	Leaf Weight, g/Plant		
	1 *	2 *	3 *
Peat + fiber of pine wood	14.55 ± 2.29 *ab*	13.26 ± 2.67 *a*	13.08 ± 2.55 *a*
Peat + fiber of spruce wood	16.41 ± 3.14 *a*	13.70 ± 1.71 *a*	9.31 ± 2.89 *b*
Peat + fiber of pine bark	13.43 ± 2.00 *a*	4.17 ± 1.93 *b*	3.03 ± 1.45 *b*
Peat + fiber of spruce bark	7.05 ± 3.11 *a*	3.33 ± 1.44 *b*	1.07 ± 0.35 *cd*
Peat + perlite	13.78 ± 3.09 *ab*	13.50 ± 2.25 *ab*	14.63 ± 2.52 *a*

* Substrate variants: 1—15% *v*/*v*; 2—30% *v*/*v*; 3–45% *v*/*v*. Values followed by different lowercase letters, within the line, indicate statistically significant differences by Duncan's test, $p = 0.05$.

It can be summarized that blueberry saplings grown in substrates with pine and spruce wood fiber or perlite additions reached a height ranging from 40.57 ± 5.52 cm to 52.59 ± 8.28 cm during the first year of vegetation, which is important in order to produce high-quality planting material for blueberry plantations [10,11].

Corresponding differences were determined in terms of leaf weight when the average leaf weight per plant was only 1.07 ± 0.35 g at 45% *v*/*v* of spruce bark fiber in the substrate (Figure S2, Table 4). The blueberry plants were more vigorous in the substrate with even 45% *v*/*v* of perlite compared to the substrate with 15% *v*/*v* of perlite. Consequently, leaf weight per plant did not differ significantly among the substrates with different perlite additions. Perlite is widely preferred because it reduces the risk of damping off, provides a balance between air and water in root zone and stimulates faster root growth [31]. Comparison of equal amounts of different additions confirmed that plant growth was very poor in substrates with 15% *v*/*v*, 30% *v*/*v*, and 45% *v*/*v* of spruce bark fiber (Tables S1 and S2). Accordingly, the saplings achieved the minimum leaf weight per plant in these substrates. Statistically significant differences found when comparing the same amount of different additions confirmed the necessity of choosing the most suitable variants and quantities of the additions.

3.2. Effect of Different Substrate Mixes on the Chlorophyll Fluorescence in the Leaves of Blueberry Saplings

The determined values of chlorophyll florescence showed no significant differences among 1–3 variants of each substrate. Therefore, the various amounts of pine and spruce wood or bark fiber and perlite did not significantly affect the Fv/Fm ratio (Table 5, Figure S3). On the other hand, the significant differences among substrates with the same amount of particular additions were determined (Table S3). The lowest values of Fv/Fm were detected for mixes of peat with 15% *v*/*v*, 30% *v*/*v*, and 45% *v*/*v* of spruce bark fiber. The growth of saplings was also lower in these substrates (Tables S1 and S2). In this study, the highest Fv/Fm ratio was determined for the pine and spruce substrates with 15% *v*/*v* and 30% *v*/*v* of wood fiber. Moreover, the Fv/Fm values were close to or lower than 0.80. As other authors have reported, the time of measurement during the day may have influenced the

chlorophyll fluorescence [32]. In the study of Björkman and Deming [33], it was stated that Fv/Fm is virtually constant when measured under no-stress conditions, being in the range of $0.75 \leq \text{Fv/Fm} \leq 0.86$.

Table 5. Effect of mixes of peat with different amounts of wood and bark fibers and perlite on the chlorophyll fluorescence (Fv/Fm) of the blueberry saplings leaves.

Substrates	Fv/Fm		
	1 *	2 *	3 *
Peat + fiber of pine wood	0.784 ± 0.020 *a*	0.779 ± 0.021 *a*	0.759 ± 0.035 *ab*
Peat + fiber of spruce wood	0.778 ± 0.024 *a*	0.797 ± 0.010 *a*	0.791 ± 0.022 *a*
Peat + fiber of pine bark	0.774 ± 0.033 *a*	0.771 ± 0.018 *a*	0.711 ± 0.085 *ab*
Peat + fiber of spruce bark	0.689 ± 0.090 *ab*	0.738 ± 0.014 *a*	0.711 ± 0.039 *ab*
Peat + perlite	0.760 ± 0.044 *ab*	0.773 ± 0.018 *a*	0.758 ± 0.032 *ab*

* Substrate variants: 1—15% v/v; 2—30% v/v; 3—45% v/v. Values followed by different lowercase letters, within the line, indicate statistically significant differences by Duncan's test, $p = 0.05$.

A non-invasive measurement of the chlorophyll-fluorescence parameter photochemical efficiency of PSII (Fv/Fm) is a commonly used technique in plant physiology. It has been confirmed that the determination of the Fv/Fm ratio can be used to identify nitrogen deficiency [33,34]. Previous studies also showed a significant correlation between nitrogen concentration and the leaves' Fv/Fm ratio. It was determined that the Fv/Fm ratio correlates not only with low nitrogen amounts but also with low chlorophyll levels and low biomass growth [35]. Different soil pH treatments had various effects on the photosynthetic characteristics [34]. The chlorophyll fluorescence Fv/Fm ratio is correlated with the efficiency of leaf photosynthesis, and a decline in this ratio is a good indicator of photoinhibition damage when plants suffer from a wide range of environmental stresses [36]. In this study, the Fv/Fm values of plants in all substrates with the spruce bark fiber showed a decrease in the Fv/Fm ratio, which confirms that plants may have suffered from stress in some of the substrates studied [33,37].

3.3. Effect of Different Substrate Mixes on the Content of Extractable Macronutrients

The nutritional status of blueberry plants is mainly assessed on the basis of studies on the chemical composition of leaves [38]. The obtained results on the content of extractable macronutrients in blueberry leaves showed significant variation among saplings grown in different substrates (Table 6). Attention was paid to whether our data corresponded to the proper foliar concentrations of nutrients for blueberry indicated in the studies of other researchers [39–42]. In our study, the amount of nitrogen (N) in blueberry leaves ranged from 0.78% (substrate with 45% v/v of spruce bark fiber) to 1.98% v/v (substrates with 15% v/v of pine wood fiber and with 30% v/v of perlite). The data presented in Table 6 shows that the leaves of the saplings grown in substrates with 45% v/v of spruce bark fiber were distinguished by lower nitrogen content than the limits indicated in the above-mentioned references. Glonek and Komosa [43] reported that the optimum ranges of N in leaves collected in the middle of the summer should be 1.52–2.17%. Studies with other plants have shown that N-immobilization can cause nutritional imbalance on young seedlings grown in organic substrates with wood fiber. In such cases, the use of N-impregnated media and an additional supply of nutrients is necessary [44].

Compared with the sufficient or normal concentration of phosphorus in blueberry leaves determined by Hart et al. [41] and Fugua et al. [42], the results of our research showed the proper content of phosphorus (P) in all variants of substrates, while the amounts of phosphorus ranged from 0.11% (45% v/v of pine bark fiber) to 0.22% (45% v/v of spruce bark fiber). The obtained leaf N:P ratio ranged from 3.55 to 13.67 (Table 6). Dibar et al. [45] reported that plants with a higher nitrogen concentration and a low N:P ratio, especially in the photosynthetic active organs, are the best-adapted to the environment. In our study, blueberry saplings grown in substrates with 30% v/v and 45% v/v of spruce bark fibers showed a particularly weak growth, and, in addition, not only low amounts of nitrogen

but also the lowest N:P ratio were determined in the leaves. Xia et al. [46] presented the possibility of using N:P ratio as an effective indicator for the health condition and growth status of plants.

Table 6. Content of extractable macronutrients and organic carbon in the leaves of blueberry saplings according to the different additions of fiber and perlite.

Substrates	Substrate Variant	N, %	P %	K, %	Ca, %	Mg, %	Organic C, %	N:P
Peat + fiber of pine wood	1	1.98 ± 0.11 *a*	0.16 ± 0.02 *b*	0.65 ± 0.02 *b*	0.98 ± 0.01 *b*	0.25 ± 0.01 *cd*	39.01 ± 2.03 *c*	12.37
	2	1.64 ± 0.10 *c*	0.14 ± 0.02 *c*	0.55 ± 0.03 *c*	1.01 ± 0.04 *a*	0.26 ± 0.01 *c*	36.59 ± 3.02 *cd*	11.71
	3	1.49 ± 0.11 *cd*	0.14 ± 0.02 *c*	0.58 ± 0.03 *c*	1.00 ± 0.09 *a*	0.25 ± 0.01 *cd*	39.07 ± 3.56 *c*	10.64
Peat + fiber of spruce wood	1	1.92 ± 0.09 *a*	0.17 ± 0.02 *b*	0.59 ± 0.01 *c*	0.91± 0.05 *bc*	0.25 ± 0.02 *cd*	41.44 ± 3.11 *b*	11.29
	2	1.56 ± 0.10 *c*	0.15 ± 0.01 *bc*	0.58 ± 0.04 *c*	1.00 ± 0.03 *a*	0.26 ± 0.03 *c*	28.41 ± 1.25 *e*	10.44
	3	1.3 ± 0.06 *d*	0.12 ± 0.02 *d*	0.56 ± 0.03 *c*	0.95 ± 0.01 *b*	0.25 ± 0.02 *cd*	40.22 ± 2.96 *b*	10.8
Peat + fiber of pine bark	1	1.43 ± 0.04 *cd*	0.12 ± 0.02 *d*	0.58 ± 0.02 *c*	0.88 ± 0.01 *d*	0.25 ± 0.01 *cd*	36.0 ± 2.35 *cd*	12.03
	2	1.15 ± 0.05 *e*	0.12 ± 0.01 *d*	0.72 ± 0.03 *b*	0.91 ± 0.05 *bc*	0.31± 0.02 *b*	35.81 ± 3.08 *cd*	11.07
	3	1.13 ± 0.04 *e*	0.11 ± 0.01 *d*	0.61 ± 0.03 *bc*	0.93 ± 0.03 *bc*	0.28 ± 0.01 *c*	33.04 ± 2.85 *d*	10.27
Peat + fiber of spruce bark	1	1.21 ± 0.02 *d*	0.12 ± 0.03 *d*	0.64 ± 0.02 *b*	0.88 ± 0.01 *d*	0.25 ± 0.01 *cd*	41.40 ± 3.74 *b*	10.08
	2	1.09 ± 0.02 *e*	0.14 ± 0.02 *c*	0.88 ± 0.02 *b*	0.90 ± 0.01 *bc*	0.28 ± 0.03 *c*	41.06 ± 3.96 *b*	7.79
	3	0.78 ± 0.01 *f*	0.22 ± 0.03 *a*	1.75 ± 0.03 *a*	0.84 ± 0.02 *d*	0.37 ± 0.02 *a*	46.83 ± 2.87 *a*	3.55
Peat + perlite	1	1.82 ± 0.11 *b*	0.16 ± 0.01 *b*	0.57 ± 0.01 *c*	0.84 ± 0.01 *d*	0.24 ± 0.01 *d*	38.38 ± 3.01 *cd*	11.38
	2	1.98 ± 0.09 *a*	0.16 ± 0.01 *b*	0.50 ± 0.02 *d*	0.83 ± 0.01 *d*	0.22 ± 0.01 *e*	37.74 ± 3.25 *cd*	12.38
	3	1.64 ± 0.09 *c*	0.12 ± 0.02 *d*	0.49 ± 0.01 *d*	0.83 ± 0.02 *d*	0.22 ± 0.02 e	39.07 ± 2.56 *c*	13.67

Values followed by different lowercase letters, within the column, indicate statistically significant differences by Duncan's test, $p = 0.05$.

A high amount of potassium (K) was found in the leaves of blueberry saplings grown in the substrates with the addition of spruce bark fiber. Even the plants grown in the substrate with 45% v/v of spruce bark fiber had exceptionally high amounts of potassium, 1.75%, compared to the proper amounts of potassium in blueberry leaves determined by other authors [39–42].

A high content of calcium (Ca) in the leaves was found in both saplings grown in the mixes of peat with pine and spruce wood fibers, while adequate calcium amounts were determined in the leaves of plants in all variants with spruce bark fiber and in substrates with perlite additions. The content of magnesium (Mg) varied significantly in the leaves of all studied plants, and high amounts for substrates with 30% v/v of pine bark fiber and 45% v/v of spruce bark fiber were determined.

Leaf organic carbon (C) content was significantly higher in saplings grown in substrates with additions of spruce bark fiber and in substrates with 15% v/v and 45% v/v of spruce wood fiber (Table 3).

The bark of various tree species has been evaluated, highlighting not only physical-chemical properties but also the different methods of medical, energetic, and industrial utilization [18,47]. In this research, substrates of peat with mixes of pine and spruce wood and bark fibers were studied. The use of tree bark in the production of substrates could be a novelty; however, the use of tree bark for the production of peat substrate mixes needs to be investigated in more detail. This study confirmed that mixes of peat with 15–45% v/v of spruce fibers had a negative effect on the growth of blueberry saplings. As other authors have reported, it is necessary to study what toxic substances are released from the new components, which could inhibit plant growth [48,49]. In the mixes of peat with fiber additions, microorganisms that need mineral nitrogen must be also evaluated [20].

The challenges presented by climate change require a new approach to the conservation of natural resources, including peat. The search for innovative substrates, evaluating the possibilities of using renewable materials, has great potential [50,51]. This study confirmed the possibility of reducing the amount of peat in substrates using tree fibers and perlite. In the continuation of this research, it would be promising to investigate substrate compositions with mixtures of organic and mineral additions.

4. Conclusions

In this study, mixes of peat with various rates of wood and bark fibers or perlite were compared to evaluate the possibility of reducing the amount of peat. The research carried out on the growth of blueberry saplings showed that the best characteristics of plants were achieved for substrates with 15–45% *v*/*v* of pine wood fiber and with 15–30% *v*/*v* of spruce wood fiber. Different amounts of spruce bark fiber had the strongest negative effect on vegetative growth and the lowest values of chlorophyll fluorescence Fv/Fm (0.689–0.738) in the leaves of the saplings were determined. Investigations of extractable macronutrients in the leaves confirmed the qualitative and quantitative composition of peat mixes suitable for the cultivation of blueberry saplings. Nitrogen and potassium levels did not meet the accepted standards and a low N:P ratio was found in the leaves of plants grown in substrates with 30–45% *v*/*v* of spruce bark fibers. The results of the investigations corroborated that 15–45% *v*/*v* of perlite in peat substrates is suitable for the purpose of growing blueberry saplings.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9020151/s1, Figure S1: Effect of mixes of peat with different additions of wood and bark fibers and perlite on the height of blueberry saplings; Figure S2: Effect of mixes of peat with different additions of wood and bark fibers and perlite on the height of blueberry saplings; Figure S3: Effect of mixes of peat with different additions of wood and bark fibers and perlite on the chlorophyll fluorescence (Fv/Fm) of blueberry-saplings leaves; Table S1: Effect of mixes of peat with the same percentage of components on the height of blueberry saplings; Table S2: Effect of mixes of peat with the same percentage of components on the leaf weight of blueberry saplings; Table S3: Effect of mixes of peat with the same percentage of components on the chlorophyll fluorescence (Fv/Fm) of blueberry-saplings leaves.

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References

1. Prasad, M.; Chrysargyris, A.; McDaniel, N.; Kavanagh, A.; Gruda, N.S.; Tzortzakis, N. Plant Nutrient Availability and pH of Biochars and Their Fractions, with the Possible Use as a Component in a Growing Media. *Agronomy* **2020**, *10*, 10. [CrossRef]
2. Gruda, N.S.; Fernández, J.A. Optimising Soilless Culture Systems and Alternative Growing Media to Current Used Materials. *Horticulturae* **2022**, *8*, 292. [CrossRef]
3. Bohlin, C.; Holmberg, P. Peat: Dominating growing medium in Swedish horticulture. *Acta Hortic.* **2004**, *644*, 177–181. [CrossRef]
4. Kern, J.; Tammeorg, P.; Shanskiy, M.; Sakrabani, R.; Knicker, H.; Kammann, C.; Tuhkanen, E.M.; Smidt, G.; Prasad, M.; Tiilikkala, K.; et al. Synergistic use of peat and charred material in growing media—An option to reduce the pressure on peatlands? *J. Environ. Eng. Landsc.* **2017**, *25*, 160–174. [CrossRef]
5. Woolf, D.; Amonette, J.; Street-Perrott, A.; Lehman, J.; Joseph, S. Sustainable biochar to mitigate global climate change. *Nat. Commun.* **2010**, *1*, 56. [CrossRef] [PubMed]
6. Fryda, L.; Visser, R.; Schmidt, J. Biochar replaces peat in horticulture: Environmental impact assessment of combined biochar & bioenergy. *Detritus* **2019**, *5*, 132–149.
7. Jackson, B.E.; Wright, R.D.; Seiler, J.R. Changes in Chemical and Physical Properties of Pine Tree Substrate and Pine Bark during Long-term Nursery Crop Production. *Hortscience* **2009**, *44*, 791–799. [CrossRef]

8. Schmilewski, G. Growing medium constituents used in the EU. *Acta Hortic.* **2009**, *819*, 33–46. [CrossRef]
9. Wang, D.; Gabriel, M.Z.; Legard, D.; Sjulin, T. Characteristics of growing media mixes and application for open-field production of strawberry (*Fragaria ananassa*). *Sci. Hortic.* **2016**, *198*, 294–303. [CrossRef]
10. Trehane, J. *Blueberries, Cranberries and Other Vacciniums*; Timber Press: London, UK, 2004; pp. 121–126.
11. Hoover, B.; Fuglie, D.; Miller, R. Optimal soil conditions for organic highbush blueberry growth: Assessment of early results. *J. Appl. Hortic.* **2014**, *16*, 32–39.
12. Xie, Z.S.; Wu, X.C. Studies on substrates for blueberry cultivation. *Acta Hortic.* **2009**, *810*, 513–520. [CrossRef]
13. Retamales, J.B.; Hancock, J.F. *Blueberries*; CABI: Wallingford, UK, 2012; pp. 115–121.
14. Nieto, A.; Gascó, G.; Paz-Ferreiro, J.; Fernández, J.; Plaza, M.C. The effect of pruning waste and biochar addition on brown peat based growing media properties. *Sci. Hortic.* **2016**, *199*, 142–148. [CrossRef]
15. Durand, S.; Jackson, B.E.; Fonteno, W.C.; Michel, J.-C. The use of wood fiber for reducing risks of hydrophobicity in peat-based substrates. *Agronomy* **2021**, *11*, 907. [CrossRef]
16. Dittrich, C.; Pecenka, R.; Løes, A.K.; Cáceres, R.; Conroy, J.; Rayns, F.; Schmutz, U.; Kir, A.; Kruggel-Emden, H. Extrusion of Different Plants into Fiber for Peat Replacement in Growing Media: Adjustment of Parameters to Achieve Satisfactory Physical Fiber-Properties. *Agronomy* **2021**, *11*, 1185. [CrossRef]
17. Mohareb, A.; Kherallah, I.; Badawy, M.; Salem, M.; Yousef, H. Chemical composition and activity of bark and leaf extracts of Pinus halepensis and Olea europaea grown in AL-Jabel AL-Akhdar region, Libya against some plant phytopathogens. *J. Appl. Biotechnol. Bioeng.* **2017**, *3*, 331–342. [CrossRef]
18. Pásztory, Z.; Mohácsiné, I.R.; Gorbacheva, G.; Börcsök, Z. The Utilization of Tree Bark. *BioRes* **2016**, *11*, 7859–7888. [CrossRef]
19. Kemppainen, K.; Siika, M.; Pattathil, S.; Giovando, S.; Kruus, K. Spruce bark as an industrial source of condensed tannins and non-cellulosic sugars. *Ind. Crop. Prod.* **2014**, *52*, 158–168. [CrossRef]
20. Vandecasteele, B.; Muylle, H.; De Windt, I.; Van Acker, J.; Ameloot, N.; Moreaux, K.; Coucke, P.; Debode, J. Plant fibers for renewable growing media: Potential of defibration, acidification or inoculation with biocontrol fungi to reduce the N drawdown and plant pathogens. *J. Clean. Prod.* **2018**, *203*, 1143–1154. [CrossRef]
21. Samar, S.; Saxena, S. Study of chemical and physical properties of perlite and its application in India. *Int. J. Sci. Technol. Manag.* **2016**, *5*, 70–80.
22. LST 1957:2022; Peats for Horticulture and Landscape Gardening—Properties, Test Methods, Specifications (in Lithuanian). Available online: https://lsd.lrv.lt/uploads/lsd/documents/files/Darbo_programa_1-dalis_2022-12-15.pdf (accessed on 5 May 2022).
23. LST EN 13037:1999; Soil Improvers and Growing Media—Determination of pH. Available online: https://standards.iteh.ai/catalog/standards/cen/a127e814-60b7-42b0-80c7-453c28968d64/en-13037-2011 (accessed on 5 May 2022).
24. LST EN 13652:2001; Soil Improvers and Growing Media—Extraction of Water Soluble Nutrients and Elements. Available online: https://standards.iteh.ai/catalog/standards/cen/a27867c1-a6a0-426e-b4cf-e6eaea48975d/en-13652-2001 (accessed on 22 April 2022).
25. ISO 10694:1995; Soil Quality—Determination of Organic and Total Carbon after Dry Combustion (Elementary Analysis). Available online: https://www.iso.org/obp/ui/#iso:std:iso:10694:en (accessed on 4 May 2022).
26. Third Commission Directive 72/199/EEC of 27 April 1972 establishing Community methods of analysis for the official control of feeding stuffs. *Official Journal of the European Communities* **1972**, *123*, 75–86.
27. First Commission Directive 71/250/EEC of 15 June 1971 establishing Community methods of analysis for the official control of feeding stuffs. *Official Journal of the European Communities* **1971**, *155*, 484–495.
28. Second Commission Directive 71/393/EEC of 18 November 1971 establishing Community methods of analysis for the official control of feeding stuffs. *Off. J. Eur. Communities* **1971**, *279*, 7–18.
29. Fourth Commission Directive 73/46/EEC of 5 Dezember 1972 establishing Community methods of analysis for the official control of feeding stuffs. *Off. J. Eur. Communities* **1972**, *83*, 4–14.
30. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot.* **2000**, *51*, 659–668. [CrossRef]
31. Kim, J.K.; Shawon, M.R.A.; An, J.H.; Yun, Y.J.; Park, S.J.; Na, J.K.; Choi, K.Y. Influence of Substrate Composition and Container Size on the Growth of Tissue Culture Propagated Apple Rootstock Plants. *Agronomy* **2021**, *11*, 2450. [CrossRef]
32. Murchie, E.H.; Lawson, T. Chlorophyll fluorescence analysis: A guide to good practice and understanding some new applications. *J. Exp. Bot.* **2013**, *64*, 3983–3998. [CrossRef]
33. Björkman, O.; Deming, B. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* **1987**, *170*, 489–504. [CrossRef]
34. Retamal-Salgado, J.; Loor, B.; Hirzel, J.; López, M.D.; Undurraga, P.; Zapata, N.; Vergara-Retamales, R.; Olivares-Soto, H. Chlorophyll Fluorescence and Fruit Quality Response of Blueberry to Different Mulches. *Agronomy* **2022**, *12*, 1702. [CrossRef]
35. Janušauskaite, D.; Feiziene, D. Chlorophyll fluorescence characteristics throughout spring triticale development stages as affected by fertilization. *Acta Agr. Scan. Sect. B—Soil Plant Sci.* **2012**, *62*, 7–15. [CrossRef]
36. Klepeckas, M.; Januškaitienė, I. Changes in *Triticum aestivum* and *Hordeum vulgare* chlorophyll content and fluorescence parameters under impact of various sapropel concentrations. *Biologija* **2016**, *62*, 216–226. [CrossRef]
37. Gliožeris, S.; Tamošiūnas, A.; Štuopytė, L. Effect of some growth regulators on chlorophyll fluorescence in *Viola* × *wittrockiana* 'Wesel Ice'. *Biologija* **2007**, *53*, 24–27.

38. Jiang, Y.; Zeng, Q.; Wei, J.; Jiang, J.; Li, Y.; Chen, J.; Yu, H. Growth, Fruit Yield, Photosynthetic Characteristics, and Leaf Microelement Concentration of Two Blueberry Cultivars under different Long-Term Soil pH Treatments. *Agronomy* **2019**, *9*, 357. [CrossRef]
39. Ochmian, I.; Grajkowski, J.; Mikiciuk, K.G.; Ostrowska, P. Chelpinski Mineral composition of high blueberry leaves and fruits depending on substrate type used for cultivation. *J. Elementol.* **2009**, *14*, 509–516. [CrossRef]
40. Hanson, E.J.; Hancock, F.J. *Managing the Nutrition of Highbush Blueberries. Bulletin E-2011*; Michigan State University Extention: East Lansing, MI, USA, 1996.
41. Hart, J.; Strik, B.; White, L.; Yang, W. *Nutrient Management for Blueberries in Oregon*; Publication No. EM 8918; Oregon State University Extension Service: Corvallis, OR, USA, 2006.
42. Fuqua, B.; Byers, P.; Kaps, M.; Kovacs, L.; Wadstein, D. *Growing Blueberries in Missouri. Bulletin No. 44*; State Fruit Experiment Station, Missouri State University Missouri: Mountain Grove, MO, USA, 2005.
43. Glonek, J.; Komosa, A. The effect of fertigation on the nutrient status and yield of highbush blueberry cv. "Bluecrop". *Acta Hortic.* **2006**, *715*, 371–374. [CrossRef]
44. Gruda, N.; Tucher, S.V.; Schnitzler, W.H. N-immobilization of wood fiber substrates in the production of tomato transplants (*Lycopersicon lycopersicum* (L.) Karst. Ex. Farw.). *J. Appl. Bot.* **2000**, *74*, 32–37.
45. Dibar, D.T.; Zhang, K.; Yuan, S.; Zhang, J.; Zhou, Z.; Ye, X. Ecological stoichiometric characteristics of Carbon (C), Nitrogen (N) and Phosphorus (P) in leaf, root, stem, and soil in four wetland plants communities in Shengjin Lake, China. *PLoS ONE* **2020**, *15*, e0230089. [CrossRef]
46. Xia, C.X.; Yu, D.; Wang, Z.; Xie, D. Stoichiometry patterns of leaf carbon, nitrogen, and phosphorous in aquatic macrophytes in eastern China. *Ecol. Eng.* **2014**, *70*, 406–413. [CrossRef]
47. Barrett, G.E.; Alexander, P.D.; Robinson, J.S.; Bragg, N.C. Achieving environmentally sustainable growing media for soilless plant cultivation systems—A review. *Sci. Hortic.* **2016**, *212*, 220–223. [CrossRef]
48. Gruda, N.; Rau, B.J.; Wright, R.D. Laboratory Bioassay and Greenhouse Evaluation of a Pine Tree Substrate Used as a Container Substrate. *Eur. J. Hortic. Sci.* **2009**, *74*, 73–78.
49. Wrigth, R.D.; Rowder, R.F. Chipped pine logs: A potential substrate for greenhouse and nursery crops. *HortScience* **2005**, *40*, 1513–1515.
50. Gruda, N. Do soilless culture systems have an influence on product quality of vegetables? *J. Appl. Bot.* **2009**, *82*, 141–147.
51. Gruda, N.S. Advances in Soilless Culture and Growing Media in Today's Horticulture—An Editorial. *Agronomy* **2022**, *12*, 2773. [CrossRef]

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Estimating Nitrogen Release from Organic Fertilizers for Soilless Production by Analysis of C and N Pools

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Abstract: The use of organic fertilizers in soilless pot plant production has sharply increased in recent years. However, there is still a lack of methods for characterizing the N release from organic fertilizers. This bears the risk of an inadequate nutrient supply and, thus, a waste of resources. Therefore, the current research analyzed fourteen commercial organic fertilizers for various C and N pools by extraction in cold and hot water, acid hydrolysis, and thermal fractionation. Furthermore, we conducted an incubation test using a growing medium (80 vol% peat plus 20 vol% green waste compost) and fitted the nitrogen release to different kinetic models. Finally, we calculated the correlations among the best-suited kinetic model parameters and the C and N pools. The C and N pools soluble in water and weak hydrochloride acid varied significantly among the fourteen fertilizers but were closely correlated with each other. The N release from most organic fertilizers could be described very well using the Gompertz function ($R^2 > 0.9$), and the parameters of the Gompertz function showed significant correlations with the C and N pools. Hydrolyzable C and N pools provided valuable information about the N release characteristics of organic fertilizers.

Keywords: incubation experiment; growing medium; hydrolyzable C and N; kinetic models; Gompertz function

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1. Introduction

In the last two decades, organic greenhouse production has rapidly grown, mainly focusing on producing fruits and vegetables in soil. However, consumers' demand for soilless products, such as vegetables, herbs, and ornamentals, is also increasing [1–4]. A survey by Burnett and Stack [5] revealed fertilization as a significant issue in the organic cultivation of bedding plants. This is particularly true for nitrogen (N) supply, as N applied with organic fertilizers must be mineralized first, and thus, it becomes plant-available only with a delay. To ensure an adequate N supply, growers need reliable information about the time course of the N release from the applied fertilizer [6].

The decomposition pattern of organic residue has been extensively characterized in the literature using various mathematical models. These models include simple first and second-order rate equations [7–9], consecutive reaction models that combine multiple first-order rate equations [10–12], and flexible sigmoid-shaped functions, such as the Richards, Gompertz, and Weibull functions [13–18]. However, simple first-order rate models, which assume N mineralization as a simple function of the substrate N concentration, and consecutive models, which assume two or more pools with different rate coefficients (e.g., labile and refractory fractions), regularly overestimate N release at the beginning [10,11,15,17]. This initial lag phase, which might be due to inhibitory compounds, such as polyphenols [11,19], or by the initial acclimation and regrouping of microbial biomass [14], is quite well-modeled by the unitless and scale-independent shape factor in flexible functions [18].

Subsequently, kinetic parameters can be correlated to the biochemical characteristics of the material [19].

The importance of biochemical characteristics for predicting mineralization rates has been shown in various studies for crop residues and other organic input materials, such as manure [9,20–26] and, to a lesser extent, for commercial organic fertilizers [27–31]. In addition, countless studies have examined the correlations between the biochemical properties of soil organic matter (SOM) and nitrogen turnover in soils [32]. In all cases—for crop residues and organic fertilizers, as well as for SOM—the most common parameters were the total nitrogen (TN), as well as the total organic carbon (TOC) or total carbon (TC) and the resulting C/N ratios. However, these parameters only show a rough correlation to the nitrogen release of organic fertilizers [27]. A more suitable approach seems to be the characterization of individual nitrogen and carbon pools either by extraction procedures with both hot and cold water, saline, acidic, or alkaline solutions or by thermal analytical techniques, such as thermogravimetry, analysis of evolved gas, or Rock-Eval pyrolysis. Von Lützow et al. [33] gave a detailed overview of the mentioned techniques. De Neve and Hofman [9] used the sequential procedure proposed by Stevenson [34] to characterize the N and C pools of vegetable crop residues whose N mineralization was fitted to a first-order rate equation. The potentially mineralizable nitrogen (N_P) was closely correlated to the C/N ratio of the lignin, lignin content, and water-soluble N. Furthermore, Jensen et al. [24] reported close correlations between water-soluble N and early N mineralization. In addition, the N_P was well described by amounts of various N fractions, whereas the correlations of the N_P to the C/N ratio of the lignin and lignin content were relatively poor. However, they did not describe the N mineralization by kinetic models but correlated the N mineralization at defined dates to analyze the C and N pools. Moreover, De Neve and Hofman [9] and Jensen et al. [24] have reported close correlations between N mineralization and the total N content, and the rate constant was significantly correlated to the percentage of organic N. This is likely attributed to the common practice of applying residues on a dry matter basis, resulting in an increased application of nitrogen (N) as the percentage of organic N increases.

As outlined by Jensen et al. [24], the physiochemical and biochemical properties of the soil used for incubation can significantly affect mineralization patterns. Thus, the results of the listed studies with different mineral soils are hardly comparable and cannot be transferred to growing media for soilless cultivation as their physiochemical and biochemical properties are quite different from those of mineral soils. Therefore, the current research aimed to characterize the N release of various commercial organic fertilizers in a peat-based growing medium by a single kinetic model, and subsequently, to describe the kinetic parameters of C and N pools analyzed by several extraction procedures and evolved gas analysis under pyrolytic conditions.

2. Materials and Methods

2.1. Commercial Organic Fertilizers

Fourteen solid organic fertilizers were purchased from various companies. Six were unprocessed raw materials, three were of animal origin, and three were coarsely ground legumes. Four fertilizers were made of processed plant material, e.g., from the production of starch adhesives. No detailed information about the origin was available for the remaining four fertilizers. In the following section, the fertilizers and their analyzed N, P, K, Mg, and S contents (in brackets), suppliers, raw materials, and pre-processing methods—as far as they are available—are summarized.

Coarse horn meal (CHM; 13.8 + 0.6 + 0.0 + 0.1 + 2.0): CHM is made of the horns and hooves of slaughtered ungulates, mainly cattle, with minor adhesions of fur, meat, bones, blood, and manure. According to the regulations of the European Union, hygienization by heating or treatment with propionic acid is mandatory [35]. The main component of horns and hooves is keratin, a group of fibrous proteins [36]. The CHM was purchased from Beckmann & Brehm GmbH (Beckeln/Germany).

Sheep wool pellets (SWPs; 9.7 + 0.1 + 3.5 + 0.1 + 2.1): SWPs were obtained from Düngepellet Produkt und Vertriebs GmbH (Lauchammer/Germany). Raw and uncleaned wool is a waste product from sheep husbandry and it is pelletized by a thermal–mechanical process. The primary N source of SWPs is keratin. However, the usually unwashed wool contains many waxes, dirt, and feces [37].

Pig bristle pellets (PBPs; 13.9 + 0.1 + 0.1 + 0 + 2.1): PBPs, also named hair meal pellets, are slaughterhouse waste. The bristles are removed from slaughtered carcasses and typically pasteurized, dried, ground, and pelletized [35]. As with CHM and SWPs, keratin is the primary N source of PBPs. However, pig bristles are less soiled than CHM and SWPs, respectively. The pig bristles were purchased from Beckmann & Brehm GmbH (Beckeln/Germany).

Fodder pea grist (FPG; 4.3 + 0.3 + 1 + 0.1 + 0.1): The seeds of fodder peas were crushed by us in a malt mill, as this is the current practice for legumes used as fertilizers in organic agriculture [35].

Faba bean grist (FBG; 3.2 + 0.2 + 0.8 + 0.1 + 0.1): Similar to the seeds of fodder peas, faba bean seeds were also crushed by us.

Lupine grist (LG; 5.2 + 0.2 + 0.7 + 0.2 + 0.3): Similar to the fodder pea and faba bean seeds, lupine seeds were crushed by us.

Phytomalz (PHZ; 5.8 + 0.7 + 1.1 + 0.2 + 0.6): Provita® Phytomalz (Beckmann & Brehm, Beckeln/Germany) is made from protein-rich residues from the food-processing industry. The principal components are malt culms and corn, which are pressed into small pellets, with the addition of vinasse.

Phytogran (PHN; 5.3 + 0.5 + 1.7 + 0.5 + 0.5): Provita® Phytogran (Beckmann & Brehm, Beckeln/Germany) is a granulate with a particle size of 2–5 mm. The raw material (residues from the food-processing industry) is dissolved in water, steam-sterilized, and fermented by yeasts. Afterwards, the fermented biomass is dried at about 100 °C, mixed with molasses, and granulated.

Phytogrieß (PHS; 6.2 + 0.8 + 0.8 + 0.2 + 1.1): Provita® Phytogrieß (Beckmann & Brehm, Beckeln/Germany) is derived from the fermented residues and glucose of corn gluten production. The granules have a particle size between 0.2 and 2 mm.

Maltaflor (MAF; 4.9 + 0.6 + 1.2 + 0.4 + 0.7): Maltaflor® (Maltaflor EUROPA GmbH, Boppard/Germany) is made of malt culms from breweries, vinasse, and vinasse–potassium, as well as grain hulls. After the malting process, the culms are dried, pelletized, and mixed with the vinasse [35].

OPF granular (OPF; 7.0 + 1.2 + 8.8 + 0.2 + 7): According to the supplier (Plant Health Cure B.V., Oisterwijk/Netherlands), OPF is made of various herbal substances, e.g., fermented sugar beets, and is adjusted to promote the growth of beneficial soil bacteria and mycorrhizal fungi. Most of the nitrogen is amino-derived. The British Soil Association tests all raw materials.

UP—fruit and vegetables (UP; 8.3 + 1 + 4.6 + 0.2 + 3.8): UP was purchased from Umweltpionier GmbH (Perg/Austria). Similar to OPF, no specific information about the raw materials and processing was available. However, according to the supplier, the fertilizer is a mixture of plant material, clay minerals, and microorganisms. A unique feature of UP is its classification as a foodstuff.

Cuxin Xtra-1 (CX1; 10.7 + 0.1 + 3.3 + 0.7 + 4.8): DCM ECO-XTRA® 1 (Deutsche CUXIN Marketing GmbH, Telgte/Germany) is a mixture of animal residues (slaughterhouse waste according to EU ordinance No. 1069/2009), residues of the food, beverage, and feed industry, and tannins from forestry. CX1 is formulated as fine granules, with a particle size between 0.8 and 2.5 mm.

Cuxin Eco-Mix 4 (CE4; 6.3 + 0.1 + 0.3 + 0.2 + 0.6): DCM Öko-Mix® 4 (Deutsche CUXIN Marketing GmbH, Telgte/Germany), similar to CX1, is a mixture of animal residues (slaughterhouse waste according to EU ordinance No. 1069/2009), residues of the food, beverage, and feed industry, and cocoa shells or vinasse. The particle size of CE4 is similar to that of CX1.

2.2. Characterization of Carbon and Nitrogen Pools

The total nitrogen (TN) was measured by the Dumas method (TrueSpec N, LECO Cooperation, Moenchengladbach/Germany; according to the VDLUFA Methods Book II.1, No. 3.5.2.7; [38]). The total organic and total carbon (TOC and TC) were measured by combustion under oxygen at 550 and 1000 °C, respectively, and by evolved gas analysis (VDLUFA Methods Book I; No. A 4.1.3.2 and No. A 4.1.3.1; [39]) using an RC 612 elemental analyzer (LECO Cooperation, Moenchengladbach/Germany). Furthermore, the nitrogen was extracted in cold and hot water (CW_N and HW_N) according to the analysis of slow-release urea fertilizers (VDLUFA Methods Book II.1, No. 3.10; [38]). Additionally, nitrogen and carbon hydrolyzable in 0.005 M and 1 M hydrochloric acid (0.005 HA_N/HA_C and 1 HA_N/HA_C) were analyzed [40].

For all extracts, the total soluble nitrogen was measured spectrophotometrically as nitrate after UV-assisted digestion [41] on an AA3 continuous flow analyzer (Bran+Lübbe, Norderstedt/Germany). Furthermore, we analyzed the mineral nitrogen (MN) as a sum of ammonium- and nitrate-N in the cold water extract using the same AA3 continuous flow analyzer. Finally, the soluble organic N (ON) was calculated in all extracts as the difference between the total soluble N in the respective extract and the cold-water-soluble MN.

The determination of the organic carbon hydrolyzable in hydrochloric acid was carried out by ICP-OES (iCAP 6300 Duo, Thermo Scientific, Dreieich/Germany) at 193 nm after strong acidification with nitric acid and purging with high-purity argon [42]. Additionally, the organic carbon pools were characterized by stepwise combustion under pyrolytic conditions at 250, 300, 350, 400, 450, 500, 600, and 1000 °C and subsequent analysis of the evolved CO_2 (Py-TA-EGA; [43]) using a modified RC 612 elemental analyzer (LECO Cooperation, Moenchengladbach/Germany).

The TN, TOC, and TC analyses, Py-TA-EGA, and cold and hot water extractions were conducted in duplicate. Hydrolysis in hydrochloric acid, on the other hand, was performed in triplicate. Control samples (e.g., standard materials) were included in each analytical run to ensure the quality of the analysis and to maintain analytical precision.

2.3. Incubation Experiment

The incubation experiment was conducted according to the procedure proposed by the association of German agricultural analysis and research institutes (VDLUFA) for testing the N dynamics of organic growing media constituents (VDLUFA Methods Book I; No. A 13.5.1; [39]). The organic fertilizers were added on the basis of 1000 mg total N per liter of growing medium consisting of 80% peat (H3-H5 [44]) and 20% green waste compost by volume. The compost successfully fulfilled the growing media compost type II requirements of the German Federal Compost Quality Association [45]. The growing medium was limed to a pH of 5.5 (determined in a $CaCl_2$ suspension according to the VDLUFA Methods Book I, No. A 5.1.1; [39]). Due to the compost amendment, no other fertilizers except commercial organic fertilizers were added. All fertilizers were incubated in two ways to assess the influence of the particle size. Firstly, the fertilizers were used as received, and only large particles (especially the sheep wool pellets) were carefully crushed by hand to ensure their homogeneous addition to the incubation vessels. Secondly, the fertilizers were chopped in a cutting mill using a 0.5 mm bottom sieve (ZM 1, Retsch, Haan/Germany). This allowed for the evaluation of the effects of the particle size on the nitrogen release.

The Incubation ran for 58 days at 25 °C and 90% relative humidity in the dark. First, the growing medium was moistened with deionized water to 60% of the maximum water capacity (determined according to the VDLUFA Methods Book I, No. A 13.6; [39]), which is assumed to be well suited for microbial activity. Then, the water loss of the growing medium was compensated three times a week during the entire incubation period. On eight dates (days 0, 3, 7, 10, 16, 23, 37, and 58), three incubation vessels per treatment were taken from the incubator and analyzed for $CaCl_2$/DTPA-soluble ammonium- and nitrate-N photometrically (VDLUFA Methods Book I, No. A 6.1.4.1; [39]). As a basis for the N release

calculation, the controls without fertilizer and those with 500 mg N per liter as ammonium nitrate were treated similarly.

On each analysis day, the net mineralization of the fertilizers was calculated as the difference between the ammonium- and nitrate-N contents in the treatments with and without the respective fertilizers. Furthermore, the data from the control fertilized with ammonium nitrate were used to estimate the N turnover (mobilization and immobilization) in the growing media, as the results of the incubation experiment might be less reliable at high nitrogen turnover rates.

2.4. Calculations and Statistical Analyses

First, various kinetic models used in the literature to describe the decomposition of organic residues in mineral soils (Table 1) were fitted to the N release pattern of the fourteen commercial organic fertilizers. Fitting was performed by an iterative non-linear approach using the generalized reduced gradient algorithm of Microsoft's SOLVER [46,47]. The starting conditions were set by hand identically for each fertilizer, and the first fitting was calculated. The results obtained were taken as the starting conditions for a new run in the next step. This procedure was repeated four times. As the fitness-of-purpose criterion of the models, the total sum of squares (TSSQ) of the fourteen fertilizers was used.

Furthermore, fitting the fertilizer-specific equations to the N mineralization data was visually rated, and the coefficient of determination (R^2) was calculated for each fertilizer. Additionally, the parameters of the most suitable model and some selected points characterizing the time course of the N mineralization were computed by calculating the first and second derivations: (i) the turning point (T) of the original function, which defines the maximum mineralization rate, and (ii) the turning points of the first derivation, indicating the start (E_1) and the end (E_2) of the phase with the maximum mineralization rate. Furthermore, the time until the release of 90% of the potentially mineralizable nitrogen (N_{90}) was calculated. Finally, correlations of the analyzed N and C pools and the C/N ratios to the parameters of the model that best describe the course of N mineralization were computed to identify the pools that were well suited for characterizing the N release of the organic fertilizers. Thereby, correlations were calculated for the entire dataset and after removing the outliers determined by Cook's distance, with 4/n as the cut-off point [48]. The fitting and visualization of the kinetic models were carried out with MS Excel 2016 (version 16.0, Microsoft Corporation, Redmond, WA, USA). The software package Minitab21 (Minitab, LLC, State College, PA, USA) was used to visualize the C and N pools and to calculate the correlations.

Table 1. Kinetic models for characterization of net N release (N_R in mg L^{-1}) from organic fertilizers in relation to incubation period (t) in days; parameters N_P and N_E represent the potentially and mineralizable N (mg L^{-1}), respectively, parameters h, k, and k(1,2) represent the specific rate constants (mg L^{-1} d^{-1}), k_0 is the zero-order rate constant for recalcitrant N pools (mg L^{-1} d^{-1}), F is the proportion of N_P with fast turnover in a simultaneous reaction model, and d and c are unitless shape factors in Richards and Weibull functions (for details, refer to the given references).

Kinetic Model (Number of Parameters)	**Equation [Reference]**	
First-order kinetic (2)	$N_R = N_P * \left(1 - e^{-kt}\right)$	[7]
First-order kinetic plus readily available N (N_E) (3)	$N_R = N_P * \left(1 - e^{-kt}\right) + N_E$	[49]
Power function (2)	$N_R = ht^k$	[50]
Three-half-order kinetic model (3)	$N_R = N_P * \left(1 - e^{-k_1 t - 0.5(k_2 t^2)}\right)$	[51]

Table 1. *Cont.*

Kinetic Model (Number of Parameters)	Equation [Reference]	
Three-half-order kinetic model plus zero order rate constant (k_0) (4)	$N_R = N_P * \left(1 - e^{-k_1 t - 0.5(k_2 t^2)}\right) + k_0 t$	[51]
Simultaneous reaction model (4)	$N_R = N_P * F * \left(1 - e^{-ht}\right) + N_P * (1 - F) * \left(1 - e^{-kt}\right)$	[52]
Consecutive reaction model (3)	$N_R = N_P - N_P * \left(\frac{ke^{-ht} - he^{-kt}}{k-h}\right)$	[53]
Consecutive reaction model with rate constants h = k (2)	$N_R = N_P - N_P * e^{-kt} * (kt + 1)$	[53]
Gompertz function (3)	$N_R = N_P * e^{-e^{(h-kt)}}$	[14]
Gompertz function + N_E (4)	$N_R = N_P * e^{-e^{(h-kt)}} + N_E$	[14]
Gompertz function + k_0 (4)	$N_R = N_P * e^{-e^{(h-kt)}} + k_0 t$	[14]
Gompertz function + N_E + K_0 (5)	$N_R = N_P * e^{-e^{(h-kt)}} + N_E + k_0 t$	[14]
Richards function (4)	$N_R = N_P * (1 + de^{-k(h-t)})^{(-\frac{1}{d})}$	[54]
Richards function + N_E (5)	$N_R = N_P * (1 + de^{-k(h-t)})^{(-\frac{1}{d})} + N_E$	[54]
Richards function + k_0 (5)	$N_R = N_P * (1 + de^{-k(h-t)})^{(-\frac{1}{d})} + k_0 t$	[54]
Richards function + N_E + K_0 (6)	$N_R = N_P * (1 + de^{-k(h-t)})^{(-\frac{1}{d})} + N_E + k_0 t$	[54]
Weibull function (3)	$N_R = N_P * \left(1 - e^{-ktc}\right)$	[15]
Weibull function + N_E (4)	$N_R = N_P * \left(1 - e^{-ktc}\right) + N_E$	[15]
Weibull function + k_0 (4)	$N_R = N_P * \left(1 - e^{-ktc}\right) + k_0 t$	[15]
Weibull function + N_E + K_0 (5)	$N_R = N_P * \left(1 - e^{-ktc}\right) + N_E + k_0 t$	[15]

3. Results and Discussion

3.1. Characterization of Nitrogen and Carbon Pools

As fertilizers were added to the incubation experiment on the basis of the TN, the nitrogen and carbon pools were also related to the TN. As shown in Figure 1, most of the fertilizers contained nearly no mineral nitrogen (<50 mg N per g TN), whereas very high amounts of ammonium-N (419 mg N per g TN) were found in the OPF and, to a lesser extent (138 mg N per g TN), in the MAF. Except for 1 HA_ON, the soluble organic N pools differed considerably among the fertilizers. Their proportions of TN ranged from 3.8% to 38% for CW_ON and from 5.3% to 48% for HW_ON, comparable to the values reported by Rubins and Bear [55] for various raw and processed plant (7.0% to 47.5%) and animal (0.2% to 39.3%) products. Using 0.005 M hydrochloric acid gave slightly higher (7.9% to 66%) values and 1 M hydrochloric acid gave remarkably higher (42% to 94%) values, whereas the lowest values were found for the OPF (42%) and MAF (77%) due to their high contents of mineral N.

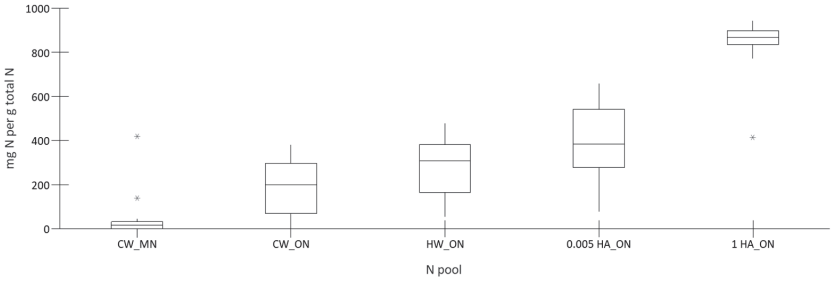

Figure 1. Variation in cold-soluble mineral nitrogen (CW_MN), cold- and hot-water-soluble organic nitrogen (CW_ON and HW_ON), and organic nitrogen hydrolyzable in 0.005 and 1 M hydrochloric acid (0.005 HA_N and 1 HA_N) among the fourteen organic fertilizers in relation to the total nitrogen (asterisks indicate outliers).

Due to the low amount of mineral nitrogen in most fertilizers, the percentages of the total soluble N of the TN were equivalent to those of the soluble organic N of the TN. In addition to their similar ranges, the cold water-, hot water-, and 0.005 hydrochloric acid-soluble N pools were highly correlated with each other but not with the 1 M_HA pool (Table 2).

Table 2. Correlations between total soluble and organic N pools for the fourteen organic fertilizers (Pearson's correlation coefficient, numbers in brackets indicate significance levels).

	Total N Soluble in			**Organic N Soluble in**		
	Cold Water	**Hot Water**	**0.005 M HCl**	**Cold Water**	**Hot Water**	**0.005 M HCl**
Hot water	0.98 (<0.01)			0.95 (<0.01)		
0.005 M HCl	0.94 (<0.01)	0.94 (<0.01)		0.92 (<0.01)	0.91 (<0.01)	
1 M HCl	0.01 (0.99)	−0.10 (0.75)	0.09 (0.76)	−0.09 (0.77)	−0.20 (0.48)	0.03 (0.91)

The variation in the organic C pools hydrolyzable in HA, and the TOC and organic carbon analyzed by Py-TA-EGA (POC), are summarized in Figure 2. The absolute values of TOC ranged between 2523 mg per g TN for the OPF and 11,514 mg per g TN for the FPG. Also, the amounts among the other organic C pools varied considerably from 19% to 88%, 57% to 96%, and 51% to 88% of TOC for 0.005 HA_C, 1 HA_C, and POC, respectively (Figure 2). Most organic carbon pools, whether analyzed by acid hydrolysis or by Py-TA-EGA, were positively correlated with each other ($r \geq 0.87$, $p < 0.01$). This indicates that Py-TA-EGA provided no more information about the organic carbon quality than acid hydrolysis.

With the exception of the TOC/TN ratio, for the calculation of the C/N ratios, only the organic N pools (CW_ON, HW_ON, 0.005 HA_ON, 1 HA_ON, TON)—calculated as the difference between the respective total N and the cold-water-soluble ammonium-N and nitrate-N—were used. The ratios of TOC to TN, TON, and 1 HA_ON, respectively, ranged from 3 to 12 and were quite similar to the values reported by Stadler et al. [30]. With the increasing strength of the extractant (CW < HW < 0.005 M HCl < 1 M HCl), the range of the TOC/ON ratios decreased. This was also true for the ratios of the organic C and organic N pools, both hydrolyzable in 0.005 (0.005 HA_C/ON) and 1 M hydrochloric acid (1 HA_C/ON), respectively (Figure 3).

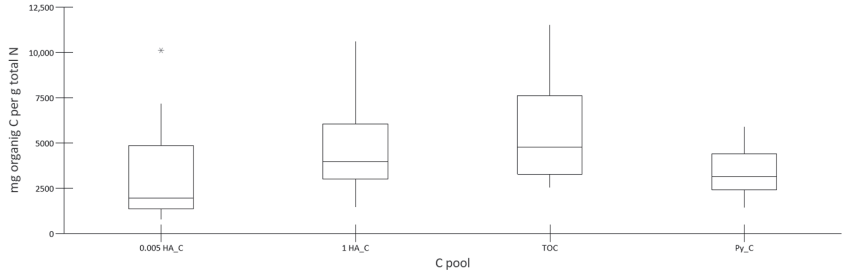

Figure 2. Variation in organic carbon hydrolyzable in 0.005 and 1 M hydrochloric acid (0.005 HA_C and 1 HA_C) and in total organic carbon (TOC) and organic carbon measured by pyrolytic combustion (POC) among the fourteen organic fertilizers in relation to the total nitrogen (asterisks indicate outliers).

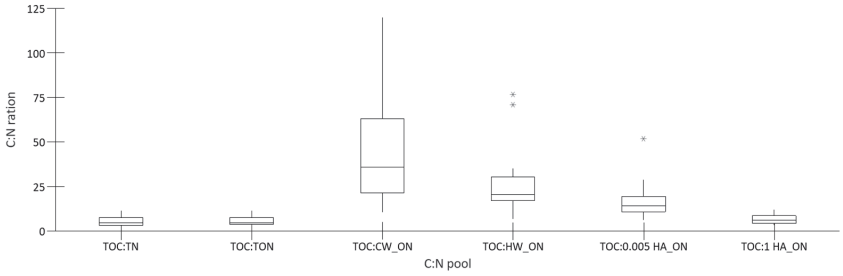

Figure 3. Variation in ratios of different carbon and nitrogen pools among the fourteen organic fertilizers (TOC = total organic carbon, TN = total nitrogen, TON = total organic nitrogen, CW_ON = cold-water-soluble organic nitrogen, HW_ON = hot-water-soluble organic nitrogen, 0.005 HA_ON = organic nitrogen hydrolyzable in 0.005 M hydrochloric acid, 1 HA_ON = organic nitrogen hydrolyzable in 1 M hydrochloric acid, 0.005 HA_C/ON = ratio of organic carbon and organic nitrogen hydrolyzable in 0.005 M hydrochloric acid, HA_C/ON = ratio of organic carbon and organic nitrogen hydrolyzable in 1 M hydrochloric acid; asterisks indicate outliers).

Due to the described correlations among the different pools of N and C and among several N and C pools whose data are not shown, the ratios of TOC to organic N pools soluble in weak extractants (CW, HW, and 0.005 M HA) were highly correlated with each other (Table 3). Furthermore, the TOC to organic N hydrolyzable ratio in 1 M hydrochloric acid (1 HA_ON) was correlated to the TOC/TN and TOC/TON ratios, respectively, which were also closely correlated. A comparably high correlation was found for the ratios of 1 HA_C/ON to TOC/TN, TOC/TON, and TOC/1 HA_ON, respectively. Additionally, a significant correlation existed between the ratios of C/ON analyzed in 0.005 and 1 M HA. This indicates that all tested weak extractants (cold water, hot water, and 0.005 M hydrochloric acid) provided more or less the same information about the organic N and C pools of the organic fertilizers and that in 1 M hydrochloric acid, the hydrolyzable organic C and N are more related to the total content than to the specific pools. This is quite different from other findings on the characterization of soil organic matter [56] but might be explainable by the higher proportion of readily degradable nitrogen-containing compounds in the organic fertilizers compared to soil organic matter. In contrast to soils, where approximately one-third of the organic nitrogen (N) remained non-hydrolyzable even after treatment with 6 M hydrochloric acid [57], the organic fertilizers contained a significantly higher proportion of hydrolyzable N (88 $\pm$ 1.5% using 1 M hydrochloric acid).

Table 3. Correlations among C/N ratios calculated from different C and N pools (TOC = total organic carbon, TN = total nitrogen, TON = total organic nitrogen, CW_ON = cold-water-soluble organic nitrogen, HW_ON = hot-water-soluble organic nitrogen, 0.005/1 HA C/ON = organic carbon/organic nitrogen hydrolyzable in 0.005/1 M hydrochloric acid) of the fourteen organic fertilizers (Pearson's correlation coefficient, numbers in brackets indicate significance levels).

	TOC/ TN	**TOC/ TON**	**TOC/ CW_ON**	**TOC/ HW_ON**	**TOC/ 0.005 HA_ON**	**TOC/ 1 HA_ON**	**0.005 HA C/ON**
TOC/TON	0.98 (<0.01)						
TOC/ CW_ON	−0.19 (0.51)	−0.29 (0.31)					
TOC/ HW_ON	0.01 (0.99)	−0.08 (0.79)	0.90 (<0.01)				
TOC/ 0.005H A_ON	0.04 (0.88)	−0.03 (0.92)	0.77 (<0.01)	0.93 (<0.01)			
TOC/ 1 HA_ON	0.95 (<0.01)	0.99 (<0.01)	−0.34 (0.24)	−0.13 (0.67)	0.08 (0.80)		
0.005 HA_C/ ON	0.76 (<0.01)	0.71 (<0.01)	0.33 (0.25)	0.54 (0.04)	0.46 (0.10)	0.66 (0.01)	
1 HA_C/ON	0.98 (<0.01)	0.98 (<0.01)	−0.25 (0.39)	−0.04 (0.96)	−0.01 (0.96)	0.96 (<0.01)	0.77 (<0.01)

3.2. Nitrogen Release

Overall, the nitrogen turnover in the control treatments without fertilizer and with 500 mg N per liter as ammonium nitrate was negligible, respectively. In the unfertilized control, the mineral N increased slightly from 14 ± 0.5 at the beginning up to 35 ± 12.0 mg L^{-1} after 58 days. Nevertheless, the N release from organic fertilizers was corrected for the mineral N in the unfertilized control at each date. No clear trend was observed in the control fertilized with ammonium nitrate. The mineral N oscillated at each date closely (484 ± 17.4 mg L^{-1}) around the target value of 500 mg N per liter. Furthermore, differences in the N release patterns between the chopped and unchopped fertilizers were rather small, and no systematic effect of particle size was found. This result was confirmed in a more detailed subsequent examination using horn shavings milled to defined grain sizes (<1 mm, 1–2 mm, and 2–4 mm). Only in the first two weeks was a slightly faster N release from finer materials observed. In the following four weeks, no differences were apparent [58]. Due to the slightly better reproducibility, only the N release from the chopped fertilizers was considered in the following.

The N release from organic fertilizers is described best by flexible sigmoid-shaped functions, in particular by the Richards and Gompertz functions. As found by Rahn and Lillywhite [59] and Nendel and Reuter [11], for brassica leaves and grape stalks, some fertilizers have shown a lag of mineralization within the first several days of incubation experiments. This was most pronounced for the SWPs, where no mineral N was found within the first seven days. Following the results of Simard and N'dayegamiye [16] for meadows, and of Hara [54] for coated urea fertilizers, this lag phase could not be described by first- or second-order rate equations or by consecutive reaction models but by flexible sigmoid Gompertz and Richards functions. As the parameter d in the Richards function was zero for most fertilizers—except for the OPF and SWPs—the Richards function is almost identical to the Gompertz function [54].

Furthermore, the TSSQ, more than twofold higher for the Gompertz compared to the Richards function (Figure 4), is nearly exclusively due to the relatively poor fitting of the

Gompertz function to the N release from OPF. In this case, the SSQ for the OPF ($226 \cdot 10^{-3}$) contributed nearly half of the TSSQ ($500 \cdot 10^{-3}$). However, due to the significant proportion of cold-water-soluble ammonium-N discussed in the previous section, it appears that OPF functioned more as a mineral rather than as an organic nitrogen fertilizer. This observation aligns with the fact that 500 out of the 1000 mg TN L^{-1} added was already present as CAT-soluble mineral N at the start of the 58-day incubation experiment. Despite this, the N mineralization only amounted to approximately 150 mg L^{-1} by the end of the experiment. Consequently, the subsequent paragraphs only minimally address the OPF.

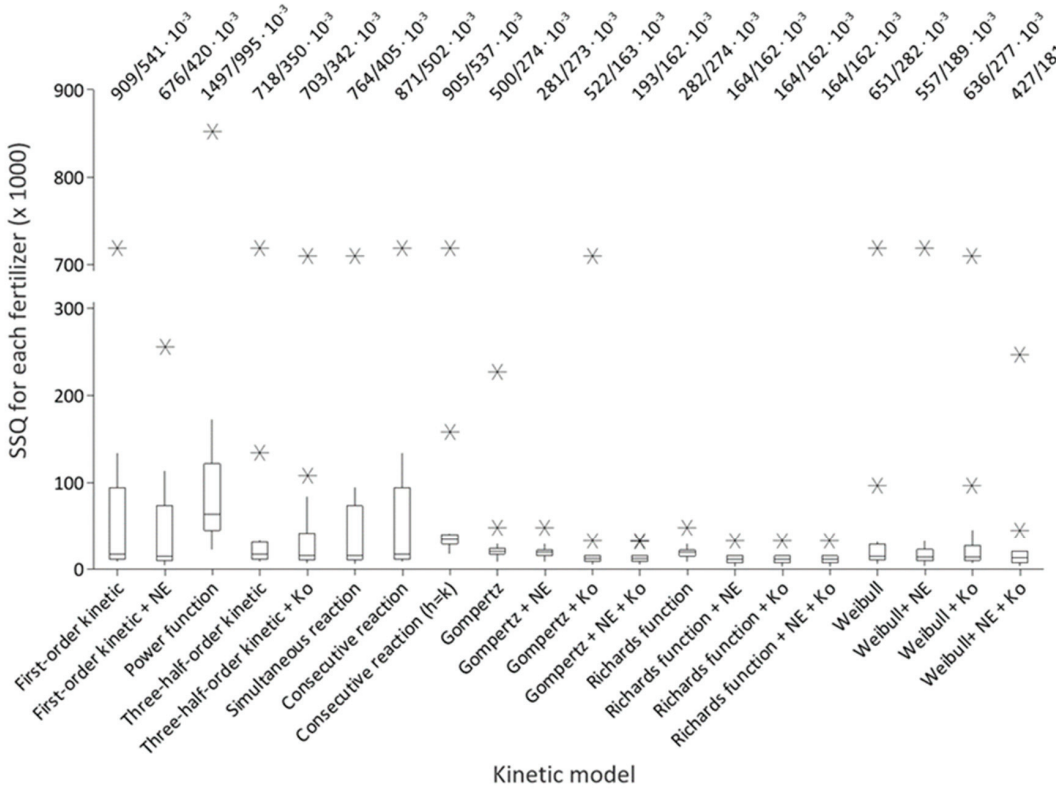

Figure 4. Sum of squares (SSQ multiplied by 1000) for fitting of different kinetic models to the N release of the fourteen organic fertilizers (for details of kinetic models, refer to Table 1; numbers above box plots indicate the total sum of squares (TSSQ) for all fertilizers except OPF for each kinetic model; asterisks indicate outliers).

As shown in Figure 4, adding a term for the easily mineralizable N pool (N_E) did not remarkably reduce the TSSQ for the remaining 13 fertilizers for the Gompertz function ($274 \cdot 10^{-3}$ to $273 \cdot 10^{-3}$) but did for the Richards function ($274 \cdot 10^{-3}$ to $162 \cdot 10^{-3}$). However, as described before, parameter d of the Richards function remained near zero for most fertilizers. This was also true when a zero-order rate constant (k_0) for less degradable N pools was added, which reduced the TSSQ remarkably for both the Gompertz and Richards functions. Finally, as the Richards function is nearly identically to the Gompertz function and the fitting was already very good for the simpler Gompertz function using only the three parameters N_P, h, and k ($R^2 > 0.91$, with an exception for OPF: $R^2 = 0.67$), this function was selected as the most suitable one. In addition, Gompertz's function also has a biological justification: The application of easily degradable organic carbon to soils triggers a rapid increase in microbial biomass [60–62]. As a result, especially the growth rate of

bacteria is significantly enhanced [62]. Concurrently, the Gompertz function has been demonstrated as a reliable model for bacterial growth. The parameters of this function have physiological significance as they can be traced back to the three phases of bacterial growth: the initial lag phase (parameter h), the phase of the maximum growth rate (parameter k), and the stationary phase in which the maximum population density (parameter N_P) is reached [63,64].

Figure 5 shows the fitting of the Gompertz function to the N release from the 14 organic fertilizers. The turning point (T) indicates the day when the maximum mineralization rate was reached, as well as the beginning (E_1) and end (E_2) of the nearly linear N release phase. Additionally, the goodness of fit (SSQ and R^2) and the period until a 90% release of N_P (N_{90}) are listed. The percentage of mineralizable organic nitrogen was around 55% of the total added N; it was the lowest for CX1, with 45%, and the highest for UP, with 63%. The same range has been reported for various commercial organic fertilizers by Prasad et al. [65] and Dion et al. [66], using peat-based growing media for incubation experiments, as well as by Müller and von Fragstein und Niemsdorff [28] and Stadler et al. [30], who conducted incubation experiments in mineral soils. Furthermore, Koch et al. [67] and Heuberger et al. [27] calculated only a slightly higher N efficiency (between 40% and 60% of the total added N) for organic fertilizer in pot experiments with pelargonium and basil, respectively. Thus, to ensure a sufficient nitrogen supply, growers have to fertilize on a total N basis, which is about twice the demand of the plants. Except for the SWPs and, to a lesser extent, the FPG and FBG, the fertilizers did not have a lag phase (indicated by parameter k), so linear mineralization started directly after adding the fertilizers ($E_1 < 1$). The maximum daily N mineralization rate (indicated by parameter h) was lowest for the SWPs, with 16, and highest for UP, with 65 mg L^{-1} d^{-1}. For the SWPs, due to the already mentioned lag phase of nearly two weeks and the low daily mineralization rate, it took nearly five weeks until two-thirds of the total mineralized nitrogen was released. For the FPG and FBG, which had a lag phase of several days and also a relatively low daily N mineralization rate (<30 mg L^{-1} d^{-1}), and for CX1, which had no remarkable lag phase but a similar low daily N mineralization rate (18 mg L^{-1} d^{-1}) to the SWPs, this point was reached within 14 to 20 days. All other fertilizers passed the 66% level within ten days. The CHM, PBPs, MAF, and UP exceeded 90% within 14 days. In addition to the relatively poor nitrogen efficiency, most organic fertilizers' high velocity of N release might be problematic for growers. For instance, in the cultivation of potted basil, if the entire N demand of about 1000 mg total N per liter [27] is applied at the date of sowing, two weeks later, the mineral N in the growing medium will be between 250 and 500 mg L^{-1}. However, by this date, the seedlings will have just emerged and might be harmed, in particular due to osmotic stress. Thus, complete stockpiling, as recommended by Heuberger et al. [27], is quite risky. Furthermore, in the case of missing nitrification, ammonium-N will accumulate, damaging plants [68]. However, in the current research—except for the OPF—nitrification already started within the first week of incubation. This was probably mainly due to the addition of compost. A similar enhancement of nitrification by compost amendment has been found, e.g., by Delics et al. [58] and Frerichs et al. [68]. However, in contrast to nitrification, neither reported a clear effect of compost amendment on the height or time course of the N mineralization. This might have been due to the fact that none of the composts used in these experiments contained remarkable amounts of mineral N [69].

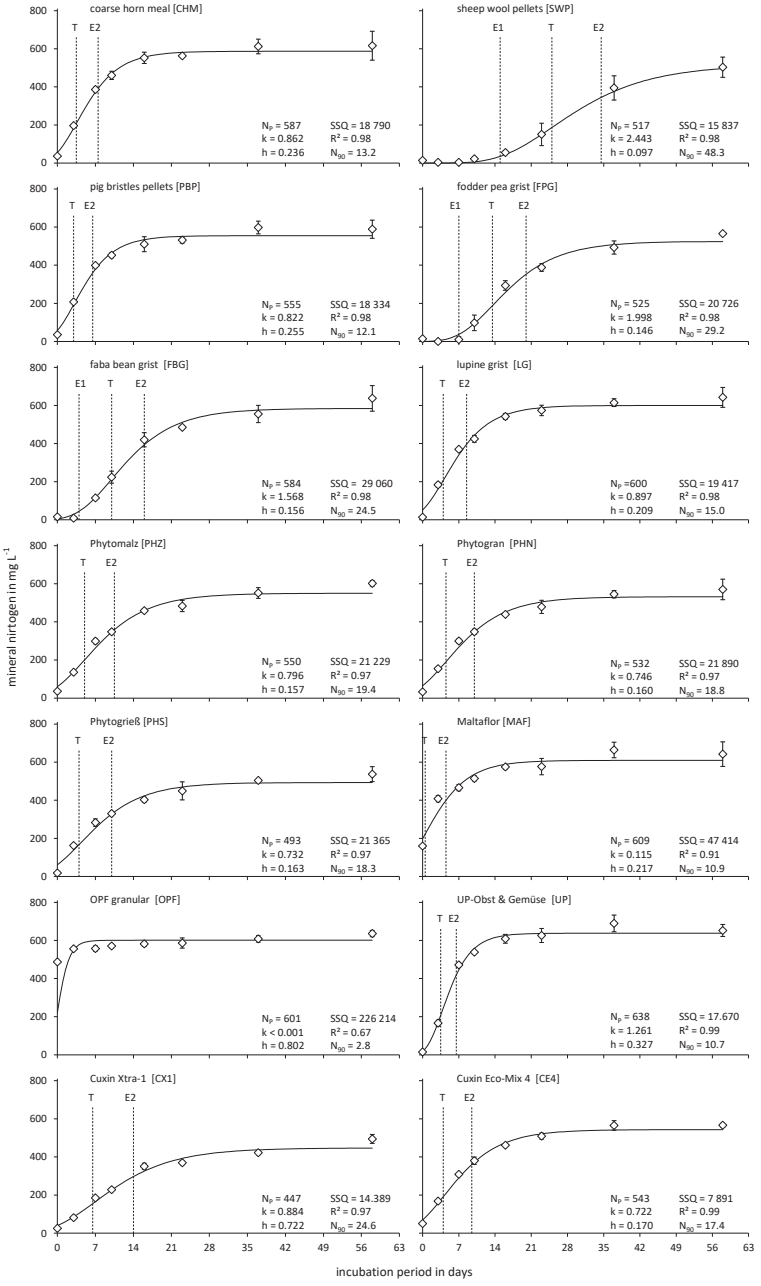

Figure 5. Fitting of Gompertz growth function to the N release of the 14 organic fertilizers (rhombs mark measures values of mineral N and error bars indicate 2·SE at each date (n = 3); vertical dashed lines mark points E_1 and E_2 = days until start and end of nearly linear mineralization, and T = days until maximum mineralization rate) and parameters of the Gompertz function (N_P = potentially mineralizable N in mg L^{-1}, k, and h = rate constants in mg L^{-1} d^{-1}), goodness of fit (SSQ = sum of the square of each fertilizer, R^2 = coefficient of determination), and days until the release of 90% of N_P (N_{90}).

3.3. Relative Importance of N and C Pools

As mentioned before, OPF is a mineral rather than an organic fertilizer. Thus, we excluded it from the following evaluation of the importance of N and C pools for N release from organic fertilizers. The level of N release to the total applied N (indicated by parameter N_P of the Gompertz function) was closely correlated to the easily soluble N pools (cold water, hot water, and 0.005 M hydrochloric acid) for most fertilizers. The only exception was UP, which had a very high N release in relation to the soluble N pool compared to all other fertilizers. As Cook's D for UP exceeded the cut-off point more than twice, correlations between the N release and the soluble N pools were calculated with and without the consideration of UP. When all fertilizers were included, the amount of mineralizable nitrogen (N_P) was not significantly correlated to the soluble N pools ($r \leq 0.48$; $p \geq 0.08$). However, omitting the UP resulted in highly significant correlations for the three named N pools ($r \geq 0.69$; $p < 0.01$), of which the relationship was closest for the total cold-water-soluble N (Figure 6a). A positive relationship between the easily soluble N pools and the mineralization potential was also shown by Iratani and Arnold [20], who found water-soluble N to be twice as influential as water-insoluble N in affecting the N release from various crop residues. Rubins and Bear [55] also emphasized a positive correlation between water-soluble N and N release. Furthermore, they reported decreasing net mineralization with an increasing C/N ratio of the non-lignin fraction. Even immobilization was observed if the C/N ratio was above 20. Coincidentally, a positive correlation between the ratios of the C and N pools hydrolyzable in weak hydrochloric acid and the lag phase of mineralization (described by parameter k) was found (Figure 6b). The hypothesis that the ratio of hydrolyzable C and N pools might be a reliable indicator for short-term net mineralization is supported by the results of Jensen et al. [24], Bushong et al. [70], and Ahn et al. [71]. All authors have reported positive relationships of soluble N and C pools with the short-term mineralization of organic matter. Thus, in products such as FBG, UP, and FPG, with higher ratios of C and N pools in the readily decomposable pools, even the net immobilization might occur within the first days, whereas for products with a lower ratio, the net N mineralization was found right from the beginning. However, the exceptionally high value of k for the SWPs (2.44 mg L^{-1} d^{-1}) and, thus, the lag phase of nearly 14 days, could not be solely attributed to the ratio of carbon and nitrogen in the easily decomposable pools. The discrepancy between the SWPs and all other fertilizers was confirmed by a Cook's $D > 4/n$ for the SWPs. One possible reason might be the high amount of wool wax—mainly lanolin—in sheep wool. As the percentages of lanolin and total N are similar in raw sheep wool [72], the amount of added lanolin in the incubation experiment was about 1 g L^{-1}. According to Arunkumar et al. [73], only certain groups of bacteria effectively degrade such wax-rich agricultural residues. Studies on wood fibers have shown that waxes can additionally act as physical and bio-chemical protection against microbial breakdown [72,74,75]. The extended lag phase of the SWPs might be related to the time needed to develop such a wax-degrading bacterial population. Furthermore, keratins—the fibrous proteins forming sheep wool—are highly resistant to hydrolysis by common proteolytic enzymes [76]. This seems, at first sight, contradictory to the fact that hooves (CHM) and pig bristles (PBPs), which also mainly consist of keratins, had no lag phase, and their daily N mineralization rates (45 and 47 mg L^{-1} d^{-1}) were among the highest of all fertilizers. However, the degradability of keratin-rich residues is closely related to the percentage of different keratin groups (α-, β-, and γ-keratins), the amino acid composition, and the amount of sulfur [76–78], which all differ among hooves, hairs, and feathers [37,77,78]. As a coincidence, the amount of easily (0.005 M hydrochloric acid) hydrolyzable organic nitrogen was about five times higher in the CHM and PBPs than in the SWPs. In contrast, the amount of organic nitrogen soluble in 1 M hydrochloric acid was the same for all three fertilizers. Moreover, as for the N_P mentioned before, the maximum daily N mineralization rate (indicated by parameter h of the Gompertz function) of easily hydrolyzable organic N pools was significantly higher for the UP than for the remaining twelve fertilizers. However, for the remaining fertilizers, quickly hydrolyzable

organic N pools were only a rough indicator of the maximum daily N mineralization rate (Figure 6c). Thus, the results present here are useful as indicators of the N release from organic fertilizers but cannot be used for a precise prediction thereof.

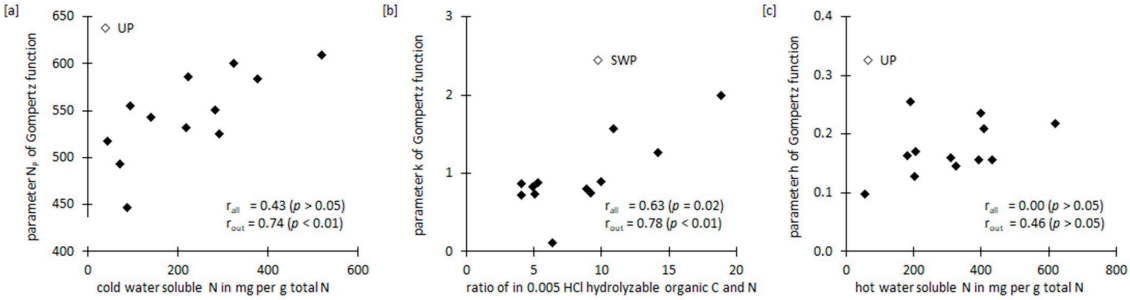

Figure 6. Correlations among parameters (**a**) N_P, (**b**) k, and (**c**) h of Gompertz function and particular C and N pools (Pearson's coefficient of correlation once calculated for all organic fertilizers with the exception of OPF ($_{all}$) and a second time additionally without outliers according to Cook's D (r_{out}), which are plotted as void symbols and labeled with acronyms).

Before transferring the results to the greenhouse, the influence of plants should be considered as an additional factor. Plant exudate and the microbiology within the rootzone can contribute to this effect [79]. For instance, the study by Gruda and Schnitzler [80] indicated that immobilized nitrogen levels were higher in experiments with plants than those without plants. In addition, root exudates affect the root and shoot growth of plants by attracting beneficial microbiota, chelating nutrients in the rootzone, modulating rootzone pH, and enhancing the availability of specific nutrient elements [79,81]. Furthermore, Helal and Sauerbeck [82] demonstrated that the activation of microorganisms, especially in proximity to the roots, might enhance carbon mobilization from the organic matter. Consequently, the demand for nitrogen increases to support the proliferation of microorganisms. Furthermore, the ongoing substitution of peat in growing media to other organic materials will make the topic more complex, as not only the N release from organic fertilizers but also N immobilization by growing media constituents, such as wood fiber, and the interaction between both processes are of increasing importance [69]. This should be addressed in detail in future research, in which the role of microbial activity and the structure of the microbial community have to be considered.

4. Conclusions

Water-soluble and easily hydrolyzable N and C pools of organic fertilizers are valuable indicators for the potentially mineralizable N and the time course of N release, which can be estimated very well by flexible-shaped sigmoid growth functions—mainly the Gompertz function. This makes organic fertilizers more comparable and can help match the N supply and demand better, thus increasing the efficiency and sustainability of organic pot plant production. In contrast to chemical composition, the particle size was less important for the time course of N release under the test conditions. Furthermore, the presented data highlight the two main challenges growers face when applying organic N fertilizers. First, growers must consider that the N release is only about half of the total applied N; thus, they must double the N supply compared to the N demand. Secondly, N release is relatively fast, which might cause salt damage and make the complete application of nitrogen before planting impossible.

Author Contributions: Conceptualization and methodology, D.L. and E.M.; formal analysis, investigation, data curation, writing—original draft preparation, D.L.; writing—review and editing, E.M. and N.S.G.; visualization, D.L.; supervision, E.M.; project administration, E.M. All authors have read and agreed to the published version of the manuscript.

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References

1. Granatstein, D.; Kirby, E.; Willer, H. Organic horticulture expands globally. *Chron. Hortic.* **2010**, *50*, 31–38.
2. Yue, C.; Dennis, J.H.; Behe, B.K.; Hall, C.R.; Campbell, B.L.; Lopez, R.G. Investigating consumer preference for organic, local, or sustainable plants. *HortScience* **2011**, *46*, 610–615. [CrossRef]
3. Reganold, J.P.; Wachter, J.M. Organic agriculture in the twenty-first century. *Nat. Plants* **2016**, *2*, 15221. [CrossRef]
4. Willer, H.; Lernoud, J. *The World of Organic Agriculture. Statistics and Emerging Trends*; Research Institute of Organic Agriculture FiBL: Frick, Switzerland; IFOAM-Organics International: Bonn, Germany, 2017; pp. 1–336.
5. Burnett, S.E.; Stack, L.B. Survey of the research needs of the potential organic ornamental bedding plant industry in Maine. *HortTechnology* **2009**, *19*, 743–747. [CrossRef]
6. Bergstrand, K.J. Organic fertilizers in greenhouse production systems–A review. *Sci. Hortic.* **2022**, *295*, 110855. [CrossRef]
7. Stanford, G.; Smith, S.J. Nitrogen mineralization potentials of soils. *Soil Sci. Soc. Am. J.* **1972**, *36*, 465–472. [CrossRef]
8. Frankenberger, W.T.; Abdelmagid, H.M. Kinetic parameters of nitrogen mineralization rates of leguminous crops incorporated into soil. *Plant Soil* **1985**, *87*, 257–271. [CrossRef]
9. De Neve, S.; Hofman, G. Modelling N mineralization of vegetable crop residues during laboratory incubations. *Soil Biol. Biochem.* **1996**, *28*, 1451–1457. [CrossRef]
10. Cabrera, M.L.; Kissel, D.E.; Vigil, M.F. Nitrogen mineralization from organic residues: Research opportunities. *J. Environ. Qual.* **2005**, *34*, 75–79. [CrossRef]
11. Nendel, C.; Reuter, S. Kinetics of net nitrogen mineralisation from soil-applied grape residues. *Nutr. Cycl. Agroecosyst.* **2007**, *79*, 233–241. [CrossRef]
12. Nakhone, L.N.; Tabatabai, M.A. Nitrogen mineralization of leguminous crops in soils. *J. Plant Nutr. Soil Sci.* **2008**, *171*, 231–241. [CrossRef]
13. Diaz-Fierros, F.; Villar, M.C.; Gil, F.; Carballas, M.; Leiros, M.C.; Carballas, T.; Cabaneiro, A. Effect of cattle slurry fractions on nitrogen mineralization in soil. *J. Agric. Sci.* **1988**, *110*, 491–497. [CrossRef]
14. Ellert, B.H.; Bettany, J.R. Comparison of kinetic models for describing net sulfur and nitrogen mineralization. *Soil Sci. Soc. Am. J.* **1988**, *52*, 1692–1702. [CrossRef]
15. White, C.S.; Marinakis, Y.D. A flexible model for quantitative comparisons of nitrogen mineralization patterns. *Biol. Fertil. Soils* **1991**, *11*, 239–244. [CrossRef]
16. Simard, R.R.; N'dayegamiye, A. Nitrogen-mineralization potential of meadow soils. *Can. J. Soil Sci.* **1993**, *73*, 27–38. [CrossRef]
17. Agbenin, J.O.; Agbaji, E.B.; Suleiman, I.; Agbaji, A.S. Assessment of nitrogen mineralization potential and availability from neem seed residue in a savanna soil. *Biol. Fertil. Soils* **1999**, *29*, 408–412. [CrossRef]
18. Deenik, J.L.; Yost, R.S. Nitrogen mineralization potential and nutrient availability from five organic materials in an atoll soil from the Marshall Islands. *Soil Sci.* **2008**, *173*, 54–68. [CrossRef]
19. Trinsoutrot, I.; Recous, S.; Bentz, B.; Linères, M.; Chèneby, D.; Nicolardot, B. Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions. *Soil Sci. Soc. Am. J.* **2000**, *64*, 918–926. [CrossRef]
20. Iritani, W.M. The Nitrogen Release of Vegetable Crop Residues During Incubation as Related to Their Chemical Composition. Ph.D. Thesis, University of Illinois at Urbana-Champaign, Champaign, IL, USA, 1958.
21. Fox, R.H.; Myers, R.J.K.; Vallis, I. The nitrogen mineralization rate of legume residues in soil as influenced by their polyphenol, lignin, and nitrogen contents. *Plant Soil* **1990**, *129*, 251–259. [CrossRef]
22. Henriksen, T.M.; Breland, T.A. Evaluation of criteria for describing crop residue degradability in a model of carbon and nitrogen turnover in soil. *Soil Biol. Biochem.* **1999**, *31*, 1135–1149. [CrossRef]
23. Pansu, M.; Thuriès, L.; Larré-Larrouy, M.C.; Bottner, P. Predicting N transformations from organic inputs in soil in relation to incubation time and biochemical composition. *Soil Biol. Biochem.* **2003**, *35*, 353–363. [CrossRef]
24. Jensen, L.S.; Salo, T.; Palmason, F.; Breland, T.A.; Henriksen, T.M.; Stenberg, B.; Pedersen, A.; Lundström, C.; Esala, M. Influence of biochemical quality on C and N mineralisation from a broad variety of plant materials in soil. *Plant Soil* **2005**, *273*, 307–326. [CrossRef]
25. Redin, M.; Recous, S.; Aita, C.; Dietrich, G.; Skolaude, A.C.; Ludke, W.H.; Schmatz, R.; Giacomini, S.J. How the chemical composition and heterogeneity of crop residue mixtures decomposing at the soil surface affects C and N mineralization. *Soil Biol. Biochem.* **2014**, *78*, 65–75. [CrossRef]
26. Noirot-Cosson, P.E.; Dhaouadi, K.; Etievant, V.; Vaudour, E.; Houot, S. Parameterisation of the NCSOIL model to simulate C and N short-term mineralisation of exogenous organic matter in different soils. *Soil Biol. Biochem.* **2017**, *104*, 128–140. [CrossRef]

27. Heuberger, H.; Kreuzmair, A.; Weh, F.; Schnitzler, W.H. Vegetabile Dünger als Stickstoffquelle für Topfbasilikum.-Freisetzung und Aufnahme von Stickstoff aus vegetabilen Düngern bei Basilikum (*Ocimum basilicum* L.) in Topfkultur. *Z. Für Arznei-Und Gewürzpflanzen* **2005**, *10*, 140–143.
28. Müller, T.; von Fragstein und Niemsdorff, P. Organic fertilizers derived from plant materials Part I: Turnover in soil at low and moderate temperatures. *J. Plant Nutr. Soil Sci.* **2006**, *169*, 255–264. [CrossRef]
29. Müller, T.; von Fragstein und Niemsdorff, P. Organic fertilizers derived from plant materials Part II: Turnover in field trials. *J. Plant Nutr. Soil Sci.* **2006**, *169*, 265–273. [CrossRef]
30. Stadler, C.; Von Tucher, S.; Schmidhalter, U.; Gutser, R.; Heuwinkel, H. Nitrogen release from plant-derived and industrially processed organic fertilizers used in organic horticulture. *J. Plant Nutr. Soil Sci.* **2006**, *169*, 549–556. [CrossRef]
31. Lü, H.; He, H.; Zhao, J.; Zhang, W.; Xie, H.; Hu, G.; Liu, X.; Wu, Y.; Zhang, X. Dynamics of fertilizer-derived organic nitrogen fractions in an arable soil during a growing season. *Plant Soil* **2013**, *373*, 595–607. [CrossRef]
32. Ros, G.H.; Hanegraaf, M.C.; Hoffland, E.; van Riemsdijk, W.H. Predicting soil N mineralization: Relevance of organic matter fractions and soil properties. *Soil Biol. Biochem.* **2011**, *43*, 1714–1722. [CrossRef]
33. Von Lützow, M.; Kögel-Knabner, I.; Ekschmitt, K.; Flessa, H.; Guggenberger, G.; Matzner, E.; Marschner, B. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biol. Biochem.* **2007**, *39*, 2183–2207. [CrossRef]
34. Stevenson, F.J. *Gross Chemical Fractionation of Organic Matter. Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*; American Society of Agronomy: Madison, WI, USA, 1965; pp. 1409–1421. [CrossRef]
35. Möller, K.; Schultheiß, U. Organische Handelsdüngemittel Tierischer und Pflanzlicher Herkunft für den Ökologischen Landbau—Charakterisierung und Empfehlungen für die Praxis. Kuratorium für Technik und Bauwesen in der Landwirtschaft (KTBL) e.V.: Darmstadt, Germany, 2013.
36. Block, R.J.; Bolling, D. The amino acid composition of keratins: The composition of gorgonin, spongin, turtle scutes, and other keratins. *J. Biol. Chem.* **1939**, *127*, 685–693. [CrossRef]
37. Górecki, R.S.; Górecki, M.T. Utilization of waste wool as substrate amendment in pot cultivation of tomato, sweet pepper, and eggplant. *Pol. J. Environ. Stud.* **2010**, *19*, 1083–1087.
38. *VDLUFA Method Book II.1: Analysis of Fertilizers*, 4th ed.; with 1–6 Supplement; VDLUFA-Verlag: Darmstadt, Germany, 2014.
39. *VDLUFA Method Book I: Analysis of Soils*, 4th ed.; with 1–7 Supplements; VDLUFA-Verlag: Darmstadt, Germany, 2016.
40. Meinken, E.; Lohr, D.; Wöck, C. Colonization of growing media by saprophytic fungi-importance of carbon and nitrogen fractions. *Acta Hortic.* **2017**, *1168*, 325–332. [CrossRef]
41. Kroon, H. Determination of nitrogen in water: Comparison of a continuous-flow method with on-line UV digestion with the original Kjeldahl method. *Anal. Chim. Acta* **1993**, *276*, 287–293. [CrossRef]
42. Oweczkin, I.J.; Kerven, G.L.; Ostatek-Boczynski, Z. Determination of dissolved organic carbon by inductively coupled plasma atomic emission spectrometry. *Commun. Soil Sci. Plant Anal.* **1995**, *26*, 2739–2747. [CrossRef]
43. Lohr, D.; Wöck, C.; Meinken, E. Pyrolytic combustion analysis of carbon-a new approach for characterizing growing media. *Acta Hortic.* **2017**, *1168*, 341–348. [CrossRef]
44. *DIN 11540:2019-03*; Peats for Horticulture and Landscape Gardening—Properties, Test Methods, Specifications. Beuth Verlag GmbH: Berlin, Germany, 2019.
45. *Methodbook for Analysis of Organic Fertilizers, Soil Improvers and Growing Media*, 1st ed.; with 1–6 Supplements; Bundesgütegemeinschaft Kompost (BGK): Köln, Germany, 2021.
46. Brown, A.M. A step-by-step guide to non-linear regression analysis of experimental data using a Microsoft Excel spreadsheet. *Comput. Methods Programs Biomed.* **2001**, *65*, 191–200. [CrossRef]
47. Kemmer, G.; Keller, S. Nonlinear least-squares data fitting in Excel spreadsheets. *Nat. Protoc.* **2010**, *5*, 267–281. [CrossRef]
48. Blatná, D. Outliers in regression. *Trutnov* **2006**, *30*, 1–6.
49. Jones, C.A. Estimation of an active fraction of soil nitrogen. *Commun. Soil Sci. Plant Anal.* **1984**, *15*, 23–32. [CrossRef]
50. Pal, D.; Broadbent, F.E. Kinetics of rice straw decomposition in soils. *J. Environ.-Qual.* **1975**, *4*, 256–260. [CrossRef]
51. Brunner, W.; Focht, D.D. Deterministic three-half-order kinetic model for microbial degradation of added carbon substrates in soil. *Appl. Environ. Microbiol.* **1984**, *47*, 167–172. [CrossRef]
52. Molina, J.A.E.; Clapp, C.E.; Larson, W.E. Potentially mineralizable nitrogen in soil: The simple exponential model does not apply for the first 12 weeks of incubation. *Soil Sci. Soc. Am. J.* **1980**, *44*, 442–443. [CrossRef]
53. Noggle, J.H. *Chapter 9 Physical Chemistry*; Little, Brown and Company: Boston, MA, USA, 1985; pp. 445–449.
54. Hara, Y. Application of the Richards function to nitrogen release from coated urea at a constant temperature and relationships among the calculated parameters. *Soil Sci. Plant Nutr.* **2000**, *46*, 683–691. [CrossRef]
55. Rubins, E.J.; Bear, F.E. Carbon-nitrogen ratios in organic fertilizer materials in relation to the availability of their nitrogen. *Soil Sci.* **1942**, *54*, 411–424. [CrossRef]
56. Silveira, M.L.; Comerford, N.B.; Reddy, K.R.; Cooper, W.T.; El-Rifai, H. Characterization of soil organic carbon pools by acid hydrolysis. *Geoderma* **2008**, *144*, 405–414. [CrossRef]
57. Leinweber, P.; Schulten, H.R. Nonhydrolyzable forms of soil organic nitrogen: Extractability and composition. *J. Plant Nutr. Soil Sci.* **2000**, *163*, 433–439. [CrossRef]
58. Delics, B.; Lohr, D.; Meinken, E. Nitrogen mineralization and subsequent nitrification of horn as affected by particle size and compost amendment to the growing medium. *DGG-Proceedings* **2017**, *7*, 1–5. [CrossRef]

59. Rahn, C.R.; Lillywhite, R.D. A study of the quality factors affecting the short-term decomposition of field vegetable residues. *J. Sci. Food Agric.* **2002**, *82*, 19–26. [CrossRef]
60. Arancon, N.Q.; Edwards, C.A.; Bierman, P. Influences of vermicomposts on field strawberries: Part 2. Effects on soil microbiological and chemical properties. *Bioresour. Technol.* **2006**, *97*, 831–840. [CrossRef] [PubMed]
61. Dinesh, R.; Srinivasan, V.; Hamza, S.; Manjusha, A. Short-term incorporation of organic manures and biofertilizers influences biochemical and microbial characteristics of soils under an annual crop [Turmeric (*Curcuma longa* L.)]. *Bioresour. Technol.* **2010**, *101*, 4697–4702. [CrossRef] [PubMed]
62. Lazcano, C.; Gómez-Brandón, M.; Revilla, P.; Domínguez, J. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function: A field study with sweet corn. *Biol. Fertil. Soils* **2013**, *49*, 723–733. [CrossRef]
63. Zwietering, M.H.; Jongenburger, I.; Rombouts, F.M.; Van't Riet, K.J.A.E.M. Modeling of the bacterial growth curve. *Appl. Environ. Microbiol.* **1990**, *56*, 1875–1881. [CrossRef] [PubMed]
64. Buchanan, R.L.; Whiting, R.C.; Damert, W.C. When is simple good enough: A comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. *Food Microbiol.* **1997**, *14*, 313–326. [CrossRef]
65. Prasad, M.; Simmons, P.; Maher, M.J. Release characteristics of organic fertilizers. *Acta Hortic.* **2004**, *644*, 163–170. [CrossRef]
66. Dion, P.P.; Jeanne, T.; Thériault, M.; Hogue, R.; Pepin, S.; Dorais, M. Nitrogen release from five organic fertilizers commonly used in greenhouse organic horticulture with contrasting effects on bacterial communities. *Can. J. Soil Sci.* **2020**, *100*, 120–135. [CrossRef]
67. Koch, R.; Emmel, M.; Lohr, D.; Frankenberg, A.; Degen, B.; Meinken, E.; Haas, H.P.; Fischinger, S. Organische Dünger in Topfkulturen auf dem Prüfstand—Wie steht es mit der Stickstofffreisetzung? In *Beiträge zu 14. Wissenschaftstagung Ökologischer Landbau, N-Düngung und Verluste*; Dr. Köster: Berlin, Germany, 2017; pp. 334–337.
68. Frerichs, C.; Daum, D.; Pacholski, A.S. Ammonia and ammonium exposure of basil (*Ocimum basilicum* L.) growing in an organically fertilized peat substrate and strategies to mitigate related harmful impacts on plant growth. *Front. Plant Sci.* **2020**, *10*, 1696. [CrossRef]
69. Vandecasteele, B.; Van Loo, K.; Ommeslag, S.; Vierendeels, S.; Rooseleer, M.; Vandaele, E. Sustainable growing media blends with woody green composts: Optimizing the n release with organic fertilizers and interaction with microbial biomass. *Agronomy* **2022**, *12*, 422. [CrossRef]
70. Bushong, J.T.; Roberts, T.L.; Ross, W.J.; Norman, R.J.; Slaton, N.A.; Wilson, C.E., Jr. Evaluation of distillation and diffusion techniques for estimating hydrolyzable amino sugar-nitrogen as a means of predicting nitrogen mineralization. *Soil Sci. Soc. Am. J.* **2008**, *72*, 992–999. [CrossRef]
71. Ahn, M.Y.; Zimmerman, A.R.; Comerford, N.B.; Sickman, J.O.; Grunwald, S. Carbon mineralization and labile organic carbon pools in the sandy soils of a North Florida watershed. *Ecosystems* **2009**, *12*, 672–685. [CrossRef]
72. Zoccola, M.; Montarsolo, A.; Mossotti, R.; Patrucco, A.; Tonin, C. Green hydrolysis as an emerging technology to turn wool waste into organic nitrogen fertilizer. *Waste Biomass Valorization* **2015**, *6*, 891–897. [CrossRef]
73. Arunkumar, N.; Gulsar Banu, J.; Gopalakrishnan, N.; Prakash, A.H. Wax degrading bacteria: Scope and applications in agriculture. *Int. J. Curr. Microbiol. Appl. Sci.* **2017**, *6*, 649–664. [CrossRef]
74. Goodrich, B.S.; Roberts, D.S. Antimicrobial factors in wool wax. *Aust. J. Chem.* **1971**, *24*, 153–159. [CrossRef]
75. Sengupta, A.; Behera, J. Comprehensive view on chemistry, manufacturing and applications of lanolin extracted from wool pretreatment. *Am. J. Eng. Res.* **2014**, *3*, 33–43.
76. Zaghloul, T.I.; Embaby, A.M.; Elmahdy, A.R. Key determinants affecting sheep wool biodegradation directed by a keratinase-producing Bacillus subtilis recombinant strain. *Biodegradation* **2011**, *22*, 111–128. [CrossRef]
77. Fellahi, S.; Zaghloul, T.I.; Feuk-Lagerstedt, E.; Taherzadeh, M.J. A Bacillus strain able to hydrolyze alpha-and beta-keratin. *J. Bioprocess. Biotech.* **2014**, *4*, 1–7. [CrossRef]
78. Sharma, S.; Gupta, A. Sustainable management of keratin waste biomass: Applications and future perspectives. *Braz. Arch. Biol. Technol.* **2016**, *59*, 1–14. [CrossRef]
79. Balliu, A.; Zheng, Y.; Sallaku, G.; Fernández, J.A.; Gruda, N.S.; Tuzel, Y. Environmental and cultivation factors affect the morphology, architecture and performance of root systems in soilless grown plants. *Horticulturae* **2021**, *7*, 243. [CrossRef]
80. Gruda, N.; Schnitzler, W.H. Influence of wood fiber substrates and application rates on the growth of tomato transplants. *Adv. Hortic. Sci.* **1999**, *13*, 20–24.
81. Vives-Peris, V.; De Ollas, C.; Gómez-Cadenas, A.; Pérez-Clemente, R.M. Root exudates: From plant to rhizosphere and beyond. *Plant Cell Rep.* **2020**, *39*, 3–17. [CrossRef] [PubMed]
82. Helal, H.M.; Sauerbeck, D. Effect of plant roots on carbon metabolism of soil microbial biomass. *Z. Für Pflanz. Und Bodenkd.* **1986**, *149*, 181–188. [CrossRef]

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Using Respirometry to Investigate Biological Stability of Growing Media in Aerobic Conditions

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Abstract: Materials used to replace peat in growing media include wood fibre (WF), often used in combination with composted bark (BC), coir (CR), green compost (GC), and anaerobic digestate fibre (AD). The physical and chemical properties of these materials are relatively well characterised; however, biological properties are less well understood. Biological stability of growing media is an important factor in plant performance. The aim of this research was to identify whether dynamic respirometry methods are suitable for measuring growing media stability and to assess the effect of blending two raw materials in a mix. Raw materials were run for 42 days in aerated conditions at 35 °C. Except for AD, individually run samples were considered stable, with CO_2 production over 7 days ranked BC < CR < WF < GC << AD in the early stages of the test. The AD was run at two moisture levels, with greater biological activity at lower moisture content. In the most active mixture, AD and WF, there was an increase of activity when nutrients were added at 28 days, indicating major elements were limiting microbial activity. There were interaction effects in sample mixtures, with the CO_2 production of WF + GC, WF + CR greater than the sum of the CO_2 production from the separate components.

Keywords: growing media; soilless culture; stability; microbial activity; wood fibre

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1. Introduction

Over the past decade there has been a shift in the use of peat within the horticulture industry from a prominent component of growing media blends to being phased out in some European countries [1–3]. As a result, a variety of alternative raw materials have been used to replace peat within the industry. The main components of peat-free blends within UK horticulture include coir, wood fibre, bark, anaerobic digestate fibre, and green composts [4]. Wood fibre is increasingly being used as a component of growing media mixes due to its useful physical properties, including water-holding capacity and ability to reduce the hydrophobicity of other mix components [5,6]. Within the literature, a number of materials, often residual materials of other industries, have also been evaluated for their potential use, such as miscanthus and bracken [7,8]. The physical and chemical properties of the main alternative components are generally well understood by the industry and amendments can be made to ensure they are suitable for use within horticulture [3,9,10]. The biological properties of peat-free raw materials are less well understood, though increasing numbers of studies have characterised the microbial populations present within some growing media raw materials [11–13].

Biological stability of growing media can be considered the lack of microbial activity. Microbial activity in growing media has the potential to alter the carefully designed physical and chemical properties during storage or within the pot during cultivation, potentially resulting in sub-optimal plant growth performance. This can include changing the physical structure, key to moisture retention and aeration, and chemical properties such as nutrient

status, mineralisation, pH, and maintaining ion exchange capacity [14]. Furthermore, this can change conditions for microbial growth over time, resulting in complex interactions.

In use, growing media are expected to be well aerated, with aerobic microbial respiration dominating. Anaerobic activity may be present in micro-sites such as in wet aggregates, but this is not considered here. Aerobic microbial activity may be limited by poor aeration (low oxygen levels), lack of available moisture, lack of key nutrients, or chemical factors including low pH. These could be considered artificial limitations that can cause "false stability" [15], rather than intrinsic to the substrate. Providing optimum, non-limiting conditions should allow determination of substrate quality, especially availability of carbon as the substrate breaks down, in defined conditions. In respirometry, either CO_2 production or O_2 consumption is used to track respiration.

Common methods for determining the stability or microbial activity of growing media include oxygen uptake rate (OUR) tests, either in vessels with tops that record the pressure changes [16] or as pre- and post-incubation comparison measurements [17]. These tests are useful to give an indication of microbial activity within a strict set of conditions. There are a few limitations with this style of test; for example, the sample size is small (typically 2 g for pressure vessels), which relies on the homogeneity of the test material. Aspray et al. [15] demonstrated that OUR tests can go out of range when the test material is highly active, which could be the case with anaerobic digestate or green compost. The test conditions are not close to the environmental conditions within which a growing medium would be stored or used to grow plants, though various authors have found reasonable correlation between OUR and respirometric tests for compost materials [18,19].

Tests measuring evolved carbon dioxide (CO_2) are less commonly used to assess the stability of growing media. Montagne et al. [11] used a modified carbon (C) mineralisation method to calculate the amount of C released as CO_2 from coir, peat, and wood fibre samples over 3 months. Vandecasteele [20] used a CO_2 respirometry test with daily CO_2 measurements, though this was a more similar to a static test, which could lead to O_2 becoming limiting.

Established dynamic respiration tests used in the UK composting industry are the four-day dynamic respiration test DR4, designed to monitor composting of waste [21], and ORG0020 [22], used to specify compost quality under the PAS100 scheme [23]. This type of test could be useful for assessing the stability of growing media in conditions similar to those used in glasshouse plant production. These tests are both solid-phase dynamic respiration tests, though the DR4 was designed for more active materials, including compost feedstocks, whereas the ORG is targeted at distinguishing between more stable composted products. Key differences between these tests are shown in Table 1.

Table 1. Comparison of DR4 and ORG0020 respirometric tests.

Parameter	ORG0020	DR4
Intended use	Composted materials	Compost process feedstock and product
Sample size	100 g fresh weight (FW)	100 g dry solids
Aeration	Flow through headspace	Air forced through sample
Aeration rate	50 ± 25 mL/min/100 g FW	400 ± 100 mL/min/400 g FW
Moisture	"hand squeeze test"	50% dry matter (DM)
Inoculum	None	100 g dry solids, mature green compost
Nutrient addition	None	NPK
Temperature	30 °C	35 °C
Data collection	Days 4–7 inclusive	Days 1–4

These tests are designed to provide robust comparisons between samples, though they use different conditions and are not directly comparable. Neither is specifically optimised for very low-activity (high-stability) growing media components. The recommended aeration rate for DR4 is higher, expected to provide sufficient aeration for relatively high-activity/low-stability samples. The DR4 is considered a "truly dynamic" test [15] as it forces

air through the sample mass, while ORG0020 passes air through the chamber headspace only, relying on diffusion to supply oxygen throughout the sample. Aspray et al. [15] found good correlation between these tests for ten composted materials, while static chamber tests, OUR, and self-heating were considered less reliable for these materials.

Moisture is expected to be a key variable, with the optimum value unknown and possibly different between sample types. A fixed gravimetric moisture content as used in the DR4 test takes no account of water-holding properties and is therefore not appropriate to the diverse materials tested here. A "hand squeeze test" approach as used in ORG0020 provides a moisture level closer to conditions used for plant growth. Other moisture conditions have been used, e.g., 75% of water-holding capacity [24], though this has been reported to be less reliable than the hand-squeeze method [22]. Temperature is also expected to be an important variable. Nutrients are added in the DR4 test with the intention that major nutrients are not limiting. This is omitted in ORG0020, apparently assuming composted materials will contain sufficient nutrients. Growing media include components with very high C:N ratio, which may require higher levels of nutrient addition, and may lack trace elements.

A further key difference is the use of an inoculum to supply a microbial population in the DR4 test. In all but recently sterilised media, there will be a microbial population present, adapted to the prevailing conditions and substrate [25]. An inoculum can provide a diverse microbial community, as well as a stable physical structure and chemical buffering, providing a more reliable test [15]. ORG0020 relies on the existing microbial population within the sample, though the initial 3-day equilibration period provides time for the existing microbial population to adapt to test conditions.

ORG0020 does not measure CO_2 production during an initial 3-day equilibration period, so that measurements cover the period from start of day 4 to end of day 7. This is explained as an initial flush of activity following disturbance that may not be related to longer-term stability [22]. It seems possible that this initial peak activity may be important, relating for instance to conditions in freshly planted growing media. Using automated data collection over the full period allows a fuller dataset and comparison of different time periods.

Previous studies have either focused on the stability of individual raw materials or of three- or four-component blends [11,20,26], without identifying the interaction effects when just two raw materials are mixed. There is the potential for one raw material with a high or diverse microbial population to act as an inoculum for another raw material with a lower microbial population. Wood fibre, for example, has been described as a stable material with a lower microbial diversity than coir [12], but has the potential to act as a readily available source of carbon when mixed with another raw material with a more diverse microbial population. A DR4-style test could be a useful tool to draw out these interactions between raw materials in a blend of growing media.

The aim of this research was to not only identify whether the dynamic respirometry tests described above (i.e., DR4 and ORG0020) are suitable methods for measuring growing media stability, but also assess the effect of blending two raw materials in a mix. As many of the samples of interest were expected to be very stable, tests were extended to observe longer-term effects that may be relevant to growing media in use.

2. Materials and Methods

2.1. Physico-Chemical Characteristics

Five types of growing media raw materials commonly used in peat-free growing media blends in the UK were tested. These were anaerobic digestate fibre (AD), bark (BC), coir (CR), wood fibre (WF), and green compost (GC). Two samples of WF were obtained from different production batches at the same source; WF2 was the primary sample reported and used in test mixtures. The WF used in the study was produced by steaming, pressure treating, and expanding wood chips. Two samples of AD were obtained, with AD1 reported in detail and used in tests on moisture and nutrients.

Initial characterisation of dry matter (DM), moisture content, and laboratory-compacted bulk density were determined according to BS EN 13040:2007 [27]. Loss on ignition was determined according to BS EN 13039:2012 [28], as a measure of organic matter. The total carbon and nitrogen content of the raw materials was analysed via an Elementar Vario EL Cube elemental analyser (Elementar Analysensysteme GmbH, Langenselbold, Germany). The main physico-chemical characteristics are summarised in Table 2.

Table 2. Characteristics of test materials used in the study. Means and (standard deviations) of three replicates except where indicated.

Sample	Bulk Density g/cm^3	Dry Matter % FW as Received	DM at "Hand Squeeze Test" % FW	Loss on Ignition % DM	C % DM	N % DM
WF1	n/a	48.77 (1.18)	23.7 (2.6)	99.99 (0.01)	45.9 (0.2)	0.08 (0.01)
WF2	0.080 (0.004)	46.58 (0.59)	* 21.9 (0.4)	100.00 (0.01)	46.2 (0.1)	0.06 (0.00)
BC	0.434 (0.003)	31.86 (0.59)	* 27.3 (0.3)	84.71 (3.1)	41.4 (0.8)	1.08 (0.02)
CR	0.320 (0.002)	22.50 (2.93)	* 18.3 (0.2)	79.04 (4.9)	39.7 (0.5)	0.75 (0.04)
AD1	0.159 (0.011)	57.02 (4.2)	* 28.3 (0.3)	88.02 (0.9)	40.1 (0.9)	1.96 (0.08)
AD2	n/a	39.25 (1.16)	28.2 (0.05)	81.40 (0.6)	35.1 (0.8)	2.57 (0.05)
GC	0.425 (0.001)	63.82 (0.97)	47.5 (0.06)	46.79 (2.9)	21.4 (0.4)	1.13 (0.03)
Reference (Cellulose)	n/a	93.19 (0.05)	n/a	99.99 (0.01)	44.44	0

n/a = not available. * Means of only two replicates.

2.2. Stability Testing

Test conditions followed the DR4 test [21], extended to 42 days. Each material was run individually, using 100 g dry weight per chamber, without inoculum, as in ORG0020 [22]. In addition, 100 g dry weight of each material was mixed with 100 g dry weight WF per chamber. A full DR4 was conducted on the WF only. In short, 100 g dry matter (DM) of WF was mixed with 100 g DM of GC inoculum. This was tested alongside a blank (100 g DM of GC) and a reference material (100 g DM of GC + 10 g α-cellulose from Sigma-Aldrich, UK).

All samples were incubated at 35 °C throughout the test. All treatments were run in triplicate. Moisture content for each sample was standardised as per the "hand squeeze test" at the start of the experimental run as per ORG0020 [22]. The "hand squeeze test" was performed by the same operative throughout to reduce the potential variability that has been noted with this measure [18]. Nutrients were included in the added water following the DR4 method [21], as NH_4Cl and KH_2PO_4, to provide 0.28 g N, 0.15 g P, and 0.19 g K in each chamber.

To evaluate the effect of moisture on the test, the AD fibre was tested in the ORG style outlined above on an "as received" basis with no additional moisture, other than the initial 10 mL of nutrient solution. The effect of nutrients on this test were determined by running samples of AD and WF with no nutrients added to the test mixture at the start of experiment. These treatments then had the addition of the nutrient solution at 28 days along with all other treatments.

The respirometer setup is shown in Figure 1. The test samples were placed into 4 L respirometer chambers and were incubated at 35 °C, with forced air flows maintained in the range of 250–300 mL/min for 42 days. Inlet air was passed through a condenser at 4 °C to standardise moisture content. Outlet air was also passed through condensers at 4 °C, and further dried before measurement of composition of the dry gas. Inlet flow rates and the CO_2 and O_2 content of inlet and exhaust gases were recorded at 2 h intervals using on-line analysers (FB8 mass flow meters, MUX3 multiplexers, CAXL CO_2 analyser, FCX oxygen analyser, data collection via UI2 interface and ExpeData v.1.8.5 software; Sable Systems International, Las Vegas, NV, USA). Analysers were calibrated using 1% and 2% gas standards in nitrogen for CO_2 (Calgaz Ltd., Stoke, UK), ambient outside air for O_2, and pure nitrogen as a zero point.

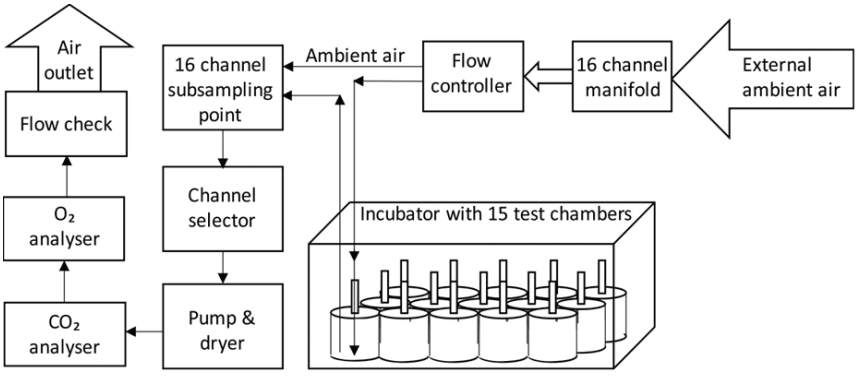

Figure 1. Schematic diagram of respirometer.

The chambers were weighed and shaken every 7 days to redistribute moisture and nutrients. Further nutrient stock solution, as specified above, was added at 28 days to all of the test chambers during the shaking procedure.

2.3. Data Analysis

Initial data processing was carried out in ExpeData® v.1.8.5, using inbuilt macros. Baseline corrections were applied using ambient outside air at three points in each 2 h run to compensate for drift in the analyser readings. Lags between flow data and CO_2 and O_2 concentrations were corrected, and the most stable flow, CO_2, and O_2 signals selected for each channel. Data were exported and further data processing was conducted using an R statistical environment (v.4.2.2). The outlet flow was not measured, and gas composition will have changed in the chamber, changing the mass flow. CO_2 production was corrected using O_2 composition of the outlet gases using gas-exchange equations [29]. The flow rate was more than adequate to maintain aerobic conditions, with outlet O_2 not falling below 18 % at peak O_2 demand.

The graphs presented below have been simplified for clarity, with error bars only marked for one point in each two days. Lines have been used rather than individual points, unless otherwise stated.

3. Results

3.1. Microbial Activity of Single Materials

When run as single materials, four out of five substrate types could be considered biologically stable (Table 3; Figure 2). The UK compost quality specification PAS100 [23] defines a threshold for stable compost using ORG0020 as 16 g/kg volatile solids(VS)/day, or 64 g/kgVS over the 4 days of ORG0020 [23]. Llewelyn [22] suggested a single threshold equivalent to 40 g/kgVS/4 days, or in more detail, respiration rate was considered very low below 32 g/kgVS/4 days, low up to 48 g/kgVS/4 days, medium up to 64 g/kgVS/4 days, high up to 80 g/kgVS/4 days, and very high above 80 g/kgVS/4 days. These are based on stability of mature green compost and are broadly related to compost age. The GC sample used here passes the PAS100 stability threshold, though is slightly above Llewelyn's [22] suggested single threshold (Table 3).

Most materials showed a distinct initial peak in the first few days of the test, followed by steadily declining CO_2 production. This forms a cumulative curve tending to an asymptotic value or linear increase (Figure 2c,d). In contrast, the composted bark did not show any initial peak and had low, though measurable, activity throughout the 42 days. The coir showed an increase in CO_2 production after a 7-day lag period, peaking at about 15 days.

Table 3. Cumulative values of CO_2 production at 4 days (equivalent to DR4), days 3–7 (equivalent to ORG0020), 7 and 28 days, 28-day O_2 consumption, and percentage C loss, means and (standard deviations) for three replicates. Samples are in rank order for DR4 results.

Sample	4-Day CO_2, g/kgVS (DR4)	Days 3–7 CO_2 g/kgVS (ORG0020)	7-Day CO_2 g/kgVS	28-Day CO_2 g/kgVS	28-Day O_2 g/kgVS	42 Days %C Loss
AD1	156.5 (7.2)	99.4 (5.8) ***	225.9 (11.2)	464.9 (21.6)	495.4 (19.2)	30.1 (1.6)
AD2	133.6 (9)	135.3 (4.4) ***	226.6 (7.5)	461.3 (13.3)	498.5 (3.2)	31.9 (0.8)
GC	32.4 (1.9)	22.2 (1.7) *	48.6 (3.1)	124.4 (5)	164.3 (61.8)	7.7 (0.1)
WF1	18.7 (1.6)	9.7 (1) *	25.5 (2.1)	43 (0.9)	47.9 (7)	3.1 (0.2)
WF2	17.4 (0.3)	11.1 (0.3) *	24.8 (0.3)	40.9 (1.2)	57.2 (40.7)	2.8 (0.2)
CR	4 (0.3)	4.8 (0.3) *	7.7 (0.5)	63 (1.6)	67 (14.3)	4.6 (0.2)
BC	3.3 (0.4)	3.6 (0.9) *	5.9 (1.1)	31.8 (13.3)	33 (12.6)	2.6 (1.3)
Mixes						
AD + WF	94.4 (6.2)	53.5 (5.3) **	131.9 (9.8)	254.3 (18)	267.9 (6.4)	19.1 (1.6)
GC + WF	34.4 (1.4)	15.5 (0.2) *	45.8 (1.4)	112.4 (1.6)	115.3 (16.2)	7.9 (0.1)
CR + WF	19.1 (0.6)	6.9 (0.2) *	23.8 (0.7)	53.8 (1.6)	56.1 (14.0)	3.9 (0.1)
BC + WF	13 (1.1)	5.9 (0.2) *	17.1 (1.1)	34.1 (0.6)	34.5 (17.7)	2.4 (0)

Assessment according to Llewelyn [22]: *** extremely high; ** medium; * very low.

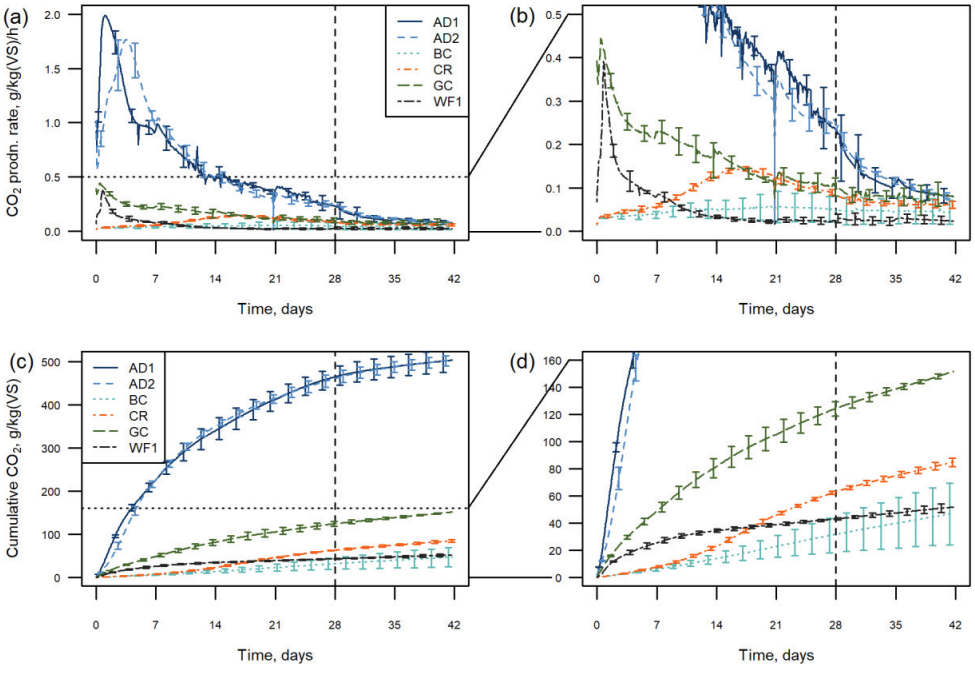

Figure 2. CO_2 production of the single raw materials' (**a**) gas production rate; note the area below the horizontal dotted line is expanded in (**b**) for visibility of the samples with lower CO_2 production; (**c**) cumulative gas production; note the area below the horizontal dotted line is expanded in (**d**) for visibility of the samples with lower CO_2 production. Graphs show means, error bars show ±1 standard deviation and every 24th point is plotted. The vertical dashed line indicates nutrient addition at 28 days.

The AD was the least stable substrate, with a large initial peak over the first two days, then declining. A comparison of the two AD batches showed a difference in the stability over the first four days (Figure 2a,c) and differing ORG0020 values (99 and 135 g/kgVS/4 days); however, by seven days the cumulative CO_2 production was the

same. There was an initial lag in the activity of AD2, with peak CO_2 production at 3 days, compared with AD1 at 1 day. A similar comparison of the WF batches showed that the biological stability of the two samples over time was almost identical, with all results being within one standard deviation of each other (Table 3).

The WF and GC samples showed a similar initial peak; however, the amount of CO_2 produced was much lower in the WF over the first four days (19 and 32 g/kgVS CO_2 respectively). The peak in the WF was very sharp and CO_2 production then rapidly decreased, whist the GC had more sustained activity over the duration of the whole test.

The BC was the most stable of all the substrate types, with very low CO_2 production for the whole time series. There was variability in this data, however, which was attributed to one replicate losing moisture during the test, resulting in higher CO_2 production. This suggests the "hand squeeze" test moisture was not optimal for this sample. As a result, CO_2 production may have been underestimated by the samples remaining at "hand squeeze" moisture, and stability overestimated in this current test.

The nutrient addition at day 28 appears to have had no obvious effect on the stability of any of the single raw materials, with no pulse in CO_2 production seen.

The percentage of carbon loss can be found in Table 3. This shows 30 to 32% carbon loss in the most active samples (AD), with next largest loss from GC at under 8%. Other single materials lost under 5% of carbon in the 42-day test, with WF and BC samples losing least, reflecting the higher stability of these samples over the full period of the test.

3.2. Moisture Effects on Test

The "as received" sample had a dry matter content of 57% compared with 28% when the sample moisture was adjusted to the "hand squeeze test" (Table 1). The moisture content of the AD substrate influenced the CO_2 production in the test (Figure 3). The chambers with added moisture had a larger initial peak than those without, with the CO_2 production becoming comparable only at the 28-day point. The cumulative CO_2 production at 28 days was less than half the "hand squeeze" moisture sample when no moisture was added to the material (216 g/kgVS CO_2 "as received"; 495 g/kgVS CO_2 adjusted moisture).

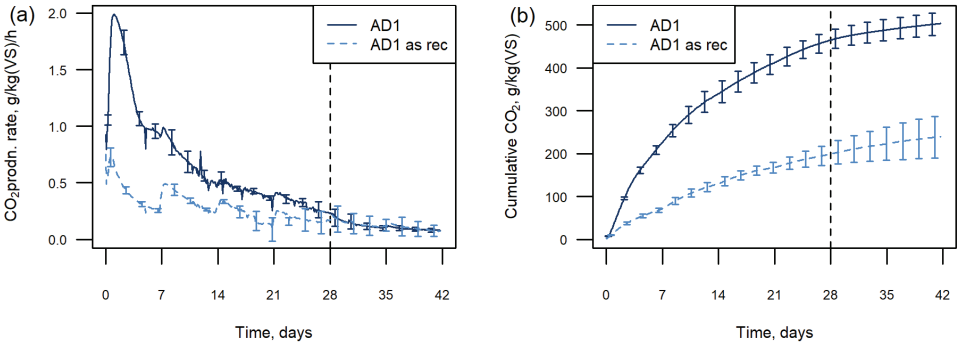

Figure 3. CO_2 production from one AD sample with added moisture and as received: (**a**) gas production rate, (**b**) cumulative data. Graphs show means, error bars show ±1 standard deviation and every 12th point is plotted. The vertical dashed line indicates nutrient addition at 28 days.

Pulses in CO_2 production were noted in the "as received" sample following each disturbance during the weekly shaking events to redistribute moisture within the chambers (Figure 3a). These pulses can also be seen in the AD sample with adjusted moisture; however, the effect is less pronounced.

3.3. Nutrient Effect on Test

The effects of nutrients on the test were investigated in the AD and WF samples. For both materials, there was a reduction in the CO_2 production from the samples when no

nutrients were added at the start of the test (Figure 4). The same trend in CO_2 production was seen both with and without nutrients, with an initial peak and then decline, but for both substrate types the production was lower in the samples without nutrient addition. The CO_2 production at 4 days was 109 g/kgVS CO_2 for AD without nutrients compared with 156 g/kgVS CO_2 with nutrient addition. For the WF, this was 7.4 g/kgVS CO_2 and 18.7 g/kgVS CO_2 respectively.

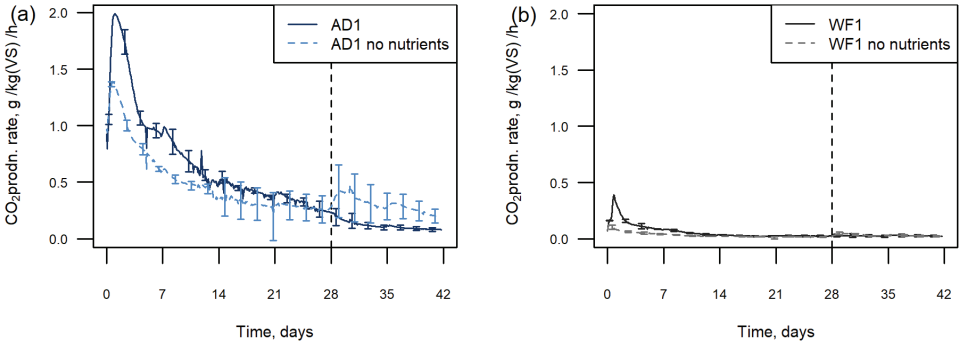

Figure 4. CO_2 production rate from an AD sample (**a**) and WF sample (**b**) with and without nutrients added at the start of the test. Graphs show means, error bars show ±1 standard deviation. The vertical dashed line indicates nutrient addition at 28 days.

When nutrients were added at 28 days, there was an initial increase in CO_2 production in both substrates, which was sustained in the AD sample. This increase was not seen in the AD fibre that had nutrients from the start (Figure 4a). There was also no nutrient effect seen in any of the single materials when nutrients were added at 28 days (Figure 2). The increase of CO_2 production in the WF was short-lived and quickly decreased back to the level seen in the WF with nutrients.

A similar nutrient effect can be seen in the AD + WF mix (Figure 5a), with an increase in CO_2 production rate once nutrients were added on day 28. The CO_2 production increased up to the end of the test at 42 days, resulting in the curve no longer tending to an asymptotic value.

3.4. Interaction Effects

Figure 5 shows the raw CO_2 production in litres from mixtures of 100 g dry matter AD, GC, BC, or CR with 100 g dry matter WF, with the results for individual components (100 g dry matter) shown for comparison. In absence of interaction effects, the mixtures may be expected to produce the sum of CO_2 of the two individual components. The sum of CO_2 production from the individual components provides a predicted value for the mixture as a range from the sum of minimum replicate values to the sum of maximum replicate values for each component (represented by the shaded area on each graph).

The CO_2 production of the AD + WF mix was very similar to the prediction based on the single materials, up until the nutrients were added at day 28 (Figure 5a). From this point, there was an increase in the CO_2 production in the mixture, with CO_2 production of 6.2 L between days 28 and end of test, 3.5 L more than the median predicted value based on CO_2 production from the single materials. This was the only mix where the addition of nutrients had an identifiable effect.

The mix containing GC + WF had the most noticeable interaction effect of all of the mixes (Figure 5b). The CO_2 production was much higher in the physical mix and was underpredicted by the simple addition of the individual components.

Initially, there was close agreement between the predicted and observed CO_2 production curves for the BC + WF mix; however, from 14 days there appears to have been a slight suppression of CO_2 production. There is some uncertainty with this as there was variability

between the BC replicates in the test. As noted above, the CO_2 production of the BC sample may have been underestimated for the wetter replicates, or the stability overestimated. The observed CO_2 production of the mixture was at the lower boundary of the predicted range.

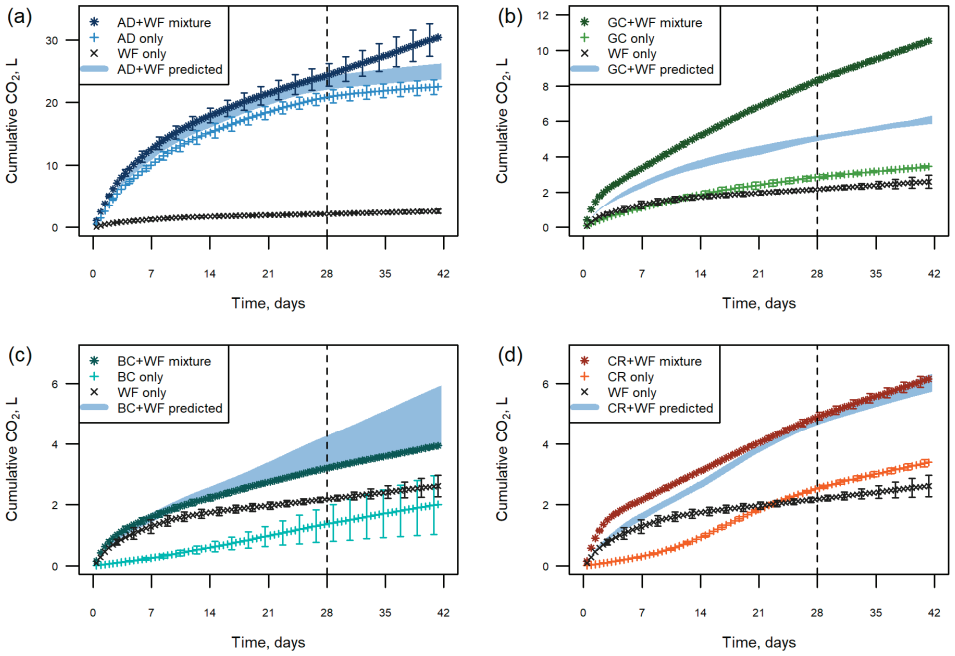

Figure 5. Raw cumulative CO_2 production in litres from 100 g dry matter of the single raw materials and mixtures: (**a**) AD + WF; (**b**) GC + WF; (**c**) BC + WF; (**d**) CR + WF. The shaded area indicates the range of CO_2 production of individual materials summed together. Note Y-axis scale differs between graphs. Graphs show every sixth mean value, and error bars show ±1 standard deviation and every 24th point is plotted. The vertical dashed vertical line indicates nutrient addition at 28 days.

The CR + WF mix had higher CO_2 production than the sum of the individual materials initially, but by day 21 of the test the predicted gas production matched the actual production.

4. Discussion

4.1. Respirometry

The biological stability of a material is a function of the material and the environmental conditions to which it is exposed. The aim of this study was to create conditions close to real-world use of growing media and assess the biological stability of commonly used materials, using aspects of existing respirometry techniques to standardise those conditions as far as possible. By removing limiting factors, such as oxygen supply, moisture, and nutrients, the CO_2 production measured in the study gives a test comparable to the intended use of materials in horticulture, but in standardised, idealised, and replicable conditions. The study was based on conditions of the DR4 test [21], adapted to incorporate single materials without inoculum as used in ORG0020 [22]. Parameters were chosen to be as robust as possible. The test is considered a good proxy for a measure of microbial activity within growing media, limited only by microbial population and substrate quality.

Aeration was not limiting in the experimental set up, with a continuous flow of external ambient air being forced through the test materials and oxygen in the outlet never falling below 17.5% at peak oxygen demand. The flow rate and flow configuration have been found to be important factors within respirometry testing [30–32]. Guillen Ferrari

et al. [33] found that optimising the aeration within the ORG0020 test improved precision within the setup. An airflow configuration that was purely within the headspace of a chamber, as used in ORG0020, may work reliably especially with more stable materials, and may be considered more realistically representative of the exchange of air over a tray or pot within a glasshouse.

The nutrient supply at the beginning of the test was sufficient not to limit microbial activity across all of the individual test materials and their mixes, except for the most active mix of anaerobic digestate fibre (AD) and wood fibre (WF). Nutrient addition at 28 days did not create a pulse in CO_2 production in the individual AD and WF samples that were given nutrients at the beginning; however, pulses in CO_2 were seen in the AD and WF samples that were not supplied with nutrients at the start. It is therefore likely that the AD + WF mix had become limited for nutrients at some point during the first 28 days of the test, resulting in an overestimation of the stability of that test mix. This may mean reduced availability of nutrients for plant growth, in competition with microbial activity (N immobilisation) [34].

Moisture was another limiting factor standardised during the test using the "hand squeeze" method. This was chosen as an acceptable level of moisture at which a plant might grow well, simulating the wetting up of a pot media when a plant is first planted. The adjusted moisture for the green compost sample (GC) was within the 40–60% moisture content range within the ORG0020 protocol [22]. The other growing media raw materials required more water to be added to reach the same physical point of water release due to squeezing, resulting in over 70% moisture content for the other four materials. Gurusamy et al. [35] found that moisture had a significant impact on stability in some compost materials during an ORG0020 test. This was seen in the moisture experiment, where the drier "as received" AD sample had higher stability compared with that of the same sample with moisture adjusted to the "hand squeeze" level. It should be noted that the "as received" AD had a moisture content of 43%, which is within the acceptable range for ORG0020 [22]. This was not optimal for this particular material and suggests that the moisture range in the ORG0020 test might not be optimal for materials with different physical properties to green compost. The "hand squeeze" test was not necessarily optimal for all of the materials tested either. The variability within the individual bark sample was identified as being related to one sample that dried out during the test and had elevated CO_2 production as a result. This suggests that the moisture within this sample may have been limiting, causing a falsely stable result.

Further investigation is required to determine the optimum water content for this kind of test. Other approaches have been used. The DR4 test specifies 50% gravimetric water content [21], which is likely to be reasonable for compost but is arbitrary. The "hand squeeze" test used by Llewelyn [22] may be considered subjective, and an alternative of 75% of water-holding capacity was tested by Adani [24]. It is likely that matric potential is a key factor [36], making any arbitrary gravimetric or volumetric moisture content questionable. This could also shed light on the likely distribution of moisture between components of a mixture, and availability of water to both microbes and plant roots. This complication can be avoided by using a water-based test such as the OUR, but at the expense of conditions for microbial growth closer to the intended real-world application. OUR tests may also be restricted to short-duration tests by supply of oxygen. This limitation has been addressed in the SOUR test [37] by periodic aeration in aqueous medium. It remains a valid question what microbial communities are supported in each of these environments.

4.2. Stability of Individual Raw Materials

The raw materials tested were a range of the most common peat-free growing media components in UK [4]. All the materials tested, except the AD, were very stable when tests were run individually. Under PAS100, compost is considered sufficiently stable if ORG0020 results are under 16 mg CO_2/g VS/d (PAS 100:2011). The wood fibre, coir, and composted bark were all well below this value. Only the testing of the wood fibre was run

as a full DR4 [21] including the standard green compost inoculum. This method has no published threshold, though from data in [15], materials under 25 mg CO_2/g VS/d may be considered stable. The reference cellulose result demonstrated the test was valid, and the result with GC contribution subtracted can be considered very stable.

Two batches of AD fibre were received and tests were run separately. These were both above the PAS100 threshold, though they differed in activity during the early stages of the test, indicating some initial variability as well as instability. After 7 days in the test conditions, there was no difference between the two samples, suggesting some initial inhibitory effect in one sample, resulting in a short lag in CO_2 production. The two batches of WF tested were from the same source and only slightly different in terms of stability. Various authors have noted that the microbial population in composts or wood fibre, for example, is dependent on the production method and source of material [11,38]. For example, Montagne et al. [11] suggests that the physical structure of wood fibre is more important than geographic origin or wood type for determining the microbial population. In this study, the wood fibre was produced by steaming, pressure treating, and expanding wood chips, which could create substrate suitable for a specific microbial community and therefore different levels of stability compared with other methods of production. Testing of batches of raw materials produced by different methods would be necessary to determine the overall variability of substrate types.

An increase in CO_2 production was seen in the coir after about 15 days, a similar effect is noted in Montagne et al. [11]. There, coir pith had a lower initial CO_2 production rate compared with other materials, such as coir fibre and wood fibre, then after 20 days the rate increased to the same as the coir fibre. The test temperature in the experiment by Montagne et al. [11] was lower than in this study (28 and 35 °C respectively), which may explain the difference in lag time. Lag periods have also been identified as correlating with biochemical composition in coir pith, as opposed to fibre [11]; this could help explain the lag identified here.

The rank order of stability of the single materials tested altered over the testing time period, with the results at 7 days different to those at 28 and 42 days. The biggest changes were that the coir became more active after day 15. At the end of the 42-day test, the order of stability was bark > wood fibre > coir > green compost >> anaerobic digestate fibre. Although not all of the same raw materials were tested, Vandecasteele [20] found a similar pattern in the ranking using respirometric CO_2 production in various growing media materials, with wood fibre and composted bark more stable and green compost ranking as one of the least stable materials. The O_2 consumption in the OUR test in that study produced a different ranking of stability, with changes in rankings of some of the more active substrate categories.

4.3. Interaction Effects of Mixing Raw Materials on Stability

The wood fibre was chosen as the common material in mixes because it potentially has a low existing microbial population [12] and high carbon content, which could be utilised by the inoculating microbial population from the other materials. As an increasingly large proportion of growing media mixes in the UK [4] include WF, any interactions in terms of biological stability with other raw materials is important to note.

The interaction experiments showed a range of responses to the mixing of individual components. Simply summing the activity of individual materials does not accurately predict the observed responses. Initially, there appears to be an effect of mixing the coir and wood fibre together. The lag that is seen in the coir on its own is no longer present when used as part of a mix. The wood fibre potentially has different forms of carbon, which may be more available than the more recalcitrant forms in the coir [11]. After the initial lag period passed at about 21 days, the predicted and observed CO_2 production matched for the rest of the test. This suggests that the overall stability of the mix was the same when compared to the component parts, but that the initial microbial activity was greater.

A large increase in the microbial activity was noted in the GC + WF mix compared with the predicted values, i.e., an overall decrease in the biological stability of the mix. A deviation from predicted microbial activity within any mix could be as a result of physical, chemical, or biological parameters, or potentially a combination of all three. For example, green compost is known to have a diverse microbial population and as such is used as an inoculum in a number of biodegradability tests, such as the DR4 test [21]. Wood fibre has been noted as having a potentially available carbon source [20] and a hydrophilic nature that enhances the moisture distribution in a mix [6], so when added to a diverse microbial population like in the GC, there is the potential for increased microbial activity compared with the raw materials alone.

The interaction effect seen in the AD and WF mix only became apparent when additional nutrients were added part way through the test. As noted above, this suggests that nutrients (most likely N) were limiting during the test. Nitrogen immobilisation (or lock up) is a common effect seen particularly in wood-based materials and can affect plant growth and quality [34].

There is a suggestion of potential suppression of microbial activity in the BC and WF mix, though this is somewhat uncertain due to the variability in the bark test samples. As noted above, this may be due to an effect of sub-optimal moisture conditions within the sample during the test. The observed microbial activity was at the bottom of the range of predicted values for these materials. It is likely that a lower moisture content would be optimal for the bark, and as a result, the microbial activity seen in this test is an overestimation of stability. If this were the case, then the predicted value would be shifted up and a real suppression effect would have been seen. Where the microbial activity is enhanced due to mixing of materials has potential implications for the use of materials as growing media. Blends are carefully constructed by growing media manufacturers to have specific chemical and physical properties when they are produced. The data presented here indicate that there is the potential for large losses of carbon over a 6-week period in optimised conditions, particularly if there is a component with low biological stability in the mix. This carbon loss has the potential to affect the structure of the growing media, particularly if fine fibres are degraded [14]. A low-stability material may not only degrade more rapidly, changing the proportions of the mixture, but also provide a means of degrading more recalcitrant materials through "priming effect" [39].

Microbial activity within growing media raw materials should not necessarily be seen as a negative issue, as there is a wealth of literature showing that microbial genera and species that are known to suppress plant pathogens are present in composted bark, wood fibre, and green composts [12,13,38].

5. Conclusions

Dynamic respirometry is a suitable tool for evaluation of existing and new growing media raw materials and their interactions in mixes. Using a respirometry technique adapted from standard methods, differences in microbial stability between different growing media were successfully identified. Furthermore, by mimicking real-world yet replicable conditions, this technique can produce realistic cultures with potential for additional microbiological or other characterisation. The specific methods DR4 and ORG0020 are not well optimised to this application. Further work is recommended to refine operational parameters in the adapted method, such as moisture status, for a standardised test.

The separate components tested ranked from most to least stable (lowest to highest CO_2 production) were BC < CR < WF < GC << AD after 7 days. This order changed through the test as CO_2 production from CR peaked in the third week.

Interactions between components were identified in simple two-component mixes. This may be due physical or chemical factors, or cross-inoculation of microbial populations native to each component. Green compost is expected to contain a wide microbial diversity.

Further work is needed to assess variability within and between sample types, and interactions present in horticulture-relevant mixes.

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References

1. Schmilewski, G. Growing Medium Constituents Used in the EU. *Acta Hortic.* **2009**, *819*, 33–46. [CrossRef]
2. Bragg, N.; Brough, W. The Development of Responsibly Sourced Growing Media Components and Mixes©. *Acta Hortic.* **2014**, *1055*, 141–144. [CrossRef]
3. Barrett, G.E.; Alexander, P.D.; Robinson, J.S.; Bragg, N.C. Achieving Environmentally Sustainable Growing Media for Soilless Plant Cultivation Systems—A Review. *Sci. Hortic.* **2016**, *212*, 220–234. [CrossRef]
4. HTA Growing Media Monitor Report. Trend in the Composition of UK Growing Media Supplied 2011 to 2022. Available online: https://projectbluearchive.blob.core.windows.net/media/Default/Research%20Papers/Horticulture/CP%20203_Final%20report%202011-2022.pdf (accessed on 12 September 2023).
5. Gruda, N.; Schnitzler, W.H. Suitability of Wood Fiber Substrate for Production of Vegetable Transplants: I. Physical Properties of Wood Fiber Substrates. *Sci. Hortic.* **2004**, *100*, 309–322. [CrossRef]
6. Durand, S.; Jackson, B.E.; Fonteno, W.C.; Michel, J.-C. The Use of Wood Fiber for Reducing Risks of Hydrophobicity in Peat-Based Substrates. *Agronomy* **2021**, *11*, 907. [CrossRef]
7. Pitman, R.M.; Webber, J. The Character of Composted Bracken (*Pteridium Aquilinum* L. Kuhn) and Its Potential as a Peat Replacement Medium. *Eur. J. Hortic. Sci.* **2013**, *78*, 145–152.
8. Nguyen, V.T.H.; Kraska, T.; Winkler, W.; Aydinlik, S.; Jackson, B.E.; Pude, R. Primary Mechanical Modification to Improve Performance of Miscanthus as Stand-Alone Growing Substrates. *Agronomy* **2022**, *12*, 420. [CrossRef]
9. Lemaire, F. Physical, Chemical and Biological Properties of Growing Medium. *Acta Hortic.* **1995**, *396*, 273–284. [CrossRef]
10. Jackson, B.E.; Wright, R.D.; Seiler, J.R. Changes in Chemical and Physical Properties of Pine Tree Substrate and Pine Bark During Long-Term Nursery Crop Production. *HortScience* **2009**, *44*, 791–799. [CrossRef]
11. Montagne, V.; Charpentier, S.; Cannavo, P.; Capiaux, H.; Grosbellet, C.; Lebeau, T. Structure and Activity of Spontaneous Fungal Communities in Organic Substrates Used for Soilless Crops. *Sci. Hortic.* **2015**, *192*, 148–157. [CrossRef]
12. Montagne, V.; Capiaux, H.; Barret, M.; Cannavo, P.; Charpentier, S.; Grosbellet, C.; Lebeau, T. Bacterial and Fungal Communities Vary with the Type of Organic Substrate: Implications for Biocontrol of Soilless Crops. *Environ. Chem. Lett.* **2017**, *15*, 537–545. [CrossRef]
13. Pot, S.; Tender, C.D.; Ommeslag, S.; Delcour, I.; Ceusters, J.; Vandecasteele, B.; Debode, J.; Vancampenhout, K. Elucidating the Microbiome of the Sustainable Peat Replacers Composts and Nature Management Residues. *Front. Microbiol.* **2022**, *13*, 1–18. [CrossRef]
14. Verhagen, H. Stability of Growing Media from a Physical, Chemical and Biological Perspective. *Acta Hortic.* **2009**, *819*, 135–142. [CrossRef]
15. Aspray, T.J.; Dimambro, M.E.; Wallace, P.; Howell, G.; Frederickson, J. Static, Dynamic and Inoculum Augmented Respiration Based Test Assessment for Determining in-Vessel Compost Stability. *Waste Manag.* **2015**, *42*, 3–9. [CrossRef]
16. Grigatti, M.; Cavani, L.; Ciavatta, C. The Evaluation of Stability during the Composting of Different Starting Materials: Comparison of Chemical and Biological Parameters. *Chemosphere* **2011**, *83*, 41–48. [CrossRef]
17. Blok, C.; Eveleens, B.; Van Winkel, A. Oxygen Use in Compost Storage as Influenced by Moisture, Temperature and Degradability. *Acta Hortic.* **2019**, *1266*, 291–300. [CrossRef]
18. Adani, F.; Gigliotti, G.; Valentini, F.; Laraia, R. Respiration Index Determination: A Comparative Study of Different Methods. *Compost Sci. Util.* **2003**, *11*, 144–151. [CrossRef]
19. Oviedo-Ocaña, E.R.; Torres-Lozada, P.; Marmolejo-Rebellon, L.F.; Hoyos, L.V.; Gonzales, S.; Barrena, R.; Komilis, D.; Sanchez, A. Stability and Maturity of Biowaste Composts Derived by Small Municipalities: Correlation among Physical, Chemical and Biological Indices. *Waste Manag.* **2015**, *44*, 63–71. [CrossRef]

20. Vandecasteele, B. Oxygen Uptake Rate versus CO_2 Based Respiration Rate for Assessment of the Biological Stability of Peat, Plant Fibers and Woody Materials with High C:N Ratio versus Composts. *Waste Manag.* **2023**, *167*, 74–80. [CrossRef]
21. Turrell, J.; Godley, A.; Agbasiere, N.; Lewin, K. *Guidance on Monitoring of MBT and Other Treatment Processes for the Landfill Allowances Schemes (LATS and LAS) for England and Wales*; Environment Agency: Bristol, UK, 2009.
22. Llewelyn, R.H. Development of Standard Laboratory Based Test to Measure Compost Stability. The Waste & Resources Action Programme: The Old Academy. 2005. Available online: https://wrap.org.uk/sites/default/files/2020-10/WRAP-DevLabTestCompostStability.pdf (accessed on 12 September 2023).
23. B.S.I. *PAS100: 2018 Specification for Composted Materials*; BSI Committee: London, UK, 2018.
24. Adani, F.; Lozzi, P.; Genevini, P. Determination of Biological Stability by Oxygen Uptake on Municipal Solid Waste and Derived Products. *Compost Sci. Util.* **2001**, *9*, 163–178. [CrossRef]
25. Grunert, O.; Reheul, D.; Van Labeke, M.-C.; Perneel, M.; Hernandez-Sanabria, E.; Vlaeminck, S.E.; Boon, N. Growing Media Constituents Determine the Microbial Nitrogen Conversions in Organic Growing Media for Horticulture. *Microb. Biotechnol.* **2016**, *9*, 389–399. [CrossRef]
26. Grunert, O.; Hernandez-Sanabria, E.; Vilchez-Vargas, R.; Jauregui, R.; Pieper, D.H.; Perneel, M.; Van Labeke, M.-C.; Reheul, D.; Boon, N. Mineral and Organic Growing Media Have Distinct Community Structure, Stability and Functionality in Soilless Culture Systems. *Sci. Rep.* **2016**, *6*, 18837. [CrossRef]
27. *BS EN 13040*; Soil Improvers and Growing Media. Sample Preparation for Chemical and Physical Tests, Determination of Dry Matter Content, Moisture Content and Laboratory Compacted Bulk Density. CEN: Brussels, Belgium, 2007.
28. *BS EN 13039*; Soil Improvers and Growing Media—Determination of Organic Matter Content and Ash. CEN: Brussels, Belgium, 2012.
29. Lighton, J.R.B.; Halsey, L.G. Flow-through Respirometry Applied to Chamber Systems: Pros and Cons, Hints and Tips. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2011**, *158*, 265–275. [CrossRef]
30. Adani, F.; Ubbiali, C.; Generini, P. The Determination of Biological Stability of Composts Using the Dynamic Respiration Index: The Results of Experience after Two Years. *Waste Manag.* **2006**, *26*, 41–48. [CrossRef]
31. Komilis, D.; Kanellos, D. A Modified Dynamic Respiration Test to Assess Compost Stability: Effect of Sample Size and Air Flowrate. *Bioresour. Technol.* **2012**, *117*, 300–309. [CrossRef]
32. Almeira, N.; Komilis, D.; Barrena, R.; Gea, T.; Sánchez, A. The Importance of Aeration Mode and Flowrate in the Determination of the Biological Activity and Stability of Organic Wastes by Respiration Indices. *Bioresour. Technol.* **2015**, *196*, 256–262. [CrossRef]
33. Guillen Ferrari, D.; Howell, G.; Aspray, T.J. Improved Precision and Efficiency of a Modified ORG0020 Dynamic Respiration Test Setup for Compost Stability Assessment. *Sustainability* **2017**, *9*, 2358. [CrossRef]
34. Vandecasteele, B.; Pot, S.; Maenhout, K.; Delcour, I.; Vancampenhout, K.; Debode, J. Acidification of Composts versus Woody Management Residues: Optimizing Biological and Chemical Characteristics for a Better Fit in Growing Media. *J. Environ. Manag.* **2021**, *277*, 111444. [CrossRef]
35. Gurusamy, N.N.; Puffer, N.; de Jongh, C.; Rodriguez Gil, C.; Aspray, T.J. Effect of Initial Moisture Content and Sample Storage Duration on Compost Stability Using the ORG0020 Dynamic Respiration Test. *Waste Manag.* **2021**, *125*, 215–219. [CrossRef]
36. Cannavo, P.; Recous, S.; Valé, M.; Bresch, S.; Paillat, L.; Benbrahim, M.; Guénon, R. Organic Fertilization of Growing Media: Response of N Mineralization to Temperature and Moisture. *Horticulturae* **2022**, *8*, 152. [CrossRef]
37. Lasaridi, K.; Stentiford, E. A Simple Respirometric Technique for Assessing Compost Stability. *Water Resour.* **1998**, *32*, 3717–3723. [CrossRef]
38. Hoitink, H.A.J. Status of Compost-Amended Potting Mixes Naturally Suppressive to Soilborne Diseases of Floricultural Crops. *Plant Dis.* **1991**, *75*, 869. [CrossRef]
39. Bréchet, L.M.; Lopez-Sangil, L.; George, C.; Birkett, A.J.; Baxendale, C.; Castro Trujillo, B.; Sayer, E.J. Distinct Responses of Soil Respiration to Experimental Litter Manipulation in Temperate Woodland and Tropical Forest. *Ecol. Evol.* **2018**, *8*, 3787–3796. [CrossRef]

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Performance Evaluation of a Cascade Cropping System

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Abstract: Minimum environmental impact and improved resource efficiency is attainable for soilless cascade systems where the nutrient solution drained from a primary (donor) crop is reused to fertigate a secondary (receiver) crop. However, it is not clear whether the nutrient solution drained from the primary crop can completely satisfy the needs of a secondary crop and if the productivity of the secondary crop is compromised. To test this hypothesis, a prototype soilless cascade system was developed and evaluated. To assess the performance of the system in terms of yield, water and nutrient productivity, a tomato crop was used as the primary crop, while lettuce, spinach and parsley were tested as secondary crops under different drainage management strategies. Measurements of plant growth, crop fresh and dry matter production, leaf chlorophyll and nutrient content, and photosynthesis rate were performed in the secondary crops. In addition, the water productivity and nutrient use efficiency for the fertigation of the primary and secondary crops were recorded. The results showed that the yield of the cascade spinach crop increased by up to 14% compared to the control treatment (monoculture of secondary crop fertigated by standard nutrient solution). The yield of the lettuce and parsley crop was not affected by the reuse of the tomato crop drainage solution. The water productivities of the lettuce, spinach and parsley plants fertigated with pure drainage solution were 50%, 30% and 14% higher than in the control treatment, respectively. The nitrogen and phosphorus use efficiency was improved by more than 50% compared to the control treatments.

Keywords: multi-cropping; drainage management; water use efficiency; nutrient use efficiency

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1. Introduction

In greenhouses, closed soilless cultivation systems provide the opportunity to increase the water and nutrient use efficiency and reduce the environmental impact of the cultivation system through the reuse of the drained water and nutrients [1,2]. However, due to the low quality of the water used in the Mediterranean countries (high concentrations of Na^+ and Cl^-, but also high Ca^{2+}, Mg^{2+}, and SO_4^{2-}, [3]), completely closed soilless systems are not feasible. Appropriate management of the drainage solution (DS) using suitable practices and models may reduce the need to discharge the drainage solution to the environment. To this end, Katsoulas et al. [1] developed a model for automatic drainage solution management in tomato crops grown in semi-closed systems. Nevertheless, partial discharge of the drainage nutrient solution when the levels of electrical conductivity (EC) or of the toxic ions in the system are reached, is still a necessity in these systems.

Many growers in the Mediterranean region operate their soilless systems as open systems, mainly because they do not have the knowledge or the capacity to manage nutrient solution drainage. One of the serious problems of open systems is the effluence of overdosed nutrient solutions from the system into the environment, resulting in eutrophication of soil and groundwater. Abd-Elmoniem et al. [4] showed that the average water consumption of plants grown in open systems was 15% to 17% higher compared to those grown in closed systems. The absolute values of water consumption in lettuce were 68.5 L and 80.5 L per plant for closed and open systems, respectively. Rufi-Salis et al. [5]

reported that closed soilless systems reduce daily water and nutrient consumption by 40% and 35–54%, respectively, when compared to open systems in green pea production. Méndez-Cifuentes et al. [6], in a similar study, concluded that 53 L and 22 L of nutrient solution were required in closed and open systems, respectively, to produce 1 kg of tomatoes. Therefore, open systems consumed 86% more water. Fayezizadeh et al. [7] found that closed systems allowed water and nutrient savings of up to 97% compared to open systems. Closed systems are therefore preferable in terms of reducing environmental pollution.

Sustainable management strategies for the reuse and discharge of DS in closed systems are needed. One of the potential strategies for enhancing the circular economy concept in the context of soilless systems is the development and implementation of soilless cascade systems. In these systems, the drainage of a primary donor crop is utilised for the fertigation of one or more secondary receiving crops that possess a higher tolerance to salinity. The reuse may also continue, with the drainage from the secondary crop being utilised for the fertigation of a tertiary, highly salinity-tolerant crop.

Some pioneer studies on cascade fertigation systems were performed more than 10 years ago [8,9]. Some interesting systems have already been developed, mostly in open-field crops, such as the system developed in San Joaquin Valley in California. However, it is not so easy to manage the drainage solution in these systems, since the solution drains directly into the underlying soil. García-Caparrós [10] studied a pilot cascade system in the facilities of the University of Almeria in Spain, in which the drainage solution from melon cultivation was used to cover the needs of rosemary, with encouraging results.

However, the recent advancements in drainage management and the increased need for sustainable production systems with low environmental impact have increased the interest in further research on soilless cascade systems [5,11–17]. In addition to their excellent utilisation of drained water and nutrients, cascade systems may lead to improvements in the quality characteristics of secondary and tertiary crops due to the increased salinity levels in the system. Incrocci et al. [8] showed that fruit dry matter increased, while Avdouli et al. [11] showed that the content of several compounds associated with the organoleptic, nutritional and nutraceutical qualities of many fruits and leafy vegetables increased when they were cultivated as secondary crops in a cascade system. Nevertheless, the efficient management of a cascade system requires knowledge of the salt tolerance levels [17] and of the fertigation needs of the crops in the loop. Furthermore, the drainage solution of a crop may include phytotoxic root exudates [10] or other metabolites, and may impose recirculation plant protection products [16] in the system. However, advanced cascade research needs to focus on the yield quality of secondary and tertiary crops, and to date, there are scant scientific articles referring to this concept.

Additionally, to obtain a sufficient cascade system, optimal management may require correction with respect to pH, EC and macronutrients, since the drained solution may have abnormal mutual nutrient ratios. However, it is not clear whether the drainage solution collected from the main soilless greenhouse crops can be directly utilised for the fertigation of secondary and then tertiary crops. Moreover, it is not clear which management practices are needed in order to fulfil the fertigation needs of secondary and tertiary crops while increasing the water and nutrient use efficiency of the cascade system.

The aim of this work is to test whether some common leafy vegetables (lettuce, spinach and parsley) can be used as secondary receiving crops in soilless cascade systems with tomato as the primary crop in greenhouses in the Mediterranean region. The specific secondary crops selected for testing were chosen due to their high consumer demand, short cultivation cycle, high nutritional value, flexible growth adaptation to soilless facilities [18–20] and high salt tolerance and ability to accumulate sodium as an osmoregulating resistance mechanism [21]. In this sense, lettuce, spinach, and parsley plants were cultivated under different cascade systems.

In this study, we aim to provide knowledge about the progress of the nutrient concentrations in the different parts of cascade systems and on the utilisation/absorption of different nutrients in soilless cascade systems. The amount of water irrigated to, uptaken

by and drained from the plants is also presented. Additionally, yield characteristics (plant height, number of leaves, chlorophyll content index, and photosynthesis rate) of the secondary crops are reported. The evaluation of the different cascade systems was performed on the basis of water productivity (WP) and fertiliser use efficiency (FUE) calculations. With this research, the gap in scientific knowledge with respect to the secondary crop response in cascade systems is decreased.

2. Materials and Methods

2.1. Greenhouse Facilities and Cascade System Set Up

The cascade system was installed in a gothic multitunnel greenhouse belonging to the University of Thessaly, located in Velestino, Central Greece (Latitude 39°22′, longitude 22°44′ and altitude 85 m). The greenhouse was oriented north–south, and the total ground area was 1500 m^2, separated into six compartments. The first compartment was used to host the fertigation and control equipment. Four out of the other five compartments were used for cultivation purposes in this work. In each of the culture compartments, six channels, 20 m in length, and carrying 19 rockwool slabs each (Grodan Delta, NL 100 × 15 × 7.5 cm, 0.18 g cm^{-3}, 90% water retention capacity, Roermond, The Netherlands), were installed.

All the compartments were covered by a polyethylene film in the roof, while the side walls were covered by polycarbonate sheets. Each compartment was further equipped with a roof vent, a pad and fan system, and a thermal/shading screen, and all the systems/compartments were controlled by a climate control computer (SERCOM, Automation SL, Lisse, The Netherlands). The roof vent was opened when the air temperature within the greenhouse was higher than 20 °C or the relative humidity exceeded 87%. The pad-and-fan system was in operation whenever the air temperature inside the greenhouse was higher than 26 °C. The shading screen was used when the outdoor solar radiation was higher than 750 W m^{-2}. The transmittance coefficient of the cover material was 0.75, while the screen's transparency was 0.50.

Within the hydroponic head (500 L), the nutrient solution (NS) was made by mixing different amounts of nutrients, stored within five stock solution tanks (capacity of 120 L each), with tap water or drainage solution (DS). The amounts of nutrients and water or drainage solution used for the preparation of the NS were based on the desired targeted concentration. The system can prepare five different recipes, stored in different nutrient storage tanks (capacity of 500 L) each time. In the current research, two nutrient solution tanks were used for irrigating the primary crop and two for the secondary crops. Therefore, each of the solution tanks was linked with its own injection pump, thus making it possible to automatically prepare fresh NS separately for each crop level (primary, secondary and tertiary). This operation was accomplished by the fertigation system, which generally works using volumetric or electronic injectors. The quality of raw water is a key factor, and must be known from the outset in order to check whether the water can be utilised as is, or if it needs specific treatment in order to calculate the amount of fertilisers required for the preparation of the nutrient stock. When the nutrient solution had reached the optimum concentration values set by the operator, it was transferred from the mixing tank to one of the eight irrigation tanks. The reused NS was first disinfected using a UV-light disinfection system before being supplied to the plants. Figure 1 presents a schematic diagram of the system.

The nutrient solution drained from the crops was collected in the DS tanks. In each culture compartment, two drainage tanks with a capacity of 300 L were used. In the compartments of the secondary crops, the DS was collected in four tanks with a capacity of 100 L, individually for each treatment, before ending in the final tanks (Figure 1). The pH of each NS preparation was set at 5.8. Correction was performed through the addition of NO_3 solution. Through this setup, each compartment of the secondary crop could host two different crop species (two channels 10 m in length), fertigated by two different treatments (two sets of three channels/repetitions). The final DS collected was further reused in the process of preparing a fresh nutrient solution.

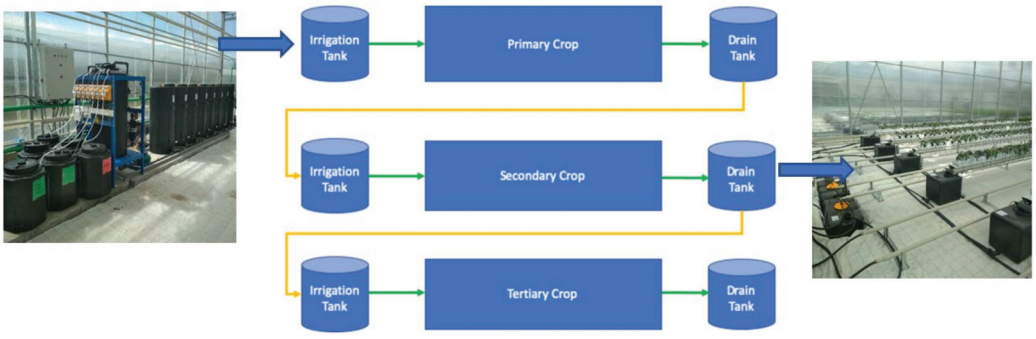

Figure 1. Scheme of the cascade system setup and the flow of the nutrient solution from the irrigation to the drainage tanks. The system is designed to operate in three consecutive circulation levels, but only the first two were used in the present work.

2.2. Crop Management and Experimental Setup

The experiments were carried out from March 2019 to October 2019. Tomato plants (*Solanum lycopersicum* cv. Elpida, hybrid F1) were cultivated to serve as the primary crop in two compartments of the greenhouse. The tomato plants were transplanted in a density of 3 plants m^{-2} at the stage of five extended leaves and 25 cm height. The plants were transplanted on the 29 March 2019, while the cultivation period lasted for six months.

The NS drained from the tomato crop was used for the fertigation of the secondary crops, which were cultivated in two experimental periods. The first period occurred during the vegetative stage of tomato crop, from April to May. During that period, spinach (*Spinacia oleracea* cv. Matador) and lettuce (*Lactuca sativa* cv. Batavia, type iceberg) were cultivated at a density of 4 plants m^{-2}. The plants were transplanted at the stage of three true leaves, 19 days after transplanting of the primary crop (DATp). Their cultivation cycle lasted 42 days. The second period occurred during the fruit stage of the primary crop, from August to October. During that period, parsley (*Petroselinum crispum* cv. Mill.) was cultivated at a density of 4 plants m^{-2}. The plants were transplanted at the stage of two true leaves, 137 DATp. The cultivation cycle of the parsley crop lasted 54 days. The cultivation cycle of each secondary crop corresponds, according to local practice, to that of a commercial production. The time interval between the two experiments (June–July) was used to clean the irrigation system and change the cultivation of the secondary crop, so no data were recorded.

To achieve a randomised block design for the secondary cultivation, in each of the six lines of each compartment, 36 plants of each species were cultivated (4 plants per slab; 72 plants per line; 432 plants per species in total). In each secondary crop, in both periods, a total of four irrigation treatments were applied in three repetitions (36 plants per treatment and repetition), in which the plants were supplied with: (i) fresh nutrient solution (FS) comprising the control treatment (T1 treatment: 0%DS + 100%FS); (ii) drainage solution of the primary crop diluted with water (W) at a ratio of 50–50 (T2 treatment: 50%DS + 50%W); (iii) drainage solution of the primary crop diluted with water at a ratio of 75–25 (T3 treatment: 75%DS + 25%W); and (iv) drainage solution of the primary crop without any dilution (T4 treatment: 100%DS + 0%W). In all systems of secondary crops, the drainage was collected, but was not recycled, in order to simulate the conditions of an open-loop system.

The tap water used for the NS preparation had a pH of 7.1, an EC of 0.8 dS m^{-1} and an Na^+ concentration of 1.3 mM L^{-1}. The composition of the nutrient solution used for both the primary and secondary crops was similar to that applied in Mediterranean climatic conditions, and was modified according to the plant stage. The pH, EC set points and nutrient composition supplied to the crops according to their growth stage are shown in Table 1. The irrigation dose for the primary crop was set to cover at least the 30% of the

leaching fraction. The daily dose for the secondary crop was around 0.345 L per plant in lettuce and spinach and 0.199 L per plant in parsley. The amount of water was added in the NS supplied to the secondary crop, and the pH and EC value variations according to the treatment are reported analytically as results.

Table 1. The targeted nutrient composition, pH and electrical conductivity (EC) of the nutrient solution supplied to the plants of primary and secondary crop according to growth stage.

Parameter	Unit	Primary Crop Vegetative Stage	Primary Crop Fruit Stage	Secondary Crop Vegetative Stage
pH		5.8	5.8	5.8
EC	(dS m^{-1})	1.3–3.5	1.3–3.5	2.2–2.6
Ca^{+2}	(mM L^{-1})	4.1	3.1	4.8
Mg^{2+}	(mM L^{-1})	2.0	2.0	1.9
K^{+1}	(mM L^{-1})	4.5	3.1	6.5
NO_3^-	(mM L^{-1})	9.5	8.2	12.9
NH_4^+	(mM L^{-1})			0.3
H_2PO_4	(mM L^{-1})	0.6	0.4	1.1
Fe	(µM L^{-1})	17.4	17.1	15.0
Mn	(µM L^{-1})	3.8	1.7	2.9
Zn	(µM L^{-1})	5.9	1.3	2.8
Cu	(µM L^{-1})	0.7	0.6	0.8
B	(µM L^{-1})			30.0
Mo	(µM L^{-1})			0.5

2.3. Measurements

Air temperature (Ta, in °C) and relative humidity (RH, in %) were measured using a temperature–humidity sensor (model HD9008TR, Delta Ohm, Italy), which was calibrated before the experimental period and placed 1.8 m above ground level. The irradiance (Rg, i, in W m^{-2}) inside the greenhouse was recorded using a solar pyranometer (model SKS 1110, Skye instruments, Powys, UK) located 1.8 m above ground.

Plant height, number of leaves, and chlorophyll content index were obtained in the plants of the secondary crop twice a week. Plant height (H) was measured by placing a calibrated ruler on the top edge of the slab and measuring to the tip of the last open leaf of the plant (number of samples (n) = 30 per treatment). The number of leaves was measured for 30 plants per treatment. Chlorophyll content index (CCI) was recorded using non-destructive sensing by means of an Opti-Science sensor, performing measurements in contact with the leaf (CCM 200, Opti-Science, Hudson, NH, USA). CCI index is the ratio of the chlorophyll's reflectance in the NIR band over the reflectance in the red band. The measurements were performed in young and fully developed leaves during the morning to avoid the effect of direct sunlight on the chlorophyll meter (n = 10 per treatment).

Photosynthesis rate (P_N) (µmol CO_2 m^{-2} s^{-1}) was measured weekly in young and fully developed leaves (n = 5 per treatment) using a portable photosynthesis system (LCpro+ 1.0 ADC, Bioscientific Ltd., Hoddesdon, Hertfordshire, UK).

Two destructive samplings were performed during both periods in order to estimate the fresh matter (FM), dry matter (DM) and nutrient leaf content for each secondary crop. During the first period, destructive sampling was performed 15 and 42 days after each secondary crop had been transplanted (DATs), and in the second period, 36 and 54 DATs (n = 9 per treatment). The samples were dried in a forced-air oven for 72 h at 70 °C. The data were initially calculated in kg $plant^{-1}$ and subsequently adjusted in kg m^{-2}. Total yield of the primary crop was also measured at the end of the seven-month cultivation period to estimate the total biomass expressed in kg m^{-2}.

Dried samples of parsley plants were subsequently ground to powder in order to perform mineral analyses of the main macro-micronutrients (N, P, K, Ca, Mg, Fe, Zn, Mn and Cu). In total, 36 plants per destructive process were used to determine the uptake nutrient concentrations of each secondary crop. The extraction was performed using the Kjeldahl Nitrogen method (TKN) based on the Kjeldahl protocol [22]. Nutrient elements

were determined by ICP (ICP-OES, SPECTRO Analytical Instruments GmbH, 180 Kleve, Germany).

EC (dS m^{-1}) and pH values of the irrigation and drainage solution (IS) were measured automatically with sensors (type GPHU 014 MP-BNC, Greisinger, Regenstauf, Germany) placed within the hydroponic head tank. The volume (V, L) of the drained nutrient solution was automatically recorded using water pressure gauges (Klinkerbeg, Graben-Neudorf, Germany) placed in each drainage tank. Furthermore, during the second period, samples of the irrigation (IR) and drainage (DR) solution were collected manually for quantitative assessment of NO_3, P, K, Ca, Na, Mg, Fe, Zn, Mn and Cu content on two sampling dates (DAT 36 and DAT 54). Extraction was performed using the Kjeldahl Nitrogen method (TKN) based on the protocol described by Kjeldahl [22], while the nutrient elements were determined by ICP (ICP-OES, SPECTRO Analytical Instruments GmbH, Kleve, Germany).

2.4. Calculations

The daily and nightly average Ta and RH were calculated for the periods from 6:00 to 18:00 and from 18:00 to 6:00 (local time), respectively, during the respective cultivation period of primary and secondary crops.

In the primary crop, the total volume of NS applied (V_{IR}), expressed in L m^{-2}, was the sum of the volume of water added in each irrigation event (L) for a six-month cultivation period divided by the total cultivated area (m^2). The total volume of NS drained (V_{DR}) from the plants (L m^{-2}) was the sum of the volume drained after each irrigation event for a six-month cultivation period divided by the total cultivated area (m^2).

In the secondary crops, the V_{IR} and V_{DR} data were adjusted in the primary crop cultivation period. To achieve this, the total volume of each secondary crop was divided by the number of the days in each cultivation period, and then multiplied by the number of days for which the primary crop was cultivated. Here, the data for water and primary crop DS added during NS are presented separately. The total volume uptaken (V_{up}) by the plants consists of the amount applied minus the amount drained from the plants. To evaluate the final impact of each system to the environment, the above data for both primary and secondary crop are summarised.

The crop uptake concentration (C_{up}), defined as the amount of nutrient absorbed by the plants, was estimated based on the following equation:

$$C_{IR} \times V_{IR} + C_{up} \times V_{up} = C_{DR} \times V_{DR}, \quad (1)$$

where (C_{IR}) and (C_{DR}) correspond to the concentration of the nutrient element in the irrigation and drainage solutions, respectively, expressed in mg L^{-1}.

The cumulative volume irrigated to and drained from the crop throughout the whole cultivation period for each crop and treatment was used in order to determine the water productivity. The water productivity (WP, in kg m^{-3}) and the fertiliser use efficiency (FUE, in kg kg^{-1}) of the primary and secondary crops were estimated by dividing the total yield FM (which is the sum of the primary and the secondary crop) with the total volume of the fresh water or fertiliser applied over the six-month cultivation period. Similarly, the nitrogen (NUE) and phosphorus (PUE) use efficiency were calculated by dividing the sum FM by the total amount of nitrogen or phosphorous added, adjusted to the six-month cultivation period. The total amounts of nitrogen and phosphorus were calculated by multiplying the N and P content showed in Table 1 by the V_{IR}.

2.5. Statistical Analysis

Comparison of means was performed by applying one-way ANOVA at a confidence level of 95% ($p \leq 0.05$) using SPSS (Statistical Package for Social Sciences, IBM, Armonk, NY, USA). Additionally, least significant difference (LSD) at the 5% level of significance was used to determine whether the drainage solution management affected the quality (yield) and quantity (marketable products) of the production. The mean values and standard deviations (±SD) of the measured parameters are reported.

3. Results

3.1. Climatic Conditions

The average daily air temperature and relative humidity in the cultivation area of the tomato crop were 21 °C (with standard deviation SD ± 4.0) and 63% (SD ± 18), respectively. In the compartments of the secondary crop, the respective average values were 22 °C (SD ± 4.1) and 53% (SD ± 19), respectively. The respective maximum and minimum daily average values measured were 26.5 °C and 18 °C and 75% and 50%, respectively. During the night, the values remained stable at around 15 °C (SD ± 3.9) and 75% (SD ± 19) for both primary and secondary crops. The maximum outdoor global radiation was around 1000 W m^{-2} (SD ± 280), and the daily mean radiation varied around 420 W m^{-2}. The daily mean radiation inside the greenhouse was around 315 W m^{-2}, indicating an average transmission of the greenhouse to solar radiation of about 75%. The consistent daily climatic conditions occurred across all the treatments had no significant impact on the assessment of the results.

3.2. Nutrient Solution Quality

To assess the NS quality, it is necessary to examine the status of EC and pH at each stage of the system. As already mentioned, the EC values of the NS supplied to the tomato crop varied from 1.3 to 3.5 dS m^{-1} over the total cultivation period (Table 1). The values were changed according to the growth stage of the crop, where at the vegetative stage the values were higher than at the fruit stage. The EC values of the DS were about 30% higher than those of the NS supplied. Similar to the EC, the pH values increased from 5.8, recorded in the supplied NS (Table 1), to more than 6.2, and up to 7.4 (SD ± 0.7). Comparable progress was observed in the EC and pH values of the secondary crop control treatment (T1 treatment) during both periods.

For the other cascade treatments, the EC and the pH values were changed according to the quality of the DS of the primary crop and the amount of water added in the NS preparation process. At the primary crop vegetative stage (first period), where the EC values of the NS supplied were high, the respective values of the cascade treatments were also high, and were higher than those of the control treatment ($p < 0.05$). Only the values of the T2 treatment were lower than those of the control treatment ($p < 0.05$). At the primary crop fruit stage (second period), where the EC values of the NS supplied were low, the three cascade treatments demonstrated values lower than the control ($p < 0.05$). Similar to the primary crop, the EC values of the DS increased by about 30% and the pH values increased from 5.8 to more than 6.2.

The above data of EC variation in the NS supplied to and drained from the plants are presented in Figures 2 and 3, according to the treatment received by each secondary crop. The pH variations of the DS according to the respective treatment are presented in Figure 4. The pH values in the supplied NS are not presented, since the pH was set at 5.8 for each treatment.

3.3. Water Consumption

The total volumes of NS supplied to and drained from the primary crop during the six-month cultivation period were 882 L m^{-2} and 472 L m^{-2}, respectively. In the secondary crop, an amount of water and DS was added to the total amount of the supplied NS, changed according to the treatment and for each cultivation period. However, in order to be able to compare the treatments and their efficiency, the secondary crop data of the NS supplied to, uptaken by and drained from the plants were adjusted to a six-month cultivation period, equal to that of the primary crop. The resulting data are presented in Table 2.

According to the adjusted calculations, as was expected, the maximum amount of primary crop DS reused was observed in the T4 treatment. In this case, and for the same cultivation period with tomato, 290 L m^{-2} was reused to irrigate the lettuce plants. The same amount was reused in spinach plants, while in parsley plants, the amount was

167 L m^{-2}. For the other cascade treatments, the amounts of primary crop DS reused were 50% and 75% lower than the T4 treatment under T2 and T3, respectively.

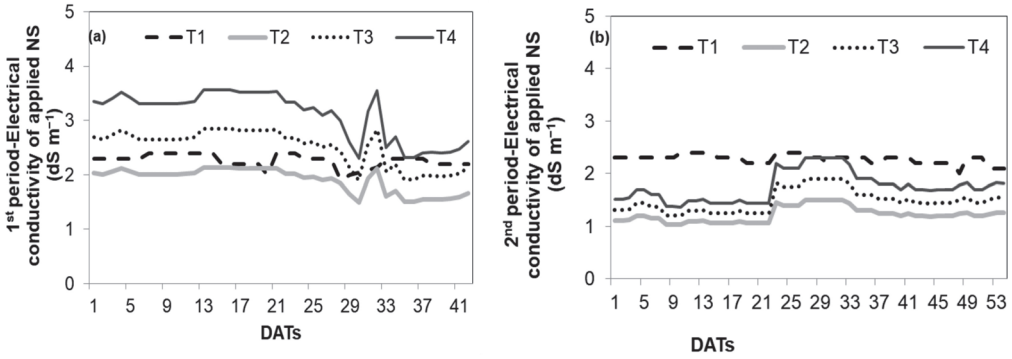

Figure 2. Evolution of EC values of the NS supplied to the secondary crops during (**a**) the first experimental period (19–60 DATp; 1–42 DATs) and (**b**) the second experimental period (137–190 DATp; 1–54 DATs). T1 (0%DS + 100%FS); T2 (50%DS + 50%W); T3 (75%DS + 25%W); T4 (100%DS + 0%W).

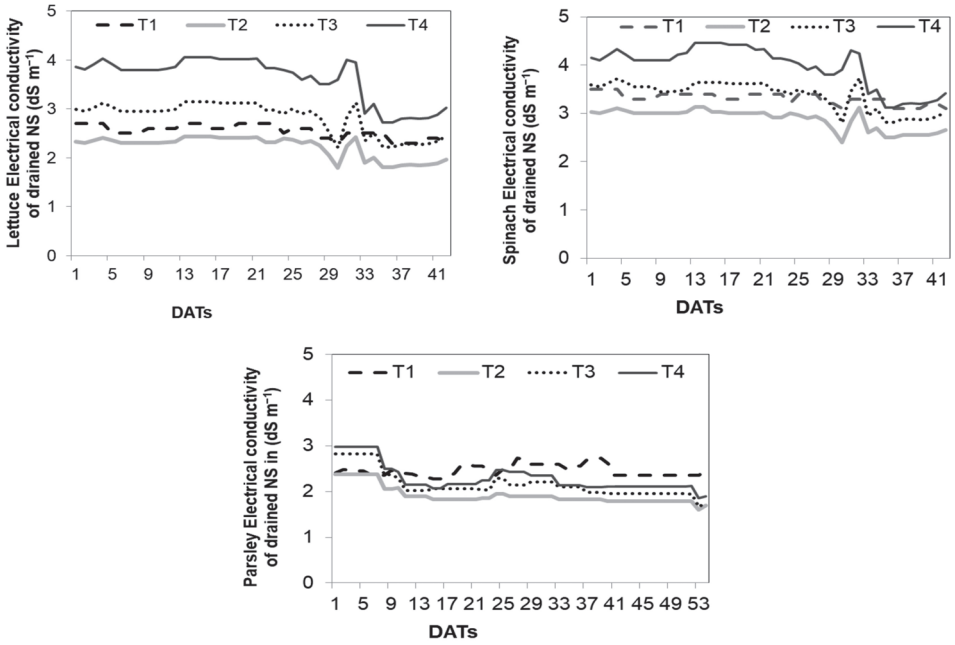

Figure 3. Evolution of EC values of the DS of the secondary crops. T1 (0%DS + 100%FS); T2 (50%DS + 50%W); T3 (75%DS + 25%W); T4 (100%DS + 0%W).

The amount of water added in each treatment affected the amount of NS was uptaken by the plants in different ways. The lettuce and parsley plants in the monoculture system absorbed at least 18% more than the plants in the cascade system receiving any of the treatments ($p < 0.05$). In spinach, the plants receiving the T3 and T4 treatments absorbed similar amounts to with the control treatment, with no significance differences among them ($p > 0.05$). Here, only the plants fertigated with 50% DS and 50% water were not able to absorb the necessary amount provided in the control treatment.

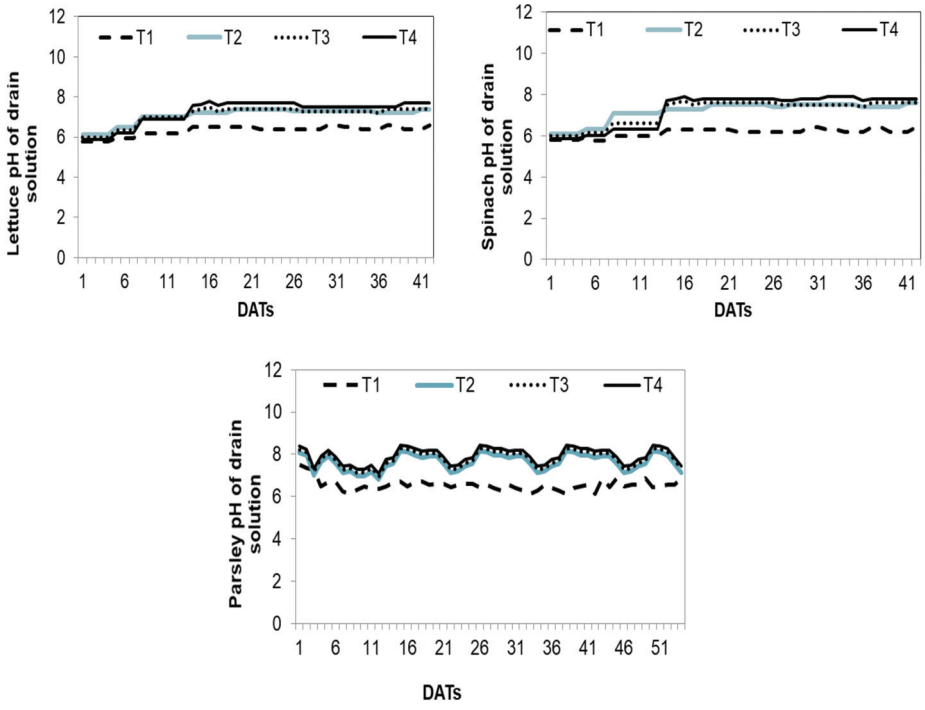

Figure 4. Evolution of pH values of the DS of the secondary crops. T1 (0%DS + 100%FS); T2 (50%DS + 50%W); T3 (75%DS + 25%W); T4 (100%DS + 0%W).

Table 2. Total amount of nutrient solution supplied to, drained from and uptaken by primary and secondary crops (L m^{-2}) for the same cultivation period, according to the treatment. The nutrient solution supplied is presented separately from the amount of water and drainage solution added.

Crop Species	Volume of NS Applied (L m^{-2})		Volume of NS Drained (L m^{-2})	Water Uptake (L m^{-2})
Tomato	882		472	410
Lettuce	Water	DS		
T1	290	0	155	135
T2	145	145	180	110
T3	215	75	195	95
T4	0	290	185	105
Spinach	Water	DS		
T1	290	0	85	205
T2	145	145	130	160
T3	215	75	95	195
T4	0	290	105	185
Parsley	Water	DS		
T1	167	0	89	105
T2	84	84	86	86
T3	124	43	109	74
T4	0	167	93	82

T1 (0%D + 100%FS); T2 (50%D + 50%W); T3 (75%D + 25%W); T4 (100%D + 0%W).

Because the plants absorbed different amounts of NS, they also drained different amounts of DS each time. The tomato and lettuce crops provided a total of 605 L m^{-2} DS. In the tomato–spinach system layout, the collected DS was 545 L m^{-2} and in the tomato–parsley system, 549 L m^{-2}. According to these data, all three cascade systems collected an amount of DS at least 48% lower than that observed in the corresponding monoculture systems. The lowest impact on the environment was considered to be exhibited by the T4 treatment, with amounts equal to 382 L m^{-2}, 313 L m^{-2}, 409 L m^{-2} for lettuce, spinach and parsley, respectively.

The above values correspond to a primary crop cultivation area equal to that of the secondary crop (1:1 cultivation ratio). Increasing the cultivation area of the secondary crop, these rates could be lower. For instance, at a higher cultivation ratio like 1:2, where the cultivation area of secondary crop is doubled, the unused DS of the primary crop was calculated to be less than 40%. In spinach, a 1:3 cultivation ratio could further reduce the amount of unused DS of the primary crop by 10%.

3.4. Nutrient Concentration

In Tables 3 and 4, the nutrient concentrations, measured in the laboratory, of the NS supplied to, uptaken by and drained from the plants in the primary and secondary crops on 36 DATs and 54 DATs, respectively, are presetned. For technical reasons, nutrient analysis was performed only for the parsley plants. As expected, the nutrient concentration of the NS supplied to the tomato and control parsley plants was similar to the system settings (Table 1). It is likely that any differences occurred due to the concentrations of nutrients remaining in the tube network.

Table 3. The average nutrient concentration in the supplied to, uptaken by and drained from the tomato and parsley plants according to treatment, on 36 DATs of the second experimental period. The concentrations of NO_3, P, K, Ca, Na, Mg are expressed in mmol L^{-1} and those of Fe, Zn, Mn, Cu in μmol L^{-1}.

Crop	Treatment	NO_3	P	K	Ca	Na	Mg	Fe	Zn	Mn	Cu	Drainage Percentage (%)
	36 DATs					Irrigation Solution						
Tomato		9.50	0.19	4.50	4.14	1.30	2.03	17.42	5.92	3.80	0.70	
Parsley	T1	11.00	0.36	6.49	4.80	1.27	1.91	15.00	2.80	2.94	0.80	
	T2	7.17	0.16	2.72	4.09	1.22	2.86	13.33	3.16	3.30	0.84	
	T3	9.53	0.24	3.96	4.29	1.26	2.98	15.37	4.73	4.60	0.97	
	T4	11.90	0.32	5.20	4.50	1.30	3.10	17.42	6.30	5.90	1.10	
	36 DATs					Drainage Solution						
Tomato		11.90	0.32	5.20	4.50	1.30	3.10	17.42	6.30	5.90	1.10	44
Parsley	T1	18.39	0.30	8.32	8.05	2.01	3.03	21.85	2.00	3.46	1.09	36
	T2	6.97	0.08	1.35	5.56	1.53	4.08	16.26	0.10	0.13	1.76	44
	T3	10.61	0.19	1.09	4.69	2.02	3.32	8.98	1.51	0.85	0.90	45
	T4	10.75	0.21	4.20	5.51	1.44	4.03	17.17	0.15	8.02	1.94	42
	36 DATs					Uptaken Solution						
Tomato		7.61	0.09	3.95	3.86	1.30	1.19	17.42	5.62	2.15	0.39	
Parsley	T1	9.77	0.35	5.46	2.97	0.85	1.28	11.15	2.30	2.64	0.50	
	T2	5.44	0.09	0.77	2.83	1.12	1.71	6.78	0.52	0.50	0.13	
	T3	7.24	0.24	3.33	2.66	1.20	2.00	11.43	3.88	4.14	0.66	
	T4	9.03	0.31	4.38	2.78	1.20	2.08	12.95	5.17	5.31	0.75	

T1 (0%DS + 100%FS); T2 (50%DS + 50%W); T3 (75%DS + 25%W); T4 (100%DS + 0%W).

Table 4. The average nutrient concentration in the supplied to, uptaken by and drained from the tomato and parsley plants, according to the treatment, on 54 DATs of the second experimental period. The concentrations of NO_3, P, K, Ca, Na, and Mg are expressed in mmol L^{-1} and those of Fe, Zn, Mn, and Cu in μmol L^{-1}.

Crop	Treatment	NO_3	P	K	Ca	Na	Mg	Fe	Zn	Mn	Cu	Drainage Percentage (%)
	54 DATs					Irrigation Solution						
Tomato		8.20	0.14	3.10	3.10	1.17	2.02	17.11	1.30	1.69	0.55	
Parsley	T1	12.41	0.26	6.36	4.60	1.27	1.79	14.68	1.10	2.77	0.60	
	T2	5.83	0.13	1.75	3.53	1.15	2.32	13.17	1.60	1.75	0.74	
	T3	7.52	0.05	0.41	4.80	0.67	2.79	24.51	0.02	2.49	1.35	
	T4	9.21	0.26	3.26	3.38	1.17	2.02	17.11	1.45	2.80	0.90	
	54 DATs					Drainage Solution						
Tomato		9.21	0.26	3.26	3.38	1.17	2.02	17.11	1.45	2.80	0.90	42
Parsley	T1	12.10	0.06	2.88	7.86	2.20	3.29	26.10	0.20	2.56	1.07	29
	T2	2.86	0.04	0.74	5.20	0.69	3.13	20.09	0.03	2.25	1.29	39
	T3	3.57	0.05	0.41	4.80	0.67	2.79	24.51	0.02	2.49	1.35	39
	T4	4.56	0.05	0.49	6.03	0.59	2.72	24.96	0.80	1.30	1.33	40
	54 DATs					Uptaken Solution						
Tomato		7.47	0.06	2.98	2.90	1.17	2.02	17.11	1.19	0.88	0.30	
Parsley	T1	9.42	0.26	5.35	2.85	0.85	1.20	10.91	0.90	2.49	0.41	
	T2	4.42	0.13	1.48	2.18	1.10	1.56	9.79	1.31	1.58	0.50	
	T3	5.71	0.19	2.11	2.14	1.08	1.46	11.26	0.90	2.05	0.56	
	T4	6.99	0.25	2.75	2.09	0.80	1.36	12.72	1.20	2.52	0.61	

T1 (0%DS + 100%FS); T2 (50%DS + 50%W); T3 (75%DS + 25%W); T4 (100%DS + 0%W).

The nutrient concentrations were similar between the DS for the primary crop and the NS supplied to the plants receiving the T4 treatment, at both the 36 and 54 DATs sampling dates. On the other hand, the nutrients supplied to the cascade treatments were different from the target concentration, and varied according to the sampling date. On 36 DATs, most of the macronutrient concentrations of T4 were similar to the targeted concentration applied to the plant of the control treatment (Table 3). Significant differences were observed with respect to micronutrient concentration, with higher values in the DS applied to the primary crop ($p < 0.05$). The macronutrient concentrations of the other treatments—T2 and T3—were between 13% and 59% lower than in the control treatment ($p < 0.05$), and the micronutrient concentrations were between 21% and 69% higher than in the control treatment ($p < 0.05$).

On 54 DATs, the nutrients in the DS primary crop were lower than those collected on 36 DATs, affecting the synthesis of NS supplied to the cascade treatments (Table 4). Therefore, none of the cascade treatments were able to be irrigated with nutrient concentrations close to those targeted. In the T4 treatment, most of the element contents were 25–49% lower than the target.

The synthesis of the NS supplied to the plants affected the amount of NS absorbed by the secondary crops and the resulting synthesis of the DS in different ways. On 36 DATs, the plants receiving T1, T3 and T4 absorbed between 11% and 38% of the nutrients of the supplied NS. In contrast, for the T2 treatment, where the NS supplied was poor, the plants absorbed the majority of the nutrients. On 54 DATs, no significant differences were observed among the treatments ($p < 0.05$).

All the cascade treatments presented lower nutrient concentrations in the DS compared to the control treatment. The DS with the lowest concentrations of NO_3, P, Zn and Mn was that used in the T2 treatment. The contents of most of the elements in that treatment were 24–96% lower (depending on the element) than in the control plants. The T3 treatment

presented the lowest concentrations of K, Ca, Mg, Fe, and Cu. The differences between the cascade treatments and the control treatment were similar on both sampling dates. However, on the second sampling date (54 DATs), the contents of most of the macronutrients, except Ca, in the final DS were 25–65% lower than on the first sampling date.

Table 5 presents the nutrient content (N, P, K, Ca and Mg expressed in g per 100 g DM, and microelements Fe, Zn, Mn, Cu expressed in mg kg^{-1} DM) in the leaf tissues of lettuce, spinach and parsley plants subjected to the different treatments on the different sampling dates. In lettuce leaves, the lowest concentrations of most of the macronutrients were found for the T4 treatment. In the other treatments, no significant differences were observed. In spinach leaves, the highest concentrations of N, P and K were observed in T1, while Ca and Mg were higher with the T4 treatment. In parsley plants, the concentration of most of the nutrients was lower in the cascade than in the control treatment.

Table 5. Nutrient content (N, P, K, Ca and Mg expressed in g per 100 g DM, and microelements Fe, Zn, Mn, Cu expressed in mg kg^{-1} DM) in leaf tissues of lettuce, spinach and parsley plants of the different treatments and sampling dates (n = 9).

Crop	Treatment	N	P	K	Ca	Mg	Fe	Zn	Mn	Cu
Lettuce										
15 DATs	T1	4.15 ^{aA,1}	0.67 ^{aA}	7.06 ^{aA}	1.03 ^{aA}	0.49 ^{aA}	148.7 ^{aA}	31.7 ^{aA}	26.8 ^{aA}	5.89 ^{aA}
	T2	4.11 ^{aA}	0.67 ^{aA}	8.18 ^{bA}	1.00 ^{aA}	0.39 ^{bA}	233.47 ^{bA}	40.1 ^{bA}	33.9 ^{bA}	4.82 ^{aA}
	T3	4.01 ^{aA}	0.60 ^{bA}	7.14 ^{aA}	1.06 ^{aA}	0.54 ^{cA}	141.4 ^{aA}	29.8 ^{aA}	26.8 ^{aA}	4.33 ^{aA}
	T4	3.61 ^{aA}	0.55 ^{cA}	6.67 ^{aA}	0.92 ^{aA}	0.45 ^{aA}	156.8 ^{aA}	28.6 ^{aA}	28.7 ^{aA}	4.99 ^{aA}
41 DATs	T1	3.54 ^{aA}	0.64 ^{aA}	6.03 ^{aA}	1.29 ^{aB}	0.65 ^{aB}	122.5 ^{aA}	28.5 ^{aA}	26.8 ^{aA}	7.01 ^{aA}
	T2	3.62 ^{aA}	0.58 ^{aB}	5.25 ^{abB}	1.26 ^{aB}	0.63 ^{aB}	160.5 ^{bB}	37.2 ^{bA}	36.4 ^{aA}	4.11 ^{aA}
	T3	3.38 ^{aA}	0.49 ^{bB}	4.78 ^{abB}	1.28 ^{aB}	0.69 ^{aB}	126.2 ^{aA}	28.9 ^{aA}	33.4 ^{aB}	4.08 ^{aA}
	T4	3.30 ^{aA}	0.44 ^{bB}	4.56 ^{bB}	1.23 ^{aB}	0.66 ^{aB}	119.7 ^{aA}	27.5 ^{aA}	31.9 ^{aA}	3.86 ^{aA}
	Optimal levels	4.32	0.89	4.91	0.76	0.65	82.8	24.8	39.7	4.84
Spinach										
15 DATs	T1	4.17 ^{aA}	0.71 ^{aA}	6.78 ^{aA}	1.47 ^{aA}	1.10 ^{aA}	69.42 ^{aA}	31.9 ^{aA}	18.6 ^{aA}	3.33 ^{aA}
	T2	4.28 ^{aA}	0.74 ^{aA}	8.48 ^{bA}	1.13 ^{bA}	1.29 ^{bA}	83.75 ^{bA}	44.9 ^{bA}	26.8 ^{bA}	4.94 ^{bA}
	T3	4.22 ^{aA}	0.59 ^{bA}	6.69 ^{aA}	1.34 ^{cA}	1.23 ^{bA}	66.88 ^{aA}	31.0 ^{aA}	18.1 ^{aA}	3.56 ^{aA}
	T4	4.25 ^{aA}	0.58 ^{bA}	6.90 ^{aA}	1.34 ^{aA}	1.23 ^{bA}	66.96 ^{aA}	31.4 ^{aA}	19.8 ^{aA}	3.10 ^{aA}
41 DATs	T1	4.54 ^{aA}	0.67 ^{aA}	7.80 ^{aA}	1.85 ^{aB}	1.37 ^{aB}	85.34 ^{aB}	38.6 ^{aB}	26.7 ^{aB}	3.74 ^{aA}
	T2	4.37 ^{aA}	0.62 ^{aB}	6.62 ^{bB}	1.63 ^{bB}	1.50 ^{bB}	86.55 ^{aB}	51.45^{bB}	28.6 ^{aA}	5.38 ^{bA}
	T3	4.25 ^{aA}	0.50 ^{bA}	5.51 ^{cB}	2.07 ^{aB}	1.60 ^{bB}	90.26 ^{aB}	38.5 ^{aB}	24.7 ^{aB}	5.32 ^{bB}
	T4	4.21 ^{aA}	0.51 ^{bA}	6.17 ^{bA}	1.93 ^{aB}	1.57 ^{bB}	85.54 ^{aB}	38.9 ^{aB}	25.3 ^{aB}	4.76 ^{bB}
	Optimal levels	4.61	0.60	5.27	1.40	1.00	60.35	unspecified		
Parsley										
36 DATs	T1	3.78 ^{aA}	0.84 ^{aA}	6.00 ^{aA}	0.97 ^{aA}	0.27 ^{aA}	105.7 ^{aA}	25.9 ^{aA}	35.4 ^{aA}	5.83 ^{aA}
	T2	3.99 ^{aA}	0.42 ^{bA}	2.48 ^{bA}	1.27 ^{bA}	0.60 ^{bA}	87.3 ^{bA}	32.6 ^{aA}	48.4 ^{bA}	8.19 ^{aA}
	T3	3.57 ^{aA}	0.34 ^{bA}	2.16 ^{cA}	1.17 ^{bA}	0.63 ^{bA}	108.7 ^{aA}	29.5 ^{aA}	43.9 ^{bA}	7.82 ^{aA}
	T4	3.69 ^{aA}	0.39 ^{bA}	2.68 ^{bA}	1.23 ^{bA}	0.64 ^{bA}	81.39 ^{bA}	24.6 ^{aA}	45.7 ^{bA}	7.16 ^{aA}
54 DATs	T1	3.43 ^{aA}	0.62 ^{aB}	5.63 ^{aA}	1.09 ^{aA}	0.28 ^{aA}	76.33 ^{aB}	19.6 ^{aA}	28.8 ^{aB}	4.53 ^{aB}
	T2	2.97 ^{aB}	0.24 ^{bB}	1.12 ^{bB}	1.32 ^{bA}	0.80 ^{bB}	64.61 ^{bB}	20.4 ^{aB}	35.7 ^{aB}	5.56 ^{aB}
	T3	3.17 ^{aA}	0.27 ^{bB}	1.46 ^{cB}	1.29 ^{bA}	0.71 ^{bB}	71.91 ^{aB}	21.0 ^{aB}	30.8 ^{aB}	4.4 ^{aB}
	T4	3.02 ^{aB}	0.23 ^{bB}	1.19 ^{bB}	1.33 ^{bA}	0.73 ^{bB}	69.87 ^{aB}	19.2 ^{aA}	34.6 ^{aB}	5.3 ^{aB}
	Optimal levels	4.7	0.72	5.8	0.84	0.40	75	57	107	10

[1] Different uppercase letters (A, B) indicate statistically significant differences between sampling dates and different lowercase letters (a, b) indicate statistically significant differences between the different (T1–T4) treatments ($p < 0.05$). T1 (0%DS + 100%FS); T2 (50%DS + 50%W); T3 (75%DS + 25%W); T4 (100%DS + 0%W). The optimal level was defined by El-Shinawy and Gawish [23] for lettuce, Öztekin et al. [24] for spinach, and Currey et al. [25] for parsley.

3.5. Yield Performance of Secondary Crops

To assess the sustainability of each cascade system, it is necessary to study the yield performance of the secondary crops. In this sense, a series of yield characteristics including plants height, number of leaves, chlorophyll content index, photosynthesis rate, FM and DM were analysed. The data presented here correspond to the last day of each secondary crop cultivation period (42 DATs for lettuce and spinach; 54 DATs for parsley). The measurements collected in the earlier pre-harvest cultivation period are not considered, since no significant differences among the treatments were noticed.

Figure 5a presents the average plant height of each secondary crop, according to the treatment, measured during the last day of each cultivation period. According to the results, the nutrient solution applied to the lettuce and parsley did not affect the height ($p > 0.05$) of the plants. Spinach plants showed a final plant height 14% higher for the T4 treatment ($p < 0.05$) compared to the rest of the treatments.

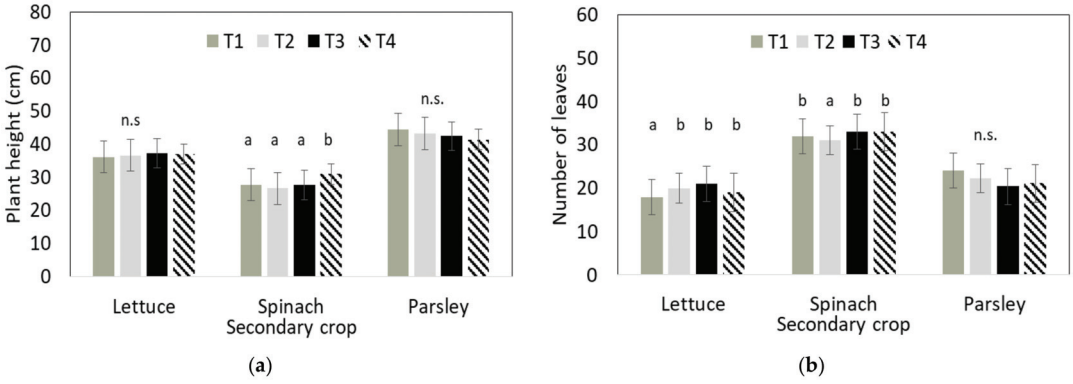

Figure 5. Mean values and standard deviations of (**a**) plant height (cm), and (**b**) number of leaves measured on the last day of each secondary cultivation period grown under the different treatments ($n = 9$ samples/treatment). T1 (0%DS + 100%FS); T2 (50%DS + 50%W); T3 (75%DS + 25%W); T4 (100%DS + 0%W). Different lowercase letters (a, b) indicate statistically significant differences ($p < 0.05$) of the different treatments within each crop, n.s. indicates no significant difference ($p > 0.05$).

The number of leaves per plant for the different treatments during the last day of each secondary crop cultivation period is presented in Figure 5b. Lettuce plants fertigated with the targeted NS (T1 treatment) had significantly lower numbers of leaves compared to the other treatments. Spinach plants with the T2 treatment presented significantly lower numbers of leaves compared to the other treatments. No treatment effect was in the number of leaves of parsley plants ($p > 0.05$).

Figure 6 presents the variation in CCI for each secondary crop according to the treatment measured during the last day of the cultivation period of each secondary crop. The average values of chlorophyll content observed during the measurement period were 32 mg cm^{-2}, 55 mg cm^{-2}, and 45 mg cm^{-2} for lettuce, spinach, and parsley, respectively. No treatment effects were observed on the CCI ($p > 0.05$).

Figure 7 presents the P_N variation of each secondary crop according to the treatment during the last day of the cultivation period of each secondary crop. The average P_N values observed in the different secondary crops were 12 μmol CO_2 m^{-2} s^{-1} for lettuce and spinach and 10 μmol CO_2 m^{-2} s^{-1} for the parsley crop. Similar to CCI, no treatment effects were observed on P_N ($p > 0.05$).

The values of FM and DM production observed in the different treatments are shown in Table 6. In the lettuce crop, no significant difference was observed among the treatments ($p > 0.05$). The moisture content of the samples ranged from 93% to 95%. In the spinach crop, only the FM with the T2 treatment was less than the FM of the control treatment, by

about 22% ($p < 0.05$). The moisture content of the samples ranged from 88 to 90%. Parsley presented the highest yield in the control treatment and the reuse of the tomato drainage solution imposed a decrease in yield by about 30%. The moisture content of the parsley samples was 83%.

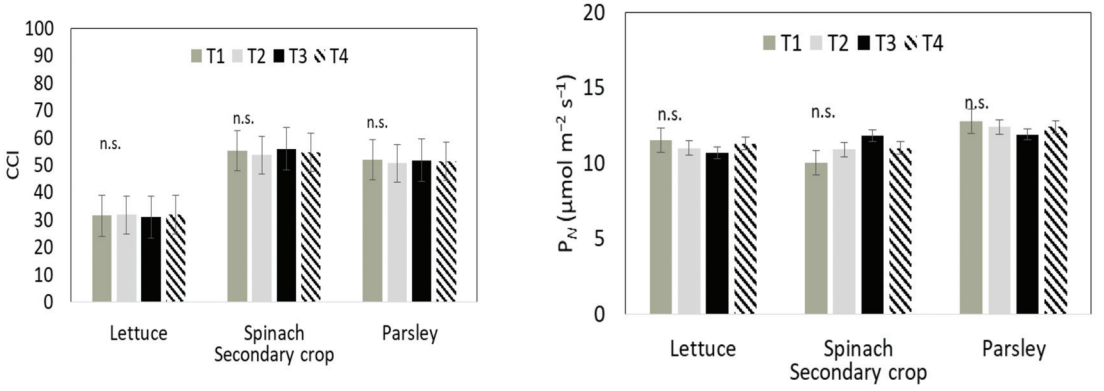

Figure 6. Mean values and standard deviations of (**a**) CCI and (**b**) P_N (µmol CO_2 m^{-2} s^{-1}), measured on the last day of each secondary cultivation period under the different treatments ($n = 9$ samples/treatment). T1 (0%DS + 100%FS); T2 (50%DS + 50%W); T3 (75%DS + 25%W); T4 (100%DS + 0%W). n.s. indicates no significant difference ($p > 0.05$).

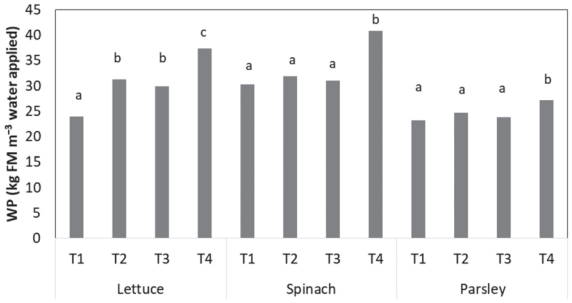

Figure 7. Water productivity in kg of fresh mass per kg of water applied based on the systems layout in combination with the primary crop for a six-month cultivation period. Different lowercase letters (a, b, c) within each secondary crop indicate statistically significant differences ($p < 0.05$).

Table 6. Mean value (and standard deviation) of fresh mass (FM) and dry mass (DM) in g m^{-2} of the secondary crops on the two sampling dates.

Crops	Lettuce		Spinach		Parsley	
	FM (g m^{-2})	DM (g m^{-2})	FM (g m^{-2})	DM (g m^{-2})	FM (g m^{-2})	DM (g m^{-2})
DAT	28		28		36	
T1	966 ± 11 ^{a,1}	42 ± 0.8 ^{a}	1300 ± 37.7 ^{a}	128 ± 3.8 ^{a}	67 ± 10.7 ^{b}	11 ± 1.7 ^{b}
T2	1011 ± 8.8 ^{b}	46 ± 1.0 ^{b}	1192 ± 24.9 ^{a}	120 ± 2.5 ^{a}	56 ± 9.2 ^{ab}	11 ± 1.7 ^{b}
T3	1134 ± 9.1 ^{b}	47 ± 1.0 ^{b}	1392 ± 36.4 ^{ab}	140 ± 3.6 ^{ab}	48 ± 7.2 ^{a}	10 ± 1.2 ^{a}
T4	1045 ± 9.4 ^{b}	47 ± 0.8 ^{b}	1548 ± 58.8 ^{b}	156 ± 5.9 ^{b}	45 ± 4.7 ^{a}	8 ± 0.8 ^{a}
DAT	42		42		54	

Table 6. *Cont.*

Crops	Lettuce		Spinach		Parsley	
	FM (g m^{-2})	**DM (g m^{-2})**	**FM (g m^{-2})**	**DM (g m^{-2})**	**FM (g m^{-2})**	**DM (g m^{-2})**
T1	2354 ± 39.3 ^{a}	98 ± 2.8 ^{b}	2904 ± 45.3 ^{b}	292 ± 4.5 ^{b}	329 ± 33.6 ^{b}	46 ± 8.6 ^{b}
T2	2117 ± 33.9 ^{a}	90 ± 2.9 ^{a}	2272 ± 67.5 ^{a}	216 ± 5.5 ^{a}	207 ± 21.9 ^{a}	32 ± 3.4 ^{a}
T3	2276 ± 19.1 ^{a}	94 ± 2.0 ^{ab}	2576 ± 26.2 ^{ab}	260 ± 5.6 ^{ab}	229 ± 18.6 ^{a}	37 ± 3.1 ^{a}
T4	2318 ± 47.4 ^{a}	100 ± 2.7 ^{b}	3040 ± 82.0 ^{b}	284 ± 5.3 ^{b}	226 ± 27.8 ^{a}	35 ± 4.2 ^{a}

[1] Different lowercase letters (a, b) within a row for FM or for DM indicate statistically significant differences ($p < 0.05$).

3.6. Water Productivity and Fertiliser Use Efficiency

Figure 7 presents the WP (kg FM m^{-3} water applied) of each cultivation system. To ensure comparability of data among the systems, the calculations were performed for a growing period equal to that of the primary crop. The WP value estimated for the tomato crop was 26.5 kg m^{-3}. The WP values of the secondary crops with the control treatment were equal to 24 kg m^{-3}, 30 kg m^{-3} and 23 kg m^{-3} for lettuce, spinach and parsley, respectively. In the cascade system with T4, the WP values were significantly higher than in the monoculture system, by 50%, 30% and 14% for lettuce, spinach and parsley, respectively. In the cascade systems with other treatments, the WP values of parsley and spinach were equal to that of the monoculture system ($p > 0.05$), while in the lettuce crop, WP was slightly higher ($p < 0.05$).

The FUE (kg FM kg^{-1} fertiliser applied) of tomato during the six-month cultivation period was 20.9 kg FM kg^{-1} fertiliser applied. For the same cultivation period, the FUE was calculated to be equal to 18 kg FM kg^{-1} for lettuce, 20 kg FM kg^{-1} for spinach and 17 kg FM kg^{-1} for parsley. In the cascade system where only DS was used, the FUE was significantly higher than in the monoculture system (T1), by 62% for lettuce and spinach and 22% for parsley. The FUE values of the other cascade treatments were also higher than in the monoculture system. The NUE and PUE showed similar trends (Figure 8). The NUE varied from 42 kg FM kg^{-1} to 74 kg FM kg^{-1}, with the maximum values being found for the T4 treatment. The PUE values were significantly higher than those of NUE, since the amount of P added to the irrigated NS was quite low, almost 90% less than the N concentration. Therefore, PUE varied from 463 kg FM kg^{-1} to 1270 kg FM kg^{-1}, with the maximum values being observed for the T4 treatment. The other cascade treatments resulted in NUE and PUE values close to those of the monoculture system.

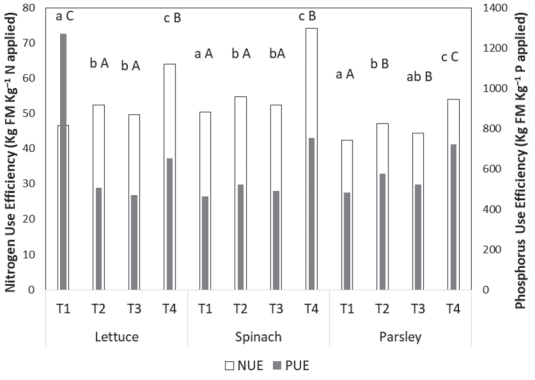

Figure 8. Nitrogen and phosphorus use efficiency in kg of fresh mass per kg of nutrient applied based on the system layout in combination with the primary crop for six-month cultivation period. Different lowercase letters (a, b, c) indicate statistically significant differences ($p < 0.05$) in NUE and uppercase letters (A, B, C) indicate statistically significant differences ($p < 0.05$) in PUE.

4. Discussion

The correct choice of crop combinations and system layouts is the key to the efficient functioning of cascade systems. In the current research, three different cascade systems with three different crop species were compared with the respective monoculture system.

Among the cascade systems, the NS that used pure Ds of the primary crop had a macronutrient concentration closer to the targeted concentration. The micronutrients, on the other hand, were closer to the targeted concentration in the cascade systems where the DS was diluted with water. In the case of Fe concentrations, although the values were much higher at both sampling dates for recycling treatments than when operating as an open system, they were still clearly above critical levels, as suggested by [26,27]. These results were confirmed by the nutrient concentration estimated in leaf tissues.

Spinach and parsley seem to be more adjustable than the lettuce crop in secondary crop cultivation systems, since they are more salt tolerant. Usually, lettuce is considered to be more moderately sensitive to salinity compared to spinach and parley crops, with a threshold electrical conductivity (EC) of 1.3 dS m^{-1}, and a negative relative yield decrease slope of 13%. The respective values for spinach and parsley were 2.0 dS m^{-1} with a slope of 7.6% per dS m^{-1} and 1.8 dS m^{-1} with a slope of 6.2% per dS m^{-1} [28].

However, in this study, all crops were able to grow sufficiently under the cascade system, although the amounts of nutrients absorbed were different. The CCI and P_N values of all crops were similar in all cultivation systems. The lettuce plants in the cascade systems were sufficiently tall and heavy, with a greater number of leaves than in the monoculture system. The spinach plants subjected to the T4 cascade treatment were taller and heavier than those in the other systems, while the parsley plants demonstrated similar growth progress to that of the monoculture system, but were less heavy. It seems that all of the studied crops could be used as secondary crops in cascade cultivation systems, given that they exhibit sufficient yield performance. The final evaluation of cascade systems, however, should be undertaken in consideration of water productivity and nutrient use efficiency.

4.1. Evaluation of Cascade System Based on Water Productivity

In the primary crop, the WP values observed (26.5 kg FM m^{-3}) were similar to those reported in [6]. In Katsoulas et al. [1], the WP of tomato crop varied from 20 kg FM m^{-3} to 35 kg FM m^{-3}. Nikolaou et al. [29] mentioned that the ratio of product yield to water use increased from 3 kg m^{-3} to 17 kg m^{-3} in an unheated greenhouse and reached 45 kg m^{-3} in a soilless growing system.

In secondary crops, the WP values for the monoculture system were 25 kg m^{-3}, 31 kg m^{-3} and 24 kg m^{-3} for lettuce, spinach and parsley, respectively. Bozkurt et al. [30] found an FM of 23 kg m^{-3} for lettuce plants cultivated in soil under greenhouse conditions. Here, the lettuce plants with T4 exhibited a WP performance 50% than that of the monoculture system. Moreover, the plants in the cascade system subjected to T2 and T3 also had a WP performance 26% higher than in the monoculture system.

Kuslu et al. [31] found a WP of 9.7 kg m^{-3} in spinach crops after a cultivation period of about 45 days. Here, for the same cultivation period, the WP of the monoculture system exhibited similar values. During the primary crop cultivation period of six months, the WP was almost tripled. In the cascade system with T4, the WP was even higher, with an increase of about 30% with respect to the monoculture system.

In parsley plants, Martins et al. [32] found WP values ranging from 3.7 kg m^{-3} to 4.73 kg m^{-3} under greenhouse conditions with a coconut substrate. Here, for the same cultivation period, the WP was 34% higher. During the primary crop cultivation period of six months, WP was about 24 kg m^{-3} in the monoculture system. In the cascade system with T4, the WP was 14% higher than that of the monoculture system.

Accordingly, the two-level cascade cultivation system was demonstrated to be most efficient from an agronomical point of view, since the net water input was restricted, and the WP was significantly higher than in the open system. Understanding and further improving WP under cascade system side yield is the primary focus of developing water

productive plants in greenhouses. The improvement in WP could impart tolerance to drought and salinity stress, while still accumulating sufficient biomass to make their production commercially viable. According to Damerum et al. [33], it is imperative to develop systems for improving crop WP, particularly in the case of crops such as lettuce, where over 75% of the total production in the US is dominated by the state of California. The combination of DS with fresh nutrient solution may allow this goal to be achieved, and should be further investigated.

4.2. Evaluation of Cascade System Based on Nutrient Use Efficiency

Compared to open (free drainage) soilless systems, cascade systems can be considered that has higher fertiliser use efficiency, since they make complete use of the drainage produced from the primary (and potentially the secondary) crop.

In the current research, the FUE values of the cascade parsley and lettuce systems (18 kg FM kg^{-1}) were 18% and 62% higher than in the monoculture (control) treatment. The FUE values of the lettuce monoculture system were similar to the values found by Santamaria et al. [34] in lettuce plants cultivated in soilless growth chambers.

In the spinach crop, the FUE value was 0.20 kg FM kg^{-1} for the 42-day cultivation period. Chan-Navarrete et al. [35] reported FUE values ranging from 0.14 kg DM kg^{-1} to 0.18 kg DM kg^{-1} for a 28-day cultivation period. For a six-month cultivation period, the FUE, expressed in FM, was calculated to be equal to 15 kg FM kg^{-1}. The FUE of the T4 treatment was 66% higher than in the monoculture system. For spinach to maintain a satisfactory yield under low nitrogen conditions, high NUE is necessary. This is because spinach is not very efficient at either nitrogen uptake or utilisation, and requires considerable amounts of nitrogen for growth and the establishment of its dark green colour [36,37]. The combination of high nitrate input and low nitrate reduction by spinach leads to high levels of nitrate in the marketable product [35]. In cascade systems, spinach plants can accumulate substantial amounts of nitrate in the leaves, because the extra mineralisation of DS gives a surplus of nitrate to the plant.

Here, the maximum NUE value occurred in cascade with the 100% DS treatment. The NUE in that system was higher than those reported in a currently available commercial hydroponic system. Zhang et al. [38] found that NUE values varied from 44 kg FM kg^{-1} to 74 kg FM kg^{-1} for a 5-month cultivation period in a microalgae and crop co-cultivation system. Similar to the present study, lower NUE values were found in the simple hydroponic system, while the maximum values were found in the co-cultivated system. The PUE values were also higher than those found for a commercial soilless system. The PUE values were more than double those reported by Zhang et al. [38], however, due to low values of P concentration added to the system. Usually, PUE is a complex trait for plant breeding, with many potential interactions and trade-offs with other factors affecting crop yield, such as water use efficiency and energy balance [39]. The effectiveness of cascade approaches in cascade systems based on traits that affect P absorption rates is due to the deeper layer of water productivity occurring.

Our results are in agreement with previous reports by Elvanidi et al. [12] and Muñoz et al. [40], which mentioned that the nitrogen balance in cascade systems shows an important decrease in nutrient leachate. According to Muñoz et al. [40], the adoption of a cascade crop system reduced the environmental impact by 21%. Additionally, García-Caparrós et al. [10] concluded that the establishment of sequential irrigation systems can result in water savings and the removal of nitrates, which are of great advantage in arid and semi-arid regions.

Cascade farming systems represent a promising sustainable alternative cultivation system compared to monoculture systems. Likewise, due to climate change and the increasing population, it is becoming a challenge to balance demand and supply, leading to negative economic externalities. However, cultivation in cascade systems, especially in hydroponic ones, can provide valuable ecosystem services, such as savings in terms of fertilisers, the consumption of less water, the minimisation of energy needs and the

maximisation of yield productivity. A well-developed soilless cascade system represents a substantial competitive advantage in overcoming the challenges outlined.

However, to further assess the sustainability of cascade systems, the use of a life cycle assessment (LCA) systems analysis methodology is required for the assessment of their environmental impacts.

5. Conclusions

Three secondary crops were tested under different treatments in a soilless cascade system using a tomato crop as the primary donor crop. It was found that among the secondary crops, spinach was the most appropriate secondary receiver crop among those considered in this study. The use of the tomato drainage solution for the fertigation of the spinach crop positively affected crop yield. In the case of the other secondary crop species tested, lettuce and parsley, yield was not negatively affected when fertigated by the tomato crop drainage solution. The reuse of the drainage solution significantly increased the water productivity and nutrient use efficiency of the cascade crops. The water productivity in the plants irrigated with pure drainage solution was 50%, 30% and 14% higher for lettuce, spinach and parsley, respectively, than in the monoculture system. The nitrogen and phosphorus use efficiency were improved more than 50% with respect to their values in the monoculture system. The current research gives small holder farmers the ability to convert their cultivations to more sustainable systems, minimising construction costs and environmental impact while maximising yield.

Author Contributions: Conceptualisation, N.K.; methodology, A.E. and N.K.; formal analysis, E.K., S.F., I.N. and A.E.; investigation, E.K., A.E., S.F., I.N. and N.K.; resources, N.K.; data curation, E.K., S.F., I.N. and A.E.; writing—original draft preparation, E.K., A.E. and N.K.; writing—review and editing, A.E. and N.K.; supervision, N.K.; project administration, N.K.; funding acquisition, N.K. All authors have read and agreed to the published version of the manuscript.

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References

1. Katsoulas, N.; Savvas, D.; Kitta, E.; Bartzanas, T.; Kittas, C. Extension and evaluation of a model for automatic drainage solution management in tomato crops grown in semi-closed hydroponic systems. *Comput. Electron. Agric.* **2015**, *113*, 61–71. [CrossRef]
2. Massa, D.; Magán, J.J.; Montesano, F.F.; Tzortzakis, N. Minimizing water and nutrient losses from soilless cropping in southern Europe. *Agric. Water Manag.* **2020**, *241*, 106395. [CrossRef]
3. Katsoulas, N.; Voogt, W. Recent trends in salinity control for soilless growing systems management. *Acta Hortic.* **2013**, *1034*, 433–442. [CrossRef]
4. Abd-Elmoniem, E.M.; Abdrabbo, M.; Farag, A.; Medany, M. Hydroponics for Food Production: Comparison of Open and Closed Systems on Yield and Consumption of Water and Nutrient. In Proceedings of the 2nd International Conference on Water Resources & Arid Environment, Riyadh, Saudi Arabia, 2–5 December 2006.
5. Rufí-Salís, M.; Parada, F.; Arcas-Pilz, V.; Petit-Boix, A.; Villalba, G.; Gabarrell, X. Closed-loop crop cascade to optimize nutrient flows and grow low-impact vegetables in cities. *Front. Plant Sci.* **2020**, *11*, 596550. [CrossRef]
6. Méndez-Cifuentes, A.; Valdez-Aguilar, L.A.; Cadena-Zapata, M.; González-FUEntes, J.A.; Hernández-Maruri, J.A.; Alvarado-Camarillo, D. Water and fertilizer use efficiency in subirrigated containerized tomato. *Water* **2020**, *12*, 1313. [CrossRef]
7. Fayezizadeh, M.R.; Ansari, N.A.Z.; Albaji, M.; Khaleghi, E. Effects of hydroponic systems on yield, water productivity and stomatal gas exchange of greenhouse tomato cultivars. *Agric. Water Manag.* **2021**, *258*, 107171. [CrossRef]
8. Incrocci, L.; Pardossi, A.; Malorgio, F.; Maggini, R.; Campiotti, C.A. Cascade cropping system for greenhouse soilless culture. *Acta Hortic.* **2003**, *609*, 297–301. [CrossRef]
9. Muñoz, P.; Paranjpe, A.; Montero, J.I.; Antón, A. Cascade crops: An alternative solution for increasing sustainability of greenhouse tomato crops in Mediterranean zone. *Acta Hortic.* **2012**, *927*, 801–805. [CrossRef]

10. García-Caparrós, P.; Espino, C.V.; Lao, M.T. Comparative behavior of Dracaena marginata plants integrated into a cascade cropping system with the addition of hydrogen peroxide. *Agronomy* **2021**, *11*, 218. [CrossRef]
11. Avdouli, D.; Max, J.F.J.J.; Katsoulas, N.; Levizou, E. Basil as secondary crop in cascade hydroponics: Exploring salinity tolerance limits in terms of growth, amino acid profile, and nutrient composition. *Horticulturae* **2021**, *7*, 203. [CrossRef]
12. Elvanidi, A.; Reascos, C.M.B.; Gourzoulidou, E.; Kunze, A.; Max, J.F.J.; Katsoulas, N. Implementation of the circular economy concept in greenhouse hydroponics for ultimate use of water and nutrients. *Horticulturae* **2020**, *6*, 83. [CrossRef]
13. Faliagka, S.; Elvanidi, A.; Spanoudaki, S.; Kunze, A.; Max, J.F.J.; Katsoulas, N. Effect of NaCl or macronutrient-imposed salinity on basil crop yield and water use efficiency. *Horticulturae* **2021**, *7*, 296. [CrossRef]
14. García-Caparrós, P.; Llanderal, A.; Velasquez, C.; Lao, M.T. Water and nutrient balance in an ornamental cascade cropping system. *Agronomy* **2021**, *11*, 1251. [CrossRef]
15. Katsoulas, N.; Demmelbauer-Benitez, C.M.; Elvanidi, A.; Gourzoulidou, E.; Max, J.F.J. Reuse of cucumber drainage nutrient solution in secondary crops in greenhouses: Initial results. *Acta Hortic.* **2020**, *1296*, 767–773. [CrossRef]
16. Santos, M.G.; Moreira, G.S.; Pereira, R.; Carvalho, S.P. Assessing the potential use of drainage from open soilless production systems: A case study from an agronomic and ecotoxicological perspective. *SSRN Electron. J.* **2022**, *273*, 107906. [CrossRef]
17. Garcia-Caparros, P.; Contreras, J.I.; Baeza, R.; Segura, M.L.; Lao, M.T. Integral Management of Irrigation Water in Intensive Horticultural Systems of Almería. *Sustainability* **2017**, *9*, 2271. [CrossRef]
18. Maboko, M.M.; Du Plooy, C.P. Evaluation of crisphead lettuce cultivars (*Lactuca sativa* L.) for winter production in a soilless production system. *Afr. J. Plant Sci.* **2008**, *2*, 113–117.
19. Brandenberger, L.; Cavins, T.; Payton, M.; Wells, L.; Johnson, T. Yield and quality of spinach cultivars for greenhouse production in Oklahoma. *HortTechnology* **2007**, *17*, 269–272. [CrossRef]
20. Sonneveld, C.; Voogt, W. Plant nutrition of greenhouse crops. In *Plant Nutrition of Greenhouse Crops*; Springer: Dordrecht, The Netherlands, 2009. [CrossRef]
21. Kumar, R.R.; Cho, J.Y. Reuse of hydroponic waste solution. *Environ. Sci. Pollut. Res.* **2014**, *21*, 9569–9577. [CrossRef]
22. Kjeldah. A new method for the determination of nitrogen in organic matter. *Z. Anal. Chem.* **1883**, *486*, 22–366.
23. El-shinawy, M.; Gawish, S.M. Effect of Commercial Organic Nutrient Solutions on Growth and Chemical Composition of Lettuce under Agricultural Soilless System. *Egypt. J. Hortic.* **2006**, *33*, 19–28.
24. Öztekin, G.B.; Uludağ, T.; Tüzel, Y. Growing spinach (*Spinacia oleracea* L.) in a floating system with different concentrations of nutrient solution. *Appl. Ecol. Environ. Res.* **2018**, *16*, 3333–3350. [CrossRef]
25. Currey, C.; Walters, K.; Flax, N. Nutrient Solution Strength Does Not Interact with the Daily Light Integral to Affect Hydroponic Cilantro, Dill, and Parsley Growth and Tissue Mineral Nutrient Concentrations. *Agronomy* **2019**, *9*, 389. [CrossRef]
26. Sularz, O.; Smoleń, S.; Koronowicz, A.; Kowalska, I.; Leszczyńska, T. Chemical Composition of Lettuce (*Lactuca sativa* L.) Biofortified with Iodine by KIO3, 5-Iodo-, and 3.5-Diiodosalicylic Acid in a Hydroponic Cultivation. *Agronomy* **2020**, *10*, 1022. [CrossRef]
27. Kennard, N.; Stirling, R.; Prashar, A.; Lopez-Capel, E. Evaluation of Recycled Materials as Hydroponic Growing Media. *Agronomy* **2020**, *10*, 1092. [CrossRef]
28. Maas, E.V.; Hoffman, G.J. Crop salt tolerance—Current assessment. *ASCE J. Irrig. Drain. Div.* **1977**, *103*, 115–134. [CrossRef]
29. Nikolaou, G.; Neocleous, D.; Christou, A.; Polycarpou, P.; Kitta, E.; Katsoulas, N. Energy and Water Related Parameters in Tomato and Cucumber Greenhouse Crops in Semiarid Mediterranean Regions. A Review, Part II: Irrigation and Fertigation. *Horticulturae* **2021**, *7*, 548. [CrossRef]
30. Bozkurt, S.; Mansurolu, G.; Kara, M.; Önder, S. Responses of lettuce to irrigation levels and nitrogen forms. *Afr. J. Agric. Res.* **2009**, *4*, 1171–1177.
31. Kuşlu, Y.; Sahin, U.; Kiziloglu, F.M.; Sengul, M. Yield and quality responses of drip-irrigated spinach to different irrigation quantities in a semi-arid region with a high altitude. *J. Cent. Eur. Agric.* **2016**, *17*, 763–777. [CrossRef]
32. Martins, J.; Júnior, J.; Bartusch, V.; Gheyi, H.; Neto, E.; Silva, M. Water relations in parsley plants cultivated in brackish nutrient solutions of different cationic natures. *Rev. Bras. Eng. Agrícola E Ambient.* **2019**, *23*, 662–668. [CrossRef]
33. Damerum, A.; Smith, H.K.; Clarkson, G.J.J.; Truco, M.J.; Michelmore, R.W.; Taylor, G. The genetic basis of water-use efficiency and yield in lettuce. *BMC Plant Biol.* **2021**, *21*, 237. [CrossRef]
34. Santamaria, P.; Elia, A.; Serio, F. Effect of solution nitrogen concentration on yield, leaf element content, and water and nitrogen use efficiency of three hydroponically-grown rocket salad genotypes. *J. Plant Nutr.* **2002**, *25*, 245–258. [CrossRef]
35. Chan-Navarrete, R.; Kawai, A.; Dolstra, O.; Lammerts van Bueren, E.T.; van der Linden, C.G. Genetic diversity for nitrogen use efficiency in spinach (*Spinacia oleracea* L.) cultivars using the Ingestad model on hydroponics. *Euphytica* **2014**, *199*, 155–166. [CrossRef]
36. Biemond, H.; Vos, J.; Struik, P. Effects of nitrogen on accumulation and partitioning of dry matter and nitrogen of vegetables. 3. Spinach. *Neth. J. Agric. Sci.* **1996**, *44*, 227–239. [CrossRef]
37. Smolders, E.; Buysse, J.; Merckx, R. Growth Analysis of Soil-Grown Spinach Plants at Different N-Regimes. *Plant Soil* **1993**, *154*, 73–80. [CrossRef]
38. Zhang, J.; Wang, X.; Zhou, Q. Co-cultivation of Chlorella spp and tomato in a hydroponic system. *Biomass Bioenergy* **2016**, *97*, 132–138. [CrossRef]

39. van de Wiel, C.C.M.; van der Linden, C.G.; Scholten, O.E. Improving phosphorus use efficiency in agriculture: Opportunities for breeding. *Euphytica* **2016**, *207*, 1–22. [CrossRef]
40. Muñoz, P.; Flores, J.S.; Antón, A.; Montero, J.I. Combination of greenhouse and open-field crop fertigation can increase sustainability of horticultural crops in the Mediterranean region. *Acta Hortic.* **2017**, *1170*, 627–634. [CrossRef]

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Reusing Coir-Based Substrates for Lettuce Growth: Nutrient Content and Phytonutrients Accumulation

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Abstract: This research aimed to assess the influence of reusing coir-based substrates on growth, nutrient content, and phytonutrients accumulation in lettuce. The experiment included a new coir pith and four coir-based mixes (1) coir, biochar, and perlite; (2) coir, compost, and perlite; (3) coir, biochar, and pine bark; and (4) coir, compost, and pine bark. All mixes had been previously utilized to grow transplanted spinach and possessed identical ratios of 78:12:10% (*v*/*v*) among their components. Lettuce (*Lactuca sativa* L. cv. 'Godzilla') seedlings were transplanted into Styrofoam plant boxes. Each day, the planting boxes received a nutrient solution via drip irrigation. Plants grown in reused mixes had similar macronutrient concentrations as those grown in coir for the first time, except for N and K in the third mix. Plants grown in reused mixtures had similar yields as those in new coir. Lettuce heads yielded 4.6–4.9 kg/m^2, while plants grown in reused mixtures had equal or higher total phenols than those in new coir. Ascorbic acid content was higher in plants cultivated in reused mixes. Coir-based growing media can be reused for another short-cycle crop, like lettuce, without yield loss or phytonutrients decrease.

Keywords: *Lactuca sativa*; soilless system; short-cycle crops; municipal compost; biochar; total phenols; flavonoids; ascorbic acid; circular economy

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1. Introduction

In soilless culture, the reuse of substrates for cultivation is becoming a crucial issue due to the scarcity of resources, the need to reduce environmental impacts, continuous restrictions on the use of peat, rising demand for growing media components, and the increase in costs [1]. Maximizing the effective utilization of available resources offers a means to tackle their scarcity and diminish agriculture's carbon footprint [2]. One of the main objectives for agriculture in the coming ten years is to boost production system efficiency, promote sustainability, and optimize resource use efficiency [3]. Substrate culture, a widely adopted technique in vegetable crop production, is expected to increase in the future due to its numerous advantages over open-field-grown methods. These advantages include increased water and nutrient use efficiency, higher yields, and more precise pest and disease control [2]. Additionally, substrate culture offers a solution to address challenges such as reducing arable land and more frequent climate extremes [4,5]. One disadvantage of this technique is the disposal of the growing medium at the end of cultivation [6,7]. Therefore, reusing growing media is the most environmentally friendly

approach [4,6,8]. Furthermore, considering the ongoing restrictions on peat usage, reusing substrates becomes even more crucial.

The number of growing cycles during which a substrate can be reused depends on the substrate's nature and the crop [7]. The spent growing media's stability is an essential indicator for its reuse [8]. Organic substrates are subject to decomposition, interfering with their physical and chemical properties [9]. The rate of the degradation reactions largely determines the useful life of an organic substrate. It depends on the substrate type, irrigation management, time-dependent action of roots, microbial activity [10], etc. In previous studies, [11,12] reported that coir could be an alternative to peat to produce spinach. Ref. [13] reported that spinach production and quality in coir-base substrates (78%, v/v) with municipal solid organic compost, or biochar, in the percentage of 12 by volume and either with perlite or pine bark, at a volume ratio of 10%, were similar to those obtained in coir. The spinach growth period in these mixes was 32 days. Spinach is a short-cycle crop of 25 to 50 days. Thus, the time for the substrate to lose its physical stability is short. Physical stability is defined by [9] as the ability of a product to maintain its physical dimensions and properties. On the other hand, these substrates were predominantly composed of coir, whose stability varies but is generally good [14–16]. It still has the advantage of having relatively low shrinkage [17] and being slightly hydrophobic.

In mixes with biochar, due to its recalcitrant nature [18–20], decay may occur at a slower rate. On the other hand, perlite and pine bark also had good stability [14–16]. According to Lemaire et al. [21], the pine bark remained stable for eight months. Ref. [22] reported that the reutilization of substrates did not pose a problem when fertigation parameters were adjusted to the reused substrate properties.

It is of the utmost importance to meticulously evaluate and contemplate the possible hazards associated with substrate reuse, specifically in relation to the dissemination of soil-borne ailments [23]. It is imperative to adopt suitable procedures to guarantee that any reused substrate is thoroughly sterilized and devoid of any detrimental pathogens that may threaten plant growth or the ecosystem. Adding compost to mixes can help prevent soil-borne diseases. Compost has disease-suppressing properties that can be an effective strategy for reducing risk [24–26]. Biochar can also suppress plant diseases [27–29], reported that biochar mildly improved the survival of beneficial microorganisms in a mix with peat.

Strategies to reduce potential issues related to substrate stability, improve sustainability, and reduce production costs are key to reusing substrates for planting other short-term crops. Lettuce is a short-season vegetable intensively produced in Mediterranean countries and is known to be a good source of health-promoting compounds like phenols [30,31].

We hypothesize that coir-based substrate, used for spinach cultivation, can be reused to grow other short-cycle crops. We predicted that these substrates would maintain their physical and chemical properties adequate for growing lettuce, another short-cycle crop. Here, we investigated the impact of reused coir-based substrate on lettuce growth, nutrient content, and the accumulation of phytonutrients.

2. Materials and Methods

2.1. Growth Conditions and Substrates

The experiment was conducted in a greenhouse located at the "Herdade Experimental da Mitra" (38°57′ N, 8°32′ W), University of Évora, Portugal. The greenhouse was covered with polycarbonate and had no supplemental lighting or heating. Diurnal changes in air temperature inside the greenhouse at the plant canopy level ranged from 8 to 27 °C. Solar radiation ranged from 34 to 248 $W \cdot m^{-2} \cdot d^{-1}$.

The experiment included five substrates, coir (the control, used for the first time), and four mixes already used to grow transplanted spinach, whose cycle lasted 32 days.

The coir (100% coir pith) (Projar S.A., Spain) had a pH of 5.5–6.0, electrical conductivity (EC) > 1.5 dS m^{-1}, granulometry = 0–10 mm, total porosity = 95%, air (%, v/v) = 25, and CEC (meq/100 g) = 60–120.

The mixes described in detail in Machado et al. [11] were coir-based (78 percent coir by volume, 12 percent biochar or municipal organic compost collected selectively, and 10 percent perlite or pine bark). The initial physicochemical characteristics of these mixes (pH, EC, mass wetness, moisture content, total porosity, and bulk density) were also presented in [11]. Before lettuce planting, the EC in mixes with compost averaged 2.3 mS/m $\pm$ 0.3, and in other mixes and in coir, it averaged around 1.9 d*S*/m. The pH in the mix of coir, compost, and perlite was 6.9. In the other mixes and in coir, it averaged 6.3 $\pm$ 0.5. It was observed that there was no noticeable shrinkage in the mixes.

Lettuce (*Lactuca sativa* L. cv. 'Godzilla') type Batavia seedlings with green leaves were transplanted into Styrofoam plant boxes (100 $\times$ 25 $\times$ 10 cm) on 8 April 2021, 35 days after emergence. After the spinach *(Spinacia oleracea* L. cv. Tragopan) harvest, the reused mixes remained undisturbed in the boxes. The reused substrates were not subject to sanitation. The seedlings were spaced at 20 cm in a row in the center of the box, with a plant density of 16 plants m^{-2}.

Treatments were arranged in a complete randomized block design with five replicates. Two 8 L/h pressure-compensating and anti-drain emitters were placed in each planting box. The emitters were attached to four fine tubes with a 70 cm length and 5 mm diameter. Thus, two water emission points were inserted into the substrate near the plant base, one on each side of the row crop.

The irrigation schedule was optimized for coir. It was based on substrate volumetric water content at Styrofoam box control (coir), measured using a soil moisture probe (SM105T Delta devices, Burwell, UK), and the volume of water drained. The nutrient solution was applied three to eight times daily and averaged 10 to 20% drainage (leaching fraction) for each application.

A nutrient solution was injected continuously into the irrigation system throughout the growing cycle. The nutrient solution used contained 14 mmol L^{-1} NO_3-N, 6.3 mmol L^{-1} NH_4-N, 1.32 mmol L^{-1} P, 11 mmol L^{-1} K, 3.5 mmol L^{-1} Ca, 3.5 mmol L^{-1} Mg, 1.31 mmol L^{-1} S, 46 µmol L^{-1} B, 7.86 µmol L^{-1} Cu chelated by EDTA, 8.95 µmol L^{-1} Fe chelated by EDTA, 18.3 µmol L^{-1} Mn chelated by EDTA, 1 µmol L^{-1} Mo, 2 µmol L^{-1} Zn chelated by EDTA, 2.1 mmol L^{-1} Cl, and 0.7 mmol L^{-1} Na. The EC-value of the nutrient solution ranged over time: 2 $\pm$ 0.2 dS m^{-1} ((from transplanting to 12 days after transplanting (DAP)) and 2.5 $\pm$ 0.3 d*S* m^{-1} ((from 13 DAP until harvest (29 DAP)).

2.2. Measurements

The pH and EC_W of the drainage water from each box were measured weekly using a potentiometer (pH Micro 2000 Crison) and a conductivity meter (LF 330 WTW, Weilheim, Germany).

Lettuce plants (heads) were harvested at 29 DAP. Two lettuce plants (heads) from each box were washed, oven-dried at 70 °C for 2–3 days, weighed, and ground so that they would pass through a 40-mesh sieve. The ground samples were analyzed for N, P, K, Ca, Mg, Na, Fe, B, Mn, and Zn. The total N was analyzed using a combustion analyzer (Leco Corp., St. Josef, MI, USA). The K and Na were diagnosed by flame photometry (Jenway, Dunmow, UK). The P and B were analyzed using a UV/Vis spectrometer (Perkin Elmer Lamba 25). The remaining nutrients were analyzed using an atomic absorption spectrometer (Perkin Elmer, Inc., Shelton, CT, USA).

The leaf area of two plants was measured using a leaf area meter (LI-COR Model LI-3000A).

The head samples, including inner, middle, and outer leaves, were collected in a 2 cm thick disc obtained by cross-cutting at a height of 6 cm from the base and cutting with a knife. Samples of lettuce leaf discs weighing 1.000 g were macerated in a mortar and, then homogenized for 1 min in 8 mL of a methanol/water solution (80:20 (*v*/*v*), MW80 extract) [32] to determine the total content of phenolic compounds (TPC) [33], flavonoids [34], anthocyanins [35,36], ascorbate (AsA) [37], proline [38], and FRAP antioxidant activity [33].

After that, samples were centrifuged at 4 °C at 6440× *g* for 5 min in a centrífuge Hermle Z323 K. Aliquots of the methanol extracts were kept at −20 °C for further use.

Samples of lettuce leaf discs weighing 1.000 g from each treatment were macerated in a mortar and then homogenized in 8 mL of methanol:water solution (90:10 (*v*/*v*), MW90 extract) for 1 min. They were then centrifuged at 4 °C at 6440× *g* for 5 min. to determine the amount of photosynthetic pigment present. Chlorophyll a and b and carotenoids were quantified in aliquots of MW90-extract by UV-vis spectrophotometry [38].

Total phenolic compounds (TPCs) were determined following Bouayed et al. [33], using the Folin–Ciocalteau phenol reagent by reading the absorbance at 760 nm. TPC content was estimated using a calibration curve (GAE, n = 6 concentrations from 0 to 50 mg/L) and expressed as milligrams of gallic acid equivalent (GAE) per 100 g of fresh weight (FW).

A reaction mixture of 100 µL of MW80 extract, 20 µL of 10% $AlCl_3$, 500 mL of 1 M potassium acetate, and 380 µL of distilled water was prepared to determine the flavonoid content. After that, this combination was incubated for 30 min at 25 °C. Total flavonoid content was determined by measuring the absorbance at 420 nm, using an extinction coefficient of 0.004 μM^{-1} cm^{-1}, and expressed in mg of quercetin equivalent (QE) per 100 g of fresh weight [34].

A reaction mixture composed of 500 µL of MW80 extract, 500 µL of 50% ethanol (*v*/*v*), and 84 µL of 37% HCl was used to determine the total anthocyanin content [35]. After 30 min of incubation at 60°C, the absorbance of the mixture was measured at 530, 620, and 650 nm, and the absorbance of cyanidin-3-glycoside was estimated. The total anthocyanin content, expressed as mg of cyanidin-3-glycoside equivalent (C3GE) per 100 g of fresh weight, was calculated using the molar extinction coefficient of 34,300 M^{-1} cm^{-1} and the molar mass of 449.2 $gmol^{-1}$ [36].

For the determination of AsA content, each sample (extracts or standards suitably di-luted) was incubated in a mixture containing 5% TCA in ethanol, 0.4% H_3PO_4, 0.5% β-phenanthroline in ethanol, and 0.03% $FeCl_3$ in ethanol and warmed at 30 °C, for 90 min [37]. The absorbance of the Fe (II)–β-phenanthroline complex formed was read at 534 nm. AsA concentration was calculated from a calibration curve (ascorbic acid, n = 6 concentrations from 0 to 30 mg/L) freshly prepared.

The Free Pro content of MW80-extracts was determined using the acid ninhydrin reaction with the amino acid and reading the absorbance of the formed formazan at 546 nm. The concentration of proline was calculated using a calibration curve prepared from standard solutions of pure proline (L-proline, n = 6 concentrations between 0 and 20 mg/L) [38].

To determine the ferric-reducing antioxidant power (FRAP) of the lettuce extracts, a reaction mixture of 0.050 mL of the sample (plant extracts) or standards was mixed with 0.950 mL of the FRAP reagent. The absorbance change was read at 593 nm at 37 °C for 180 s. FRAP reagent was freshly prepared by mixing 300 mM acetate buffer pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM iron (III) chloride solution (10:1:1, *v/v/v*) at 37 °C. The antioxidant activity reported as milligrams of Trolox equivalents per 100 g FW was calculated using a calibration curve (Trolox solution, n = 8 concentrations from 0 to 1120 mg L^{-1}) [33].

Samples of 0.2500 g of spinach leaves were macerated in liquid N_2 and homogenized in 50 mM phosphate buffer pH 7.0 to determine the proline dehydrogenase (PDH) enzyme activity. This extract was centrifuged for 15 min at 15,000× *g* at 4 °C to obtain the supernatant, which was then collected and kept in aliquots at 20 °C (PB-extract) for later use [39,40]. PDH enzyme activity was measured following the reduction of NAD^+ at 340 nm at 30 °C for 180 s [40]. A reaction mixture containing 100 mM Na_2CO_3-$NaHCO_3$ buffer pH 10.3, 10 mM NAD^+, and PB-extract was used during the assay. The addition of 2 mM L-proline was used to initiate the reaction. Enzyme activity was estimated from the reaction curve slope (A_{340} vs. t) using the extinction coefficient of 6.22 mM^{-1} cm^{-1}. PDH activity was expressed in nmol min^{-1}/mg protein. According to the Lowry method [41],

the amount of water-soluble protein in the PB extract was evaluated using a calibration curve (bovine serum albumin, BSA; $n = 6$ concentrations from 0 to 200 mg/mL).

A Genesys 10S UV-Vis spectrophotometer was used for all spectrometric measurements.

2.3. Data Analysis

Data were analyzed using the analysis of variance (ANOVA I) using SPSS Statistics 25 software (Chicago, IL, USA), licensed to the University of Évora. Means were separated at the 5% level using Duncan's new multiple-range test.

3. Results and Discussion

3.1. Leachate pH and EC

The nutrient solution, irrigation scheduling, and substrate may affect the leachate fraction's pH and electrical conductivity (EC_W). In the present study, the differences in hydronium ions concentration and EC_W of drained water were related to substrate (Figure 1). It can affect the volume of water drained, cation exchange capacity, and pH buffering capacity. The average leachate pH was higher in mixes with perlite than in other substrates during the first three sample dates. The highest pH occurs in the coir, compost, and perlite mix.

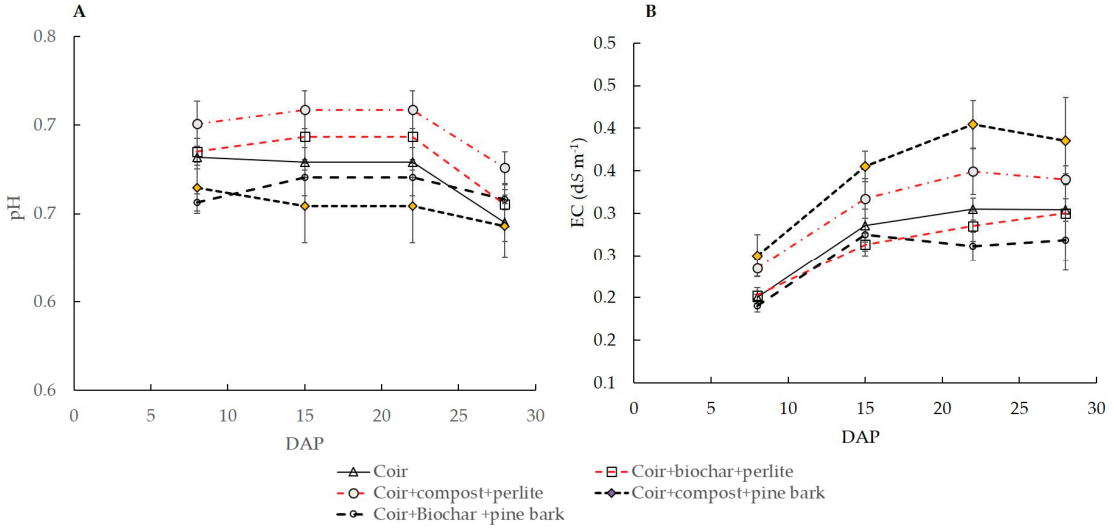

Figure 1. Effect of reused substrates on pH (**A**) and EC_W (**B**) in the drainage water. Each symbol represents the mean of five replicates, and the error bars represent ± 1 SE. DAP- days after transplanting.

The pH of this mixture ranged from 7.0 to 7.1, which is slightly higher than the nutritional solution's pH of 6.4 ± 0.3 and may negatively affect plant nutrition. In coir leachate, the pH was slightly higher than in the incoming nutrient solution. On the last sampling date, the pH of the drained water was not significantly affected by the mixes and ranged from 6.4 to 6.7.

The EC_W of leachate in the first sampling (8 DAP) was slightly higher in mixes with compost than in other mixes and coir (Figure 1). In the mix of coir, compost, and pine bark, the EC_W was 2.5 dS m^{-1}, 0.3 dS m^{-1} higher than the incoming solution (2 ± 0.2 dS m^{-1}). This slight increase could be attributed to this mix containing additional residual nutrients from the previous crop and/or a lower volume of water drainage. On the last three sampling dates in mix coir, compost, and pine bark, the EC_W was always higher than the EC of the nutrient solution (2.5 ± 0.3 dS m^{-1}). The EC_W in this mix reached

high values between 3.33 and 4.05 dS m^{-1}, i.e., 0.7 to 1.24 dS m^{-1}, which is higher than the EC of the nutrient solution (2.5 ± 0.3 dS m^{-1}).

This can indicate a tendency for salt buildup in the substrate. The EC_W was slightly lower on mixes with biochar than in coir or the incoming solution. In coir, the EC_W was 0.34 to 0.55 units higher than the nutrient solution, which may be considered adequate. As the differences may be related to the volume of water drained, the irrigation schedule must be adjusted for each mix.

3.2. Photosynthetic Pigments

Total chlorophyll, chlorophyll a and b contents of lettuce were different for different mixtures (Table 1). Plants grown in mixtures with pine bark had lower total chlorophyll and chlorophyll a content than those grown in mixtures with perlite (Table 1). Leaf chlorophyll b of the plants grown in reused substrates was not significantly different from those grown in coir. Leaf average chlorophyll b concentrations in the various plants ranged from 7.5 to 8.6 mg per 100 g of fresh weight (FW). Chlorophyll b levels were higher than chlorophyll a, which is not typical. However, this has also been observed in green and red lettuce cultivars by [42]. The Chla/Chlb ratio was high in coir, which may suggest that the plant is optimizing its photosynthetic capacity. The total chlorophyll content within this spectrum surpassed the findings of [43], who observed 1.0 to 1.5 mg.100 g^{-1} FW in lettuce cultivated via a floating culture system under varying nitrogen levels. However, the chlorophyll content remained notably inferior to the results of [44], who recorded a range of 26.8 to 52.3 mg 100 g^{-1} FW for lettuce exposed to diverse light intensities and nutrient solution concentrations. This could be due to various factors such as growing conditions, season, and genotype. For instance, the nutrient solution composition affected the chlorophyll content. [45].

Table 1. Effect of substrates on leaf photosynthetic pigments content and Chl a/Chl b ratio.

Substrate	Photosynthetic Pigments (mg 100 g^{-1} FW)				
	Total Chl	**Chl a**	**Chl b**	**Cc**	**Chl a/Chl b**
Coir	14.67 [†] a	6.51 a	8.17 ab	5.51	0.80 a
Coir + biochar + perlite	13.79 ab	5.18 ab	8.61 a	3.51	0.60 b
Coir + compost [1] + perlite	13.43 ab	5.37 b	8.06 ab	3.64	0.68 ab
Coir + biochar + pine bark	11.33 c	3.86 c	7.47 b	5.07	0.52 b
Coir + compost + pine bark	13.06 b	4.64 bc	8.42 ab	5.45	0.54 b
Significance	**	**	**	NS	**

[†] Means followed by different letters within a column are significantly different at $p \leq 0.05$. NS—nonsignificant, ** significant at $p < 0.01$ level, [1]—municipal solid organic compost collected selectively. FW—fresh weight. Total Chl—total chlorophyl; Chl a—chlorophyl a; Chl b—chlorophyl b; Cc—carotenoids; Chl a/Chl b—chlorophyl a/chlorophyl b ratio.

Leaf carotenoid content was not significantly affected by treatments. The average carotenoid content in the leaves ranged from 3.51 to 5.54 mg/100 g FW. Thus, the carotenoid content was lower than those reported by [46] (6.1–7.3 mg/100 g FW). As chlorophyll, the carotenoid content may be affected by several factors, including growing conditions, light intensity, temperature, genotype, leaf age, and position. The outer leaves generally have higher carotenoid levels than the inner leaves, which are exposed to higher light intensity, promoting carotenoid biosynthesis [47].

The lower carotenoid content observed in this study may be due to the dilution effect caused by the sample, which included inner, middle, and outer leaves.

3.3. Shoot Nutrient Concentration

Shoot macronutrient concentrations of plants from the reused mixes, except for N and K in the coir, biochar, and pine bark mix (4.34%), were not significantly different from those of plants grown in the new coir (Table 2). The low content of N and K may contribute

to lower levels of Chl a and b in mix coir, biochar, and perlite than the other substrates (Table 1). Shoot B, Zn, and Na content in plants grown in reused growing media were not significantly different from those grown in new coir (Table 2).

Table 2. Effect of reused substrates on shoot lettuce nutrient concentrations.

Substrate	Shoot Macronutrients (%)					Shoot Micronutrients ($\mu g \cdot g^{-1}$)				
	N	P	K	Ca	Mg	Fe	B	Mn	Zn	Na [1]
Coir	4.89 ab [†]	0.73	5.37 a	1.15	0.42	110.0 ab	23.3	34.4 b	59.4	0.62
Coir + biochar + perlite	4.93 a	0.68	5.57 a	1.07	0.38	107.5 ab	23.6	50.0 a	50.6	0.74
Coir + Compost [2] + perlite	4.72 ab	0.68	5.45 a	1.08	0.35	135.0 a	21.0	37.5 b	165.6	0.62
Coir + biochar + pine bark	4.78 b	0.77	4.48 b	1.20	0.42	91.3 b	23.9	55.0 a	84.4	0.74
Coir + Compost + pine bark	4.82 ab	0.74	5.57 a	1.25	0.41	58.8 c	20.9	29.4 b	45.6	0.62
Significance	*	NS	*	NS	NS	*	NS	***	NS	NS

[†] Means followed by different letters within a column are significantly different at $p \leq 0.05$. NS—nonsignificant. * and *** significant at $p < 0.05$ and 0.001 levels, respectively. [1]—Although sodium is not a micronutrient, it is included here for convenience. [2]—municipal solid organic compost collected selectively.

Plants grown in mixes that contained biochar had higher levels of Mn. Spinach, grown for the first time in these mixes, also increases shoot Mn content, as reported by [13]. Biochar may increase Mn availability in substrate solutions. Extractable Mn in biochar depends on the feedstock and the particle size [48]. Extractable Mn is high in particles smaller than 1 mm [48], and in the biochar used in this experiment, 42% of the particle, expressed as a percentage by weight, was <1 mm [49]. It could contribute to the Mn increased availability in the root medium. This emphasizes the significance of evaluating nutrient availability in the root medium of the blends for customizing nutrient solutions.

Pine bark in mixes led to a significant decrease in shoot iron content (Table 2). This could be due to iron immobilization caused by an increase in microbial activity resulting from the decomposition of pine bark, as highlighted by [50]. As previously mentioned, in future studies measuring nutrient availability in root medium is necessary. The shoot iron content was lower in plants cultivated in the coir, compost, and pine bark mix (58.8 $\mu g \cdot g^{-1}$). Despite the differences in the nitrogen, potassium, iron, and manganese concentrations, the plants grown in the different media did not show any visible signs of nutrient deficiency or toxicity.

Thus, the study suggests that reusing growing mixes can maintain shoot nutrient concentrations similar to those in coir used for the first time, with minor exceptions.

3.4. Plant Growth and Yield

Despite the low chlorophyll a in reused mixes with pine bark and low shoot K and Mn content in mix coir, biochar, and pine bark, the shoot dry weight, leaf number and area, and fresh yield were similar (Table 3). Thus, in terms of yield, the reuse of the mixes allowed yields similar to those obtained when coir was used for the first time. This is advantageous because pine bark can be locally sourced in Portugal. On the other hand, it may reduce the need for importing perlite, whose manufacturing process is resource-intensive and requires significant energy consumption [51], as well as lessen transportation-related greenhouse gas emissions. Refs. [22,52,53] also reported that the yields of some horticultural crops grown on reused organic substrates were comparable to or greater than those grown on new substrates. Lettuce plants grown on the different substrates exhibited no signs of disease during the growing cycle. Fresh yield average values ranged from 4.6 to 4.9 kg/m^2. These yields were similar to or higher than those obtained when lettuce was grown in a floating system [44], and greater than those obtained in soil in an open field and a greenhouse [54]. This finding indicates that, in terms of yield, the reuse of the mixes allowed yields similar to those obtained in coir used for the first time. On the other hand, carefully adjusting

the nutrient solution and the irrigation schedule to each mix to control the pH, EC_W, and volume of the leaching fraction could potentially lead to an increase in yield.

Table 3. Effect of reused substrates on shoot dry weight, number of leaves, leaf area, and head fresh weight yield.

Substrate	Shoot Dry Weight		Leaves	Leaf Area	Head Fresh Yield
	(g/Plant)	(%)	(n°/Plant)	(cm^2/Plant)	(kg/m^2)
Coir	11.0	3.7	25.0	5286.0	4.9
Coir + biochar + perlite	10.8	3.7	26.0	5155.5	4.8
Coir + Compost [1] + perlite	12.0	4.0	27.3	5182.2	4.7
Coir + biochar + pine bark	11.2	3.8	26.0	5122.4	4.6
Coir + Compost + pine bark	11.7	3.8	26.8	5246.3	4.8
Significance	NS	NS	NS	NS	NS

NS—nonsignificant, [1]—municipal solid organic compost collected selectively.

3.5. Phytonutrients Accumulation

The leaf total phenols of the plants grown in reused mixes were higher or equal to those grown in coir, used for the first time (Table 4). This may be due to different water availability, salinity, and pH in the root medium, as indicated by the EC_W and pH of the drained water. Water availability and salinity generally affect the total phenolic content in plants [55–57]. In lettuce, the electrical conductivity of the nutrient solution is associated with the biosynthesis of secondary metabolites, such as phenolic compounds [58,59].

Table 4. Effect of reused substrates on total phenols, anthocyanins, flavonoids, and ascorbic acid.

Substrate	TPC (mg GAE 100 g^{-1} FW) [1]	Flavonoids (mg QE 100 g^{-1} FW) [3]	Anthocyanins (mg C3GE 100 g^{-1} FW) [2]	Ascorbic Acid (mg 100 g^{-1} FW)
Coir	65.48 c[†]	3.19	0.66	1.21 c
Coir + biochar + perlite	138.9 a	3.88	0.60	1.17 c
Coir + compost + perlite	54.52 c	3.41	0.65	1.82 b
Coir + biochar + pine bark	65.38 c	3.23	0.66	2.85 a
Coir + compost + pine bark	92.25 b	3.45	0.69	1.62 b
Significance	***	NS	NS	**

[†] Means followed by different letters within a column are significantly different at $p \leq 0.05$. NS—nonsignificant. ** and *** significant at $p < 0.01$ and 0.001 levels, respectively. [1] TPC—total phenolic compounds GAE—galic acid equivalent. [2] G3GE—cianidine-3-glicoside equivalent. [3] QE—quercetine equivalente.

The highest total phenols occurred in plants cultivated in mixes of coir, biochar, and perlite (138.96 mg GAE 100 g^{-1} FW) and coir, compost, and pine bark (92.3 mg GAE 100 g^{-1} FW).

The average leaf total phenol of plants ranged from 54.52 to 138.96 mg GAE/100 g^{-1} FW. Leaf total phenol content in lettuce varies with several factors such as genotype, growing conditions, harvest time, leaf position, etc. [31,60,61]. The outer leaves have the highest phytonutrient content and antioxidant properties [42,47,60]. Despite all leaves being mixed in the study samples, the leaf total phenols values were within the range reported by Kim et al. [31] (50–270 mg GAE g^{-1} FW), Llorach et al. [30] (18.2–571.2 mg GAE g^{-1} FW) for different lettuce varieties, and Petropoulos et al. [62] for green lettuce (18 to 203 mg GAE/100 g^{-1} FW).

Leaf averages of flavonoids and anthocyanin contents of the plants grown in reused substrates did not differ significantly from those grown in the coir used for the first time (Table 4).

The leaf ascorbic acid (AsA) content of the plants grown in the reused mixes was higher or similar to those grown in coir used for the first time. Leaf AsA in the different treatments ranged from 1.17 to 2.85 mg/100 g FW). These were lower or similar to the lower end of the

range reported for lettuces with green leaves by Cozzolino et al. [61] (3.0–19.3 mg/100 g FW), Jibril et al. [63] (2.27–6.91 mg/100 g FW), and Llorach et al. [30] for lettuces of different leaf colors (2.8–9.5 mg/100 g FW). The lower values may be related to the genotype, growth conditions, and sampling method. Leaf AsA ranged with leaf position [64,65], and in the present study, leaf AsA represents the average of different leaves. The low AsA content may also be related to the environmental conditions in the greenhouse. Light intensity was low in the greenhouse, not only due to the time of year (early spring) but also because of the opacity of the plastic cover film used in our greenhouse. The condition in substrates affected leaf proline, which was lower in reused mixes than in coir used for the first time (Figure 2A).

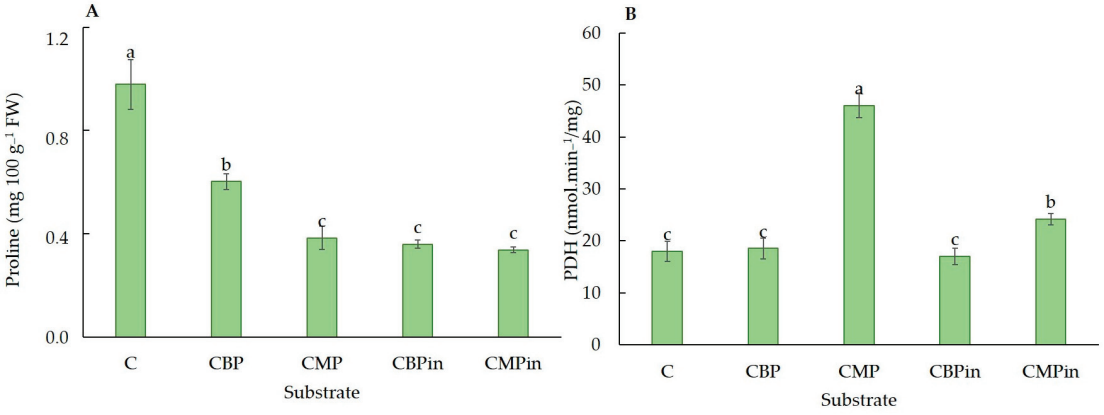

Figure 2. Effect of reused substrates on proline content (**A**) and proline dehydrogenase activity (**B**), (C), municipal compost (M), biochar (B), perlite (P), or pine bark (Pin). Each bar represents the mean of five replicates, and the error bars represent ± SE. Means with different letters are significantly different at $p < 0.05$.

Proline accumulation in plants [66], is an essential component of plant defense mechanisms [67]. One of the most effective osmoregulatory mechanisms at the molecular level involves the buildup of intracellular proline to lower water activity within the cytoplasm [68]. Overall, the water supply and the water condition in the substrate are significantly related to the proline content in plants [68,69]. In the present study, lettuce leaf proline content of the different treatments ranged from 0.38 to 1.00 mg/100 g FW (Figure 2A). These values were lower than those reported by Machado et al. [70] in leaf blades of spinach grown in the substrate (1.9 to 2.5 mg/100 g) and by Machado et al. [71] in coriander grown in soil (14.5–49.7 mg/100 g). The lower proline content in lettuce may be due to species since proline content is species-dependent [68,69]. The lower values of proline may also be linked to favorable growing conditions [72], indicating that the plants in the mixes were grown under favorable conditions.

Leaf proline dehydrogenase activity was higher in mixes with municipal compost, regardless of whether they had perlite or pine bark (Figure 2B). Proline dehydrogenase is an enzyme involved in the breakdown of proline into pyrroline-5-carboxylate (P5C). This process is part of the proline degradation pathway and is often associated with plant stress responses. Elevated proline dehydrogenase activity can also be triggered by variations in water availability and salinity [73]. Leaf PDH activity ranged from 18.00 to 47.00 nmol min^{-1}/mg protein.

3.6. Antioxidant Activity

The antioxidant activity measured by the ability of lettuce leaf extracts to reduce iron (Fe^{3+}) FRAP was higher in coir and in the mix of coir, compost, and perlite (Figure 3). Leaf FRAP values in the substrates range from 9.06 to 13.9 mg TEAC g^{-1} FW). These were much

lower than those observed by Llorach et al. [30] (98.2 to 323.4 mg TEAC g^{-1} FW) in three green varieties of lettuce.

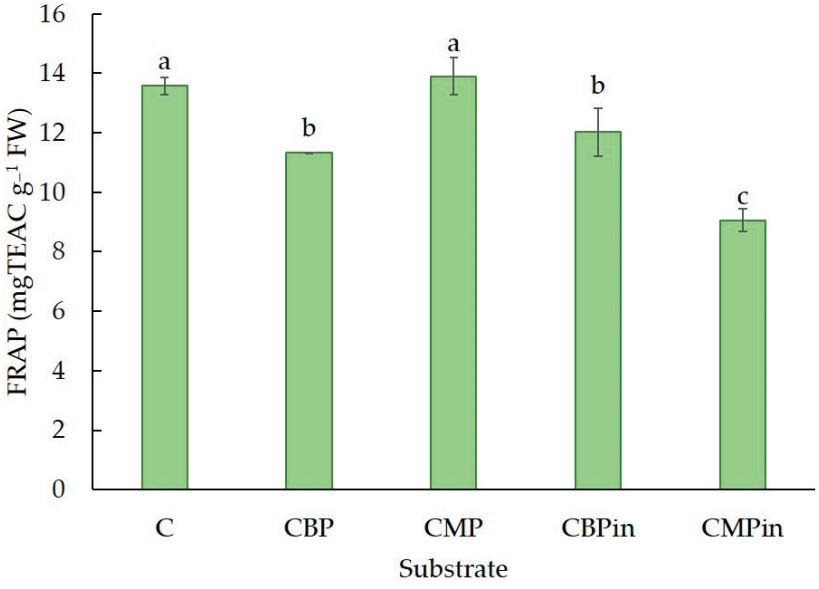

Figure 3. Effect of reused substrates on antioxidant activity estimated by FRAP, (C), municipal compost (M), biochar (B), perlite (P), or pine bark (Pin). Each bar represents the mean of five replicates and the errors bars represent ± SE. Means with different letters are significantly different at ($p < 0.05$).

Despite the observed effects on proline content, proline dehydrogenase activity, and antioxidant activity (FRAP), their magnitude was insufficient to affect the lettuce yield.

The differences observed in proline content, proline dehydrogenase activity, and antioxidant activity (FRAP) could potentially be reduced by optimizing irrigation scheduling and fertilization for each mix.

On the other hand, as the levels of total phenols, flavonoids, anthocyanins, and ascorbic acid in the lettuce leaves were either higher or comparable to those grown in coir used for the first time, this indicates that the reuse of coir-based substrates did not result in a decrease in yield and the product quality of lettuce.

4. Conclusions

The results show that coir-based growing media mixed with 12% compost or biochar and 10% perlite or pine bark, after its use in growing spinach, can still be successfully utilized for cultivating another short-cycle crop lettuce. Lettuce yield in reused substrates ranged from 4.6 to 4.8 kg/m^2, equal to the yield obtained in coir (4.9 kg/m^2) used for the first time.

The shoot nitrogen, phosphorus, potassium, calcium, and magnesium macronutrient concentrations, except in the coir, biochar, and pine bark mix, did not significantly differ between the reused and coir. Furthermore, the accumulation of total phenols, flavonoids, anthocyanins, and AsA in the leaves of plants grown on reused substrates was similar or even higher compared to those grown on coir used for the first time. Globally, the coir, biochar, and perlite mix allowed for better crop performance.

As the substrates reused did not undergo a sanitization procedure, it is strongly recommended that agricultural practitioners adopt a vigilant approach towards overseeing the growth and development of the prior crop and undertake thorough bioassays in case of

uncertainties before considering substrate reuse. This proactive measure aims to preclude any unforeseen repercussions that might arise from the reuse, thereby ensuring the optimal outcome of subsequent cultivation.

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References

1. Atzori, G.; Pane, C.; Zaccardelli, M.; Cacini, S.; Massa, D. The role of peat-free organic substrates in the sustainable management of soilless cultivations. *Agronomy* **2021**, *11*, 1236. [CrossRef]
2. Gonnella, M.; Renna, M. The Evolution of soilless systems towards ecological sustainability in the perspective of a circular economy. Is it really the opposite of organic agriculture? *Agronomy* **2021**, *11*, 950. [CrossRef]
3. Tüzel, Y.; Bertschinger, L. Future direction and opportunities of horticultural research. *Chron. Hortic.* **2020**, *60*, 9–19.
4. Gruda, N.S. Increasing sustainability of growing media constituents and stand-alone substrates in soilless culture systems. *Agronomy* **2019**, *9*, 298. [CrossRef]
5. Gruda, N.S. Advances in soilless culture and growing media in today's horticulture—An Editorial. *Agronomy* **2022**, *12*, 2773. [CrossRef]
6. Diara, C.; Incrocci, L.; Pardossi, A.; Minuto, A. Reusing greenhouse growing media. *Acta Hortic.* **2012**, *927*, 793–800. [CrossRef]
7. Pardossi, A.; Carmassi, G.; Diara, C.; Incrocci, L.; Maggini, R.; Massa, D. *Fertigation and Substrate Management in Closed Soilless Culture*; University of Pisa: Pisa, Italy, 2011.
8. Vandecasteele, B.; Blindeman, L.; Amery, F.; Pieters, C.; Ommeslag, S.; Loo, K.V.; de Tender, C.; De bode, J. Grow-Store-steam -repeat: Reuse of spent growing media for circular cultivation of *Chrysanthemum*. *J. Clean. Prod.* **2020**, *276*, 124128. [CrossRef]
9. Caron, J.; Zheng, Y. Glossary of terms and basic characteristics to be reported in scientific publications on growing media. *Acta Hortic.* **2021**, *1317*, 55–64. [CrossRef]
10. Kerloch, E.; Michel, J.-C. Pore tortuosity and wettability as main characteristics of the evolution of hydraulic properties of organic growing media during cultivation. *Vadose Zone J.* **2015**, *14*, vzj2014-11. [CrossRef]
11. Machado, R.M.; Alves-Pereira, I.; Ferreira, R.; Gruda, N.S. Coir an alternative to peat—Effects on plant growth, phytochemical accumulation, and antioxidant power of spinach. *Horticulturae* **2021**, *7*, 127. [CrossRef]
12. Barcelos, C.; Machado, R.M.; Alves-Pereira, I.; Ferreira, R.; Bryla, D.R. Effects of substrate type on plant growth and nitrogen and nitrate concentration in spinach. *Int. J. Plant Biol.* **2016**, *7*, 6325. [CrossRef]
13. Machado, R.; Alves-Pereira, I.; Morais, C.; Alemão, A.; Ferreira, R. Effects of coir-based growing medium with municipal solid waste compost or biochar on plant growth, mineral nutrition, and accumulation of phytochemicals in spinach. *Plants* **2022**, *11*, 1893. [CrossRef] [PubMed]
14. Savvas, D.; Gruda, N. Application of soilless culture technologies in the modern greenhouse industry—A review. *Eur. J. Hortic. Sci.* **2018**, *83*, 280–293. [CrossRef]
15. Gruda, N.; Bragg, N. Developments in alternative organic materials as growing media in soilless culture systems. In *Advances in Horticultural Soilless Culture*; Gruda, N., Ed.; Burleigh Dodds Science Publishing Limited: Cambridge, UK, 2021; ISBN 13 9781786764355.
16. Gruda, N.S.; Hirschler, O.; Stuart, J. Peat reduction in horticulture—An overview of Europe. *Acta Hortic.* 2023, *in print*.
17. Abad, M.; Noguera, P.; Noguera, V.; Roig, A.; Cegarra, J.; Paredes, C. Reciclado de residuos orgánicos y su aprovechamiento como sustratos de cultivo. *Acta Hortic.* **1997**, *19*, 92–109.
18. Woolf, D.; Amonette, J.E.; Street-Perrott, F.A.; Lehmann, J.; Joseph, S. Sustainable biochar to mitigate global climate change. *Nat. Commun.* **2010**, *1*, 56. [CrossRef] [PubMed]
19. Yang, Y.; Sun, K.; Han, L.F.; Chen, Y.L.; Liu, J.; Xing, B.S. Biochar stability and impact on soil organic carbon mineralization depend on biochar processing, aging and soil clay content. *Soil Biol. Biochem.* **2022**, *169*, 108657. [CrossRef]

20. Prasad, M.; Tzortzakis, N.; McDaniel, N. Chemical characterization of biochar and assessment of the nutrient dynamics by means of preliminary plant growth tests. *J. Environ. Manag.* **2018**, *216*, 89–95. [CrossRef] [PubMed]
21. Lemaire, F.; Dartigues, A.; Riviere, L.M. Properties of substrates with ground pine bark. *Symp. Substr. Hortic. Other Soils Situ* **1979**, *99*, 67–80. [CrossRef]
22. Urrestarazu, M.; Mazuela, P.C.; Martínez, G.A. Effect of substrate reutilization on yield and properties of melon and tomato crops. *J. Plant Nutri.* **2008**, *31*, 2031–2043. [CrossRef]
23. Schnitzler, W.H. Pest and disease management of soilless culture. In Proceedings of the South Pacific Soilless Culture Conference-SPSCC, Palmerston North, New Zealand, 10–13 February 2003; pp. 191–203.
24. Pascual, J.A.; Garcia, C.; Hernandez, T.; Lerma, S.; Lynch, J.M. Effectiveness of municipal waste compost and its humic fraction in suppressing *Pythium Ultimum*. *Microb. Ecol.* **2002**, *44*, 59–68. [CrossRef]
25. Raviv, M. Recent advances in soil-borne disease control using suppressive media. *Acta Hortic.* **2007**, *819*, 125–134. [CrossRef]
26. Neher, D.A.; Hoitink, H.A.; Biala, J.; Rynk, R.; Black, G. Compost use for plant disease suppression. In *the Composting Handbook*; Rynk, R., Black, G., Biala, J., Bonhotal, J., Cooperband, L., Gilbert, J., Schwarz, M., Eds.; Academic Press: London, UK, 2022; pp. 847–878. [CrossRef]
27. Yang, Y.; Chen, T.; Xiao, R.; Chen, X.; Zhang, T. A quantitative evaluation of the biochar's influence on plant disease suppress: A global meta-analysis. *Biochar* **2022**, *4*, 43. [CrossRef]
28. Lacomino, G.; Idbella, M.; Laudonia, S.; Vinale, F.; Bonanomi, G. The suppressive effects of biochar on above-and belowground plant pathogens and pests: A review. *Plants* **2022**, *11*, 3144. [CrossRef]
29. Blok, C.; Van der Salm, C.; Hofland-Zijlstra, J.; Streminska, M.; Eveleens, B.; Regelink, I.; Fryda, L.; Visser, R. Biochar for horticultural rooting media improvement: Evaluation of biochar from gasification and slow pyrolysis. *Agronomy* **2017**, *7*, 6. [CrossRef]
30. Llorach, R.; Martínez-Sánchez, A.; Tomás-Barberán, F.A.; Gil, M.I.; Ferreres, F. Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. *Food Chem.* **2008**, *108*, 1028–1038. [CrossRef] [PubMed]
31. Kim, M.J.; Moon, Y.; Tou, J.C.; Mou, B.; Waterland, N.L. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). *J. Food Compos. Anal.* **2016**, *49*, 19–34. [CrossRef]
32. Lichtenthaler, H.K.; Buschmann, C. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Curr. Protoc. Food Anal. Chem.* **1987**, *4*, 3.1–3.8. [CrossRef]
33. Bouayed, J.; Hoffmann, L.; Bohn, T. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. *Food Chem.* **2011**, *128*, 14–21. [CrossRef]
34. Pourmorad, F.; Hosseinimehr, S.J.; Shahabimajd, N. Antioxidant activity, phenol and flavonoid contents of some selected iranian medicinal plants. *Afr. J. Biotechnol.* **2006**, *5*, 1142–1145.
35. Siegelman, H.W.; Hendricks, S.B. Photocontrol of anthocyanin synthesis. *Apple Skin. Plant Physiol.* **1958**, *33*, 185–190. [CrossRef] [PubMed]
36. Paiva, E.A.S.; Isaias, R.M.D.S.; Vale, F.H.A.; Queiroz, C.G.D.S. The influence of light intensity on anatomical structure and pigment contents of *Tradescantia pallida* (Rose) Hunt. cv. purpurea Boom (*Commelinaceae*) leaves. *Braz. Arch. Biol. Technol.* **2003**, *46*, 617–624. [CrossRef]
37. Cai, W.M.; Tang, Z.C. Plant tolerance physiology. In *Experimental Guide for Modern Plant Physiology*, 1st ed.; Tang, Z.C., Ed.; Science Press: Beijing, China, 1999; pp. 315–316.
38. Bates, L.S. Rapid determination of free proline for water stress studies. *Plant Soil* **1973**, *39*, 205–207. [CrossRef]
39. Lake, B. Preparation and characterization of microsomal fractions for studies of xenobiotic metabolism. In *Biochemical Toxi-Cology: A Practical Approach*; Snell, K., Mullock, B., Eds.; IRL Press: Oxford, UK, 1987; pp. 183–215.
40. Costilow, R.N.; Cooper, D. Identity of proline dehydrogenase and delta1-pyrroline-5carboxylic acid reductase in Clostridium sporogenes. *J. Bacteriol.* **1978**, *134*, 139–146. [CrossRef]
41. Lowry, O.H.; Roseburg, N.J.; Farr, A.L.; Randell, R.J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275. [CrossRef]
42. Ozgen, S.; Sekerci, S. Effect of leaf position on the distribution of phytochemicals and antioxidant capacity among green and red lettuce cultivars. *Span. J. Agric. Res.* **2011**, *9*, 801–809. [CrossRef]
43. Petropoulos, S.A.; Chatzieustratiou, E.; Constantopoulou, E.; Kapotis, G. Yield and quality of lettuce and rocket grown in floating culture system. *Not. Bot. Horti. Agrobo.* **2016**, *44*, 603–612. [CrossRef]
44. Song, J.; Huang, H.; Hao, Y.; Song, S.; Zhang, Y.; Su, W.; Liu, H. Nutritional quality, mineral and antioxidant content in lettuce affected by interaction of light intensity and nutrient solution concentration. *Sci. Rep.* **2020**, *10*, 2796. [CrossRef] [PubMed]
45. Sapkota, S.; Sapkota, S.; Liu, Z. Effects of nutrient composition and lettuce cultivar on crop production in hydroponic culture. *Horticulturae* **2019**, *5*, 72. [CrossRef]
46. Tsouvaltzis, P.; Kasampalis, D.S.; Aktsoglou, D.C.; Barbayiannis, N.; Siomos, A.S. Effect of reduced nitrogen and supplemented amino acids nutrient solution on the nutritional quality of baby green and red lettuce grown in a floating system. *Agronomy* **2020**, *10*, 922. [CrossRef]

47. Baslam, M.; Morales, F.; Garmendia, I.; Goicoechea, N. Nutritional quality of outer and inner leaves of green and red pigmented lettuces (*Lactuca sativa* L.) consumed as salads. *Sci. Hortic.* **2013**, *151*, 103–111. [CrossRef]
48. Prasad, M.; Chrysargyris, A.; McDaniel, N.; Kavanagh, A.; Gruda, N.S.; Tzortzakis, N. Plant nutrient availability and pH of biochar and their fractions, with the possible use as a component in a growing media. *Agronomy* **2020**, *10*, 10. [CrossRef]
49. Martins, T.C.; Machado, R.M.; Alves-Pereira, I.; Ferreira, R.; Gruda, N.S. Coir-Based GrowingMedia with municipal compostand biochar and their impacts on growth and some quality parameters in lettuce Seedlings. *Horticulturae* **2023**, *9*, 105. [CrossRef]
50. Carlile, W.R.; Raviv, M.; Prasad, M. Organic soilless media components. In *Soilless Culture—Theory and Practice*, 2nd ed.; Raviv, M., Lieth, J.H., Bar-Tal, A., Eds.; Elsevier: London, UK, 2019; pp. 303–378.
51. Toboso-Chavero, S.; Madrid-López, C.; Villalba, G.; Durany, X.G.; Hückstädt, A.B.; Finkbeiner, M.; Lehmann, A. Environmental and social life cycle assessment of growing media for urban rooftop farming. *Int. J. Life Cycle Assess* **2021**, *26*, 2085–2102. [CrossRef]
52. Çelikel, G.; Caglar, G. The effects of reusing different substrates on the yield and earliness of cucumber on autumn growing period. *Acta Hortic.* **1997**, *492*, 259–264. [CrossRef]
53. Çelikel, G. Influence of reusing substrates on the yield and earliness of eggplant in soilless culture. *Acta Hortic.* **1999**, *491*, 357–362. [CrossRef]
54. Hernández, T.; Chocano, C.; Moreno, J.L.; García, C. Use of compost as an alternative to conventional inorganic fertilizers in intensive lettuce (*Lactuca sativa* L.) crops—Effects on soil and plant. *Soil Till. Res.* **2016**, *160*, 14–22. [CrossRef]
55. Giordano, M.; Petropoulos, S.A.; Rouphael, Y. Response and defense mechanisms of vegetable crops against drought, heat and salinity stress. *Agriculture* **2021**, *11*, 463. [CrossRef]
56. Kumar, M.; Tak, Y.; Potkule, J.; Choyal, P.; Tomar, M.; Meena, N.L.; Kaur, C. Phenolics as plant protective companion against abiotic stress. In *Plant Phenolics in Sustainable Agriculture*; Lone, R., Shuab, R., Kamili, A., Eds.; Springer: Singapore, 2020; Volume 1, pp. 277–308. [CrossRef]
57. Santander, C.; Vidal, G.; Ruiz, A.; Vidal, C.; Cornejo, P. Salinity eustress increases the biosynthesis and accumulation of phenolic compounds that improve the functional and antioxidant quality of red lettuce. *Agronomy* **2022**, *12*, 598. [CrossRef]
58. Kim, H.J.; Fonseca, J.M.; Choi, J.H.; Kubota, C.; Kwon, D.Y. Salt in irrigation water affects the nutritional and visual properties of romaine lettuce (*Lactuca sativa* L.). *J. Agric. Food Chem.* **2008**, *56*, 3772–3776. [CrossRef]
59. Sgherri, C.; Pérez-López, U.; Micaelli, F.; Miranda-Apodaca, J.; Mena-Petite, A.; Muñoz-Rueda, A.; Quartacci, M.F. Elevated CO_2 and salinity are responsible for phenolics-enrichment in two differently pigmented lettuces. *Plant Physiol. Biochem.* **2017**, *115*, 269–278. [CrossRef]
60. Pérez-López, U.; Sgherri, C.; Miranda-Apodaca, J.; Micaelli, F.; Lacuesta, M.; Mena-Petite, A.; Quartacci, M.F.; Muñoz-Rueda, A. Concentration of phenolic compounds is increased in lettuce grown under high light intensity and elevated CO_2. *Plant Physiol. Bioch.* **2018**, *123*, 233–241. [CrossRef]
61. Cozzolino, E.; Giordano, M.; Fiorentino, N.; El-Nakhel, C.; Pannico, A.; di Mola, I.; Mori, M.; Kyriacou, M.C.; Colla, G.; Rouphael, Y. Appraisal of biodegradable mulching films and vegetal-derived biostimulant application as eco-sustainable practices for enhancing lettuce crop performance and nutritive value. *Agronomy* **2020**, *10*, 3. [CrossRef]
62. Petropoulos, S.A.; Ferreira, C.F.R.; Barros, L. *Phytochemicals in Vegetables: A Valuable Source of Bioactive Compounds*; Bentham Science Publishers: Sharjah, United Arab Emirates, 2018; ISBN 10 1681087405.
63. Jibril, S.A.; Hassan, S.A.; Ishak, C.F.; Wahab, P.E.M. Cadmium Toxicity Affects Phytochemicals and Nutrient Elements Composition of Lettuce (*Lactuca sativa* L.). *Adv. Agric.* **2017**, *2017*, 1236830. [CrossRef]
64. Siomos, A.S.; Papadopoulou, P.P.; Dogras, C.C.; Vasiliadis, E.; Dosas, A.; Georgiou, N. Lettuce composition as affected by genotype and leaf position. *II Balkan Symp. Veg. Potatoes* **2000**, *579*, 635–639. [CrossRef]
65. Viacava, G.E.; Gonzalez-Aguilar, G.; Roura, S.I. Determination of phytochemicals and antioxidant activity in butterhead lettuce related to leaf age and position. *J. Food Biochem.* **2014**, *38*, 352–362. [CrossRef]
66. Trovato, M.; Mattioli, R.; Costantino, P. Multiple roles of proline in plant stress tolerance and development. *Rend. Lincei. Sci. Fis. Nat.* **2008**, *19*, 325–346. [CrossRef]
67. Spormann, S.; Nadais, P.; Sousa, F.; Pinto, M.; Martins, M.; Sousa, B.; Fidalgo, F.; Soares, C. Accumulation of proline in plants under contaminated soils—Are We on the Same Page? *Antioxidants* **2023**, *12*, 666. [CrossRef]
68. Gruda, N.; Schnitzler, W.H. The effect of water supply on bio-morphological and plant-physiological parameters of tomato transplants cultivated in wood fiber substrate. *J. Appl. Bot.* **2000**, *74*, 233–239.
69. Gruda, N.; Schnitzler, W.H. Schnitzler: The effect of water supply of seedlings, cultivated in different substrates and raising systems on the bio-morphological and plant-physiological parameters of lettuce. In German: Einfluss der Wasserversorgung von Jungpflanzen angezogen in verschiedenen Substraten und Anzuchtsystemen auf biomorphologische und physiologische Merkmale von Kopfsalat. *J. Appl. Bot.* **2000**, *74*, 240–247.
70. Machado, R.M.A.; Alves-Pereira, I.; Ferreira, R.M.A. Plant growth, phytochemical accumulation and antioxidant activity of substrate-grown spinach. *Heliyon* **2018**, *4*, e00751. [CrossRef] [PubMed]
71. Machado, R.M.A.; Alves-Pereira, I.; Faty, Y.; Perdigão, S.; Ferreira, R. Influence of nitrogen sources applied by fertigation to an enriched soil with organic compost on growth, mineral nutrition, and phytochemicals content of coriander (*Coriandrum sativum* L.) in two successive harvests. *Plants* **2022**, *11*, 22. [CrossRef] [PubMed]

72. Hayat, S.; Hayat, Q.; Alyemeni, M.N.; Wani, A.S.; Pichtel, J.; Ahmad, A. Role of proline under changing environments: A review. *Plant Signal Behav.* **2012**, *7*, 1456–1466. [CrossRef] [PubMed]
73. Szepesi, Á.; Szollosi, R. Mechanism of proline biosynthesis and role of proline metabolism enzymes under environmental stress in plants. In *Plant Metabolites and Regulation under Environmental Stress*; Academic Press: Cambridge, MA, USA, 2018; pp. 337–353. [CrossRef]

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Article

Biodegradable Food Packaging of Wild Rocket (*Diplotaxis tenuifolia* L. [DC.]) and Sea Fennel (*Crithmum maritimum* L.) Grown in a Cascade Cropping System for Short Food Supply Chain

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Abstract: The environmental impact of food products is significantly affected by their packaging. Therefore, this study aimed to assess the effect of PLA (polylactic acid) film, as an alternative to petroleum-based bags, on the shelf-life of fresh-cut wild rocket and sea fennel grown in a cascade cropping system (CCS). To this end, wild rocket (main crop) was cultivated using either peat or compost as a growing medium. Sea fennel (secondary crop) was subsequently grown in a floating system with leachates from the primary crop as a nutrient solution. The leaves of both crops were harvested and packaged in OPP- (oriented polypropylene) or PLA-based bags and stored for 7 days at 4 °C. The leaves of wild rocket and sea fennel showed lower dehydration and lower respiration when compost was used as a growing medium or leachate. Wild rocket in compost increased in nitrate and vitamin C contents at harvest while leachates had scarce influence on their contents in sea fennel. After storage, regardless of the crop, no relevant detrimental changes were observed on leaves packaged with PLA, being a product microbiologically safer when compared to OPP. The bag type had almost no influence on most relevant phytochemical compounds. In conclusion, the use of a PLA-based film on minimally processed wild rocket and sea fennel leaves is a sustainable alternative to petroleum-based plastic for a short food supply chain.

Keywords: sustainable agriculture; growing media; fresh-cut; postharvest; compostable package; food distribution; phytochemical compound

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1. Introduction

Nowadays, innovative urban and peri-urban plant production systems, especially in developed economies, are gaining popularity [1], particularly those that can increase resource efficiency [2], which achieves ecosystem benefits and mitigates climate change [3]. The enlarging of the supply chain that has developed over the years has produced effects from ecological, economic, and social points of view. Thus, some years ago, the short food supply chain appeared as a new concept and has expanded significantly, particularly in European countries, as it is supported by EU policies [4]. These production systems with increased circularity can both enhance food security in cities and reduce the environmental impact that results from long transportation distances, which involve elevated energy consumption for refrigeration and the use of chemical refrigerants [5].

A cascade cropping system (CCS) that collect the leachate of a substrate from a main crop to cultivate a secondary crop is of particular interest in naturally dry and nutrient-poor environments [6]. Closing the nutrient cycle in soilless systems increases resource efficiency by reducing fertiliser and water consumption [7]. In addition, any reduction in the environmental impact of agriculture also translates into a clear interest from consumers [8].

The search for organic materials that can be used as peat alternatives has become increasingly important, with the compost from agricultural and agri-food industry waste being an option as far as it is considered environmentally friendly and in agreement with the circular economy. Furthermore, compost can be an important source of biofertilisation and bio-stimulation of crops because of its nutritional elements, humic substances, and hormone-like molecules released by microorganisms [9]. Additionally, composts made from specific agro-industrial wastes have been proven to suppress a wide variety of soil-borne plant pathogens, including some oomycetes in baby leaf vegetables [10].

The commercialisation of leafy vegetables, including baby leaf vegetables and microgreens, is of increasing interest, offering customers convenient products rich in healthy bioactive compounds; one example is wild rocket (*Diplotaxis tenuifolia* (L.) DC.), an important component of ready-to-eat salads [11]. In addition to its distinct taste and peppery flavour, the potential health benefits associated with leafy phytochemicals, such as flavonoids and glucosinolates, have been recently addressed [12]. Likewise, but to a lesser extent, sea fennel (*Crithmum maritimum* L.), widely distributed throughout the Mediterranean and Atlantic seacoasts [13], has generated interest due to its desirable organoleptic attributes and health-promoting properties, including a relatively high content of fatty acids, predominantly linolenic and linoleic acids, vitamin C, and carotenoids [14,15]. Fresh leaves of this plant are versatile and can be consumed in various ways, including in salads, soups, sauces, and as a spice, particularly in fish-based dishes [16].

One of the most interesting issues concerning fresh food products is packaging, as this is one of the major factors that contribute to the overall environmental impact of products and represents one of the greatest variables affecting the sustainability of the supply chain [17]. Leafy vegetables are typical of the fresh-cut industry with petroleum-based films (e.g., polypropylene) commonly used for their packaging. However, these materials are related to global warming, ozone depletion, and non-renewable energy [18]. Millions of tons of nondegradable packages ultimately end up deposited in landfills, even when the worldwide recycling rates are increasing.

To reduce the environmental impact of plastic bags, polylactic acid (PLA) bags are being used as substitutes for packaging fresh produce due to their properties, even when they present some limitations that might prevent them from being competitive with conventional plastics [19]. PLA is a kind of biodegradable thermoplastic polymer, recyclable, compostable, produced from renewable sources (mainly from starch feedstocks), and approved for contact with food [18]. PLA is a versatile material, being thermo-sealed, a gas barrier, UV resistant, biocompatible, elastic, rigid, and hydrophobic [20,21]. PLA can retain the beneficial material characteristics of petrochemical-based packages while allowing for a transition towards a circular economy by reducing fossil resource usage. The current bioplastic market accounts for less than 1% of the entire plastic packaging market [22]. However, before large-scale systemic changes are adopted, full environmental evaluations should be considered. The physical and mechanical properties of PLA-based packages are continuously improved to meet the requirements of different commodities. Composites based on biodegradable compounds can be helpful for preserving freshness and retarding microbial spoilage. Recent research has demonstrated that porous biodegradable sodium alginate composite fortified with *Hibiscus sabdariffa* L. was appropriate for extending the shelf-life of highly perishable climacteric fruits [23]. It can be seen as a part of hurdle technology, which combines preservative techniques with synergistic effects to reduce losses and maintain nutritional value. Among the tailored PLA-based compositions, PLA coated with Kraft paper can enhance the barrier to water and air to replace non-biodegradable polymers. Nevertheless, the effectiveness of PLA in prolonging the shelf-life of fresh

salads has been scarcely analysed [24], as the possibility of storage for long periods is still unknown. Short food supply chains for vegetables open the possibility of selecting compostable and biodegradable films for modified atmosphere packaging (MAP), with foreseeable good results.

The purpose of this study was to evaluate the postharvest life of wild rocket (main crop) cultivated in peat or compost, and sea fennel cultivated in a floating system (secondary crop) using a CCS. Each vegetable was separately packaged in a PLA-based film as an alternative to petroleum-based bags for a short food supply chain. To our knowledge, there are not any previous reports evaluating a biodegradable package for the short food supply chain of leafy vegetables grown in a CCS.

2. Materials and Methods

2.1. Plant Material and Growing Conditions for Main Crop (Wild Rocket)

The characteristics of the main crop (wild rocket [*Diplotaxis tenuifolia* (L.) DC. cv. Apollo]) are described by Signore et al. [25]. Briefly, plants were cultivated in metal gutters filled with peat or an agro-industrial compost as growing media. A mix of white/black (60/40—in volume) peat was used, while the compost was composed of tomato and pepper juice waste, leek waste, and vineyard residues in dry weight percentages of 41, 43, and 16%, respectively. Fertigation was done daily with an automated system, using a nutrient solution with the following (in mM): 7.2 NO_3^-, 4.8 NH_4^+, 2 $H_2PO_4^-$, 2.5 SO_4^{2-}, 6 K^+, 1.9 Ca_2^+, and 1.5 Mg^{2+}, while micronutrients and iron were provided as a commercial solution: Nutromix® (2 mg L^{-1} for microelements—Biagro, Massalfassar, Valencia, Spain) and Sequestrene® G100 Syngenta (7% soluble Fe, 6% chelated Fe, 1.5 mg L^{-1}—Basel, Switzerland).

Harvest was performed when rocket plants had seven to eight leaves, corresponding to their appropriate commercial stage. Two harvests were carried out, 27 and 55 days after transplanting, respectively. The data presented in this work belong to the second harvest, as those from the first harvest were not significantly different from and were consistent with those from the second.

2.2. Plant Material and Growing Conditions for Secondary Crop (Sea Fennel)

Sea fennel (*Crithmum maritimum* L., Semillas Cantueso, Dunas de Artola, Málaga) seeds were sown on 4 May 2022, in styrofloat trays (0.6 m × 0.41 m) filled with peat, in a growth chamber at 20 °C for 5 days and then transferred to floating beds (1.35 × 1.25 × 0.2—L × W × H, respectively), floating on fresh tap water with an electrical conductivity (EC) of 1.1 dS m^{-1} and a pH of 7.8. Aeration was provided using a blow pump connected to a pipe trellis positioned at the bottom of each flotation bed. On 25 May 2022, thinning was done to reach a final plant density of 256 plants m^{-2}. The tap water of each bed was replaced, one month after sowing, with 200 L of the following treatments: 'Peat leach' = 100% peat leachate (pH 7.9, EC 3.6 dS m^{-1}), 'Compost leach' (pH 7.7, EC 3.1 dS m^{-1}) = 100% compost leachate (both collected from wild rocket crop), and 'Control' = 100% NS on which the concentration of every nutrient was 0.6 strength of that used for rocket crop cultivation (pH 7, EC 2.3 dS m^{-1}), in order to ensure comparability with previous results [26]. The average DLI during sea fennel cultivation was 14.9 mol m^{-2} d^{-1}, while the lowest, highest, and average air temperatures inside the greenhouse were 14.0, 43.2, and 27.1 °C, respectively. On 18 July when the plants had 3–4 true leaves, the harvest was carried out.

2.3. Processing, Packaging and Storage

After harvest, the plant material (wild rocket and sea fennel leaves) was minimally processed in a disinfected cold room at 8 °C. Leaves free of defects were sanitised by immersion for 1 min in a solution of chlorinated water (150 ppm NaOCl, pH 6.5, 4 °C) and then rinsed for 1 min in tap water (4 °C) to ensure that the final chlorine residue was below 5 ppm. After being drained for 10 min in a perforated basket, samples of about 100 g were arranged for passive MAP in 30 cm × 20 cm × 35 μm thick OPP (oriented polypropylene) plastic bags (Plásticos del Segura, Murcia, Spain) or in 30 cm × 20 cm, 20 μm thick PLA

bags. The PLA bags had one side made of transparent PLA (42.5%) and on the other side, the PLA was coated with an eco-layer of Kraft paper (57.5%) (Classpack-Nativia® NTSS, Barcelona, Spain). The transmission rates of O_2 and CO_2 at 23 °C and 0% relative humidity were similar for OPP bags, with a value of 11,000 cm^3 m^{-2} d^{-1} atm^{-1} for both gases. In contrast, for PLA bags, the transmission rates were ten times less, with a value of 1100 cm^3 m^{-2} d^{-1} atm^{-1} for both gases (data provided by the suppliers). For OPP and PLA, water vapour transmission rates (WVTR) were 18 g m^{-2} d^{-1} and 330 g m^{-2} d^{-1}, respectively. Both types of bags were then thermally sealed on the top using a thermo-sealer (Lovero Bag Sealer-SK 410, Korea).

The acid of the PLA film was obtained from corn and sugar cane and was produced according to the standards of good industrial techniques in compliance with Regulations (EU) 1935/2004 and (EU) 10/2011. Kraft paper was obtained from 100% virgin long fibre which gives cleanliness and consistency (Smurfit Kappa, Ireland), according to Forest Stewardship Council. Global migration analysed by using modified polyphenylene oxide (MPPO) as a solid food simulant for the food contact side (testing conditions: 10 d/20 °C–22 °C) was below the limit of quantification (DIN EN 1186:2002-07/2002-12). Five replicates from each treatment (peat and compost for wild rocket, and 'Peat leach', 'Compost leach', and 'Control' for sea fennel), package (OPP and PLA), and MAP storage duration (processing day = day 0, and after 7 days = day 7) were prepared and stored in darkness at 4 °C, 90% relative humidity (RH). Storage period was set at just 7 days because the packaged produce was intended only for a short food supply chain. Three replicates were randomly selected for physicochemical analysis.

2.4. Physicochemical Analyses

2.4.1. Head-Space Composition

On sampling day, before opening the bags, the atmosphere composition within the packages was assessed by an O_2/CO_2 head-space analyser (PBI-Dansensor CheckPoint, Ringsted, Denmark). The test needle of the gas analyser was inserted into each package through an adhesive silicon septum to avoid air leaking from the head-space. Data were expressed as kPa.

2.4.2. Weight Loss

Weight loss was calculated as the difference between the weight of the samples at the beginning of storage and their weight after 7 days. For data standardisation, weight loss was expressed as a percentage (%) of the initial value.

2.4.3. Microbial Quality

Microbial growth (mesophilic and psychrophilic aerobic bacteria, enterobacteria, and yeast and mould growth) was determined using standard enumeration methods. Samples of 1 g poured into a sterile stomacher bag (model 400 Bags 6141, London, UK) were homogenised with a 10 mL sterile peptone saline solution (pH 7; Scharlau Chemie SA, Barcelona, Spain) for 10 s in a masticator homogeniser (Colwort Stomacher 400 Lab, Seward Medical, London, UK). For the enumeration of each microbial group, 10-fold dilution series were prepared in 9 mL of sterile peptone saline solution. Mesophilic, enterobacteria, and psychrotrophic were pour-plated, and yeast and mould were spread-plated. Media (Scharlau Chemie, Barcelona, Spain) and incubation conditions were plate count modified agar (PCA) for mesophilic and psychrophilic aerobic bacteria (30 °C for 48 h and 5 °C for 7 days, respectively); violet-red bile dextrose agar for enterobacteria (37 °C, 48 h); and rose Bengal agar for yeasts and moulds (22 °C, 3–5 days). All microbial counts were reported as log colony forming units per gram of product (log CFU g^{-1}). Each of the three replicates was analysed by duplicate.

2.4.4. Colour

The colour of leaves was determined on three points at the upper side of 10 leaves from each replicate using a colourimeter (Minolta CR-400 Series, Ramsey, NJ, USA). Tristimulus parameters (L*, a*, b*) of the CIE Lab system were used to calculate the hue angle = arctan (b*/a*).

2.4.5. Sensory Evaluation

The sensory analysis was performed by a trained panel according to international specifications (ASTM STP 913, 1986) in a standardised room (UNE-EN ISO 8589 2007) equipped with ten testing boxes as described by Amoruso et al. [26]. The panel developed a vocabulary of sensory attributes including visual appearance, colour, dehydration, aroma, flavour, and texture. The overall quality was described as the global acceptance of the product that included visual, textural, and taste attributes. The samples were scored on a 9-point scale for colour, aroma, flavour, texture, visual appearance, and overall quality (9: excellent, 5: limit of marketability, 1: extremely bad) as well as for dehydration (9: without dehydration, 5: limit of marketability, 1: extremely dehydrated:). Sparkling mineral water was used as palate cleanser. The evaluation was done by 5 trained judges at day 0 and after 7 days of storage at 4 °C.

2.4.6. Nitrate Content

Nitrates were extracted in triplicate per treatment. The extraction from 0.2 g of dry leaf samples was carried out with 50 mL of distilled water by continuous agitation in an orbital shaker (Stuart SSL1, Stone, UK) for 45 min at 110 rpm at 50 °C. Nitrate concentration was determined by ion chromatography using a Metrosep A SUPP 5 column (Metrohm AG, Zofingen, Switzerland) at a flow rate of 0.7 mL min^{-1}, following the manufacturer's instructions.

2.4.7. Vitamin C

The vitamin C content was determined as the combined amount of ascorbic acid (AA) and dehydroascorbic acid (DHA), using high-pressure liquid chromatography as described by Zapata and Dufour [27] with slight modifications. Briefly, three grams of frozen samples were crushed, and 6 mL of an extraction solution of citric acid 0.1 M, 0.05% EDTA, and 4 nM NaF in 5% methanolic water were added. The mixture was homogenised for 30 s at high speed (Ultraturrax T25 basic, IKA, Germany). The homogenate was filtered through a sterile gauze with the pH being adjusted to 2.3–2.4 with 6 N HCl. The filtrate was transferred into tubes and centrifuged for 5 min at 13,500 rpm and 4 °C (Sorvall RC-SB series centrifuge). Subsequently, the samples were passed through a SepPak C18 cartridge (Waters Assoc.) and filtered again with a spin filter (0.45 μm). Then, 750 μL of the filtrate were placed in an HPLC vial and 250 μL of 1,2-o-phenylenediamine dihydrochloride (OPDA) (0.83 mg mL^{-1}) in methanol/water (5:95, *v*/*v*) were added. The mixture was allowed to react for 37 min at room temperature for derivatisation to form a fluorescent condensation product for detecting DHA. Then, 20 μL were injected on a Gemini NX C18-110 column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex, Torrance CA, USA), using an HPLC system (Shimadzu, Kyoto, Japan) equipped with an SPDM-20A photodiode array detector. The mobile phase was 5 mM hexadecyl trimethyl ammonium bromide, 50 mM KH_2PO_4, and 5% methanol (pH 4.59) with an isocratic flow of 1.8 mL min^{-1}. Chromatograms were recorded for 14 min at 261 nm (AA, Rt = 6.4 min) and 348 nm (DHA, Rt = 3.1 min). AA and DHA were quantified using commercial standards (Sigma, St. Louis, MO, USA). Calibration curves for quantification were made with at least eight data points from 1.25 to 0.1 mM and from 1.25 to 0.01 mM for AA and DHA, respectively. Results were expressed as the sum of AA + DHA, as g kg^{-1} fresh weight (FW).

2.4.8. Total Phenolics and Total Flavonoids Content

The total phenolic content (TPC) and the total flavonoid content (TFC) were determined as described by Martínez-Zamora et al. [28]. Briefly, 19 µL of the sample extract were mixed with 29 µL of 1 N Folin–Ciocalteu reagent and 192 µL of 0.4% Na_2CO_3 2% NaOH. After 1 h incubation in darkness, the absorbance was measured at 750 nm using a microplate reader (Tecan Infinite M200, Mannedorf, Switzerland). The TPC was expressed as mg of chlorogenic acid equivalents (ChAE) kg^{-1} FW. Each extract was analysed in triplicate. For TFC, 30 µL of extract were mixed with 80 µL of 20 g L^{-1} $AlCl_3$. After shaking and 1 h incubation in darkness, absorbance was measured at 415 nm. The TFC was expressed as mg of rutin equivalents (RE) kg^{-1} FW. Each sample extract was analysed in triplicate.

2.4.9. Total Antioxidant Capacity

Total antioxidant capacity (TAC) was analysed by the ferric reducing antioxidant power (FRAP) method according to Castillejo et al. [29] from the same extract prepared for TPC. A daily reaction solution containing sodium acetate buffer (pH 3.6), 10 mM (2,4,6-tripyridyl-S-triazine (TPTZ) solution (in 40 mM HCl), and 20 mM $FeCl_3$ was prepared in a proportion of 10:1:1 (volume) and incubated at 37 °C for 2 h in darkness. Then, 198 µL of FRAP solution were added to 6 µL of rocket extract and incubated for 30 min at room temperature in darkness. The TAC was measured by changes in absorbance at 593 nm (Tecan Infinite M200, Mannedorf, Switzerland). Obtained data were expressed as mg of Trolox equivalents (TE) kg^{-1} FW. Each sample extract was analysed in triplicate.

2.5. Experimental Design and Statistical Analyses

Experiments for the main and the secondary crop were a randomised complete block design, respectively, with three replicates ('beds'). Every bed contained 15 channels with 12 plants per channel for wild rocket and three floating trays for sea fennel. The postharvest experiment was submitted to mathematical analysis of data for each treatment: compost and peat in wild rocket and 'Peat leach', 'Compost leach', and 'Control' in sea fennel, package (OPP and PLA), and MAP storage duration (processing day and after 7 days) in both crops, and were subjected to multivariate analysis of variance (MANOVA). Means were compared by Tukey's test ($p = 0.05$) using Statgraphics Centurion (v.XIX, Stat Point Technologies, Inc., Warrenton, VA, USA).

3. Results and Discussion

3.1. Head-Space Composition

The final atmosphere composition inside the packages was balanced between the product's respiration and the permeation of the gases through the packaging material (Table 1). The highest CO_2 concentrations were observed for rocket leaves grown in peat, and for sea fennel grown in both 'peat leach' and 'control' treatments, indicating a higher respiration rate for them compared to those cultivated in compost or in 'compost leach' treatment. Lower respiration rates can be associated with a longer shelf-life. However, for brief storage periods resembling those in short food supply chains, differences in shelf-life can be unseen. PLA bags allowed for obtaining an atmosphere with less O_2 and more CO_2 than OPP for both crops and, independently of the treatment, a better suitability of PLA for MAP was indicated since a higher modification related to air was reached. Coating one side of PLA with Kraft paper reduced the gas and water vapour exchange rate and allowed a more appropriate passive atmosphere modification by compensating for the higher gas permeability and water vapour transmission of PLA when compared to OPP. The effectiveness of MAP in prolonging rocket shelf-life has been widely studied. Wild rocket is usually minimally processed and packaged in plastic, with 5 to 10 kPa CO_2 and 5 to 10 kPa O_2 as suitable compositions for convenient shelf-life [30]. In general, wild and salad rocket species have a prolonged postharvest shelf-life when exposed to levels of CO_2 around 8–10 kPa, preserving sensory and microbiological quality as well as the content of health-promoting phytonutrients compared to air [31]. There are no relevant references in

the bibliography indicating the most appropriate MAP for sea fennel. However, previous reports indicated that after 6 days of storage at 5 °C, any significant modification in O_2 and CO_2 concentrations were observed and that slight atmosphere changes were detected after 12 days [26], thus confirming a rather low respiration rate for this crop.

Table 1. Atmosphere composition within OPP or PLA bags at the end of storage for 7 days at 4 °C of fresh-cut wild rocket and sea fennel cultivated in different substrates and their leachates, respectively.

	O_2 (kPa) at Day 7 (*)		**CO_2 (kPa) at Day 7 (*)**	
	OPP	**PLA**	**OPP**	**PLA**
Wild rocket				
Peat	14.7 ± 0.8 bA	11.5 ± 2.2 aB	6.2 ± 0.8 aB	9.4 ± 2.2 aA
Compost	16.8 ± 0.9 aA	13.0 ± 1.4 aB	4.1 ± 0.9 bB	7.9 ± 1.4 aA
Sea fennel				
Peat leach	19.6 ± 0.3 aA	16.9 ± 1.1 aB	1.5 ± 0.3 abB	3.8 ± 1.0 aA
Compost leach	19.8 ± 0.3 aA	15.1 ± 0.4 aB	1.2 ± 0.2 bB	5.4 ± 0.6 aA
Control	19.1 ± 0.2 aA	16.1 ± 0.9 aB	1.9 ± 0.1 aB	4.6 ± 0.6 aA

Different lowercase letters within each column and species indicate significant differences among treatments, while different uppercase letters within each row indicate significant differences between packages at $p = 0.05$ according to Tukey's test. OPP: oriented polypropylene bags; PLA: polylactic acid bags. (*) At day 0, O_2 = 21.02 kPa and CO_2 = 0.05 kPa.

The atmospheres obtained in our experiments preserved the shelf-life of rocket and sea fennel leaves for 7 days at 4 °C. It is important to highlight the capacity of PLA for generating an atmosphere closer to that most appropriate for wild rocket storage and good for preserving the quality of sea fennel, indicating the suitability of this material as a substitute for those from typical plastic sources.

3.2. Weight Loss

Leafy vegetables are highly susceptible to water loss after harvest. Transpiration is the major cause of postharvest loss and poor quality, which causes wilt after about 3–5% water loss [32]. Values detected in our experiments were below that range. Correct packaging can prevent shriveling by ensuring high humidity inside the bag. Dehydration was higher for leaves stored in PLA than for those packaged in OPP, particularly for wild rocket, with those coming from compost and those that were OPP-packaged having the lowest values (Figure 1a). For sea fennel (Figure 1b) there were no differences between packages and growing media.

The water transmission rate for PLA was higher than for OPP (18 g m^{-2} d^{-1} vs. 330 g m^{-2} d^{-1}, respectively) and, even though several properties of PLA-based packaging material have been found to be similar to petroleum-based films, the hygroscopic nature of PLA has been found to influence product quality [33]. The elevated water vapour transmission rate of PLA is related to its chemical composition. One of the main drawbacks of PLA for various applications is its sensitivity to hydrolysis in the presence of water, leading to a drastic decrease in molecular weight and degradation of mechanical properties [34]. Hydrolysis increases at elevated temperatures, especially above the glass transition temperature [35]. The water resistance of PLA composite can be significantly improved by the addition of microencapsulated polymethyl-methacrylate (PMMA), which in combination with PLA limits water diffusion [18]. PLA bags had 57.5% of their surface coated by Kraft paper, as an affordable industrial option to reduce water loss. More studies are needed for PLA blends and copolymers and for compounds that can be applied or added to improve the physical, mechanical, and barrier properties of PLA [36].

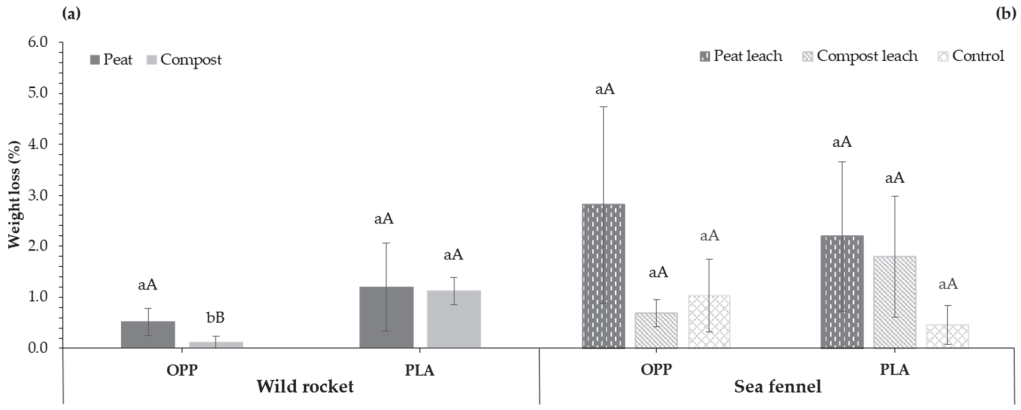

Figure 1. Weight loss of fresh-cut wild rocket (**a**) and sea fennel (**b**) cultivated in different growing media and their leachates, respectively, packaged in OPP or PLA bags for storage during 7 days at 4 °C. Different lowercase letters indicate significant differences among treatments for each vegetable, while different uppercase letters indicate significant differences between packages of each crop at $p = 0.05$ according to Tukey's test. OPP: oriented polypropylene bags; PLA: polylactic acid bags.

However, as will be discussed below, water loss measured for PLA-packaged wild rocket and sea fennel was not noticed by the trained panel; all the leaves showed a freshness aspect after 7 days at 4 °C. Independently of that, we hypothesise that water loss could have been significantly reduced by lowering the temperature during storage to reduce the vapour pressure deficit. Wilting is better prevented at 0 °C than at 4 °C or above, particularly if longer storage periods (>7 days) are intended. Moreover, breaks in the cold chain during distribution and retail must be avoided.

3.3. Microbial Quality

Before washing, microbial load was between 1.7–2.3 and 6.0–6.4 log CFU g^{-1} for total mesophilic aerobic bacteria; between 0.9–1.5 and non-detected log CFU g^{-1} for psychrophilic; and between 0.3–1.3 and 4.3–6.2 log CFU g^{-1} for yeasts and moulds for wild rocket and sea fennel, respectively, while *Enterobacteriaceae* were not detected in any vegetable or in any treatment (data not shown) (Figure 2). Initial values of microbial counts for wild rocket before disinfection were low and typical for soilless cultivated vegetables when compared with standard cultivation [37] except for psychrophiles, and with lower values when compared with sea fennel. Washing with NaClO was highly effective for decreasing counts to undetectable levels in wild rocket. However, for sea fennel, the only decrease was detected for yeast and moulds when leaves were grown in 'peat leach'. The waxy surface of sea fennel can reduce disinfection efficacy and longer washing time or higher chlorine concentrations could be needed. Amoruso et al. [26] obtained similar values for microbial load at harvest in sea fennel grown on saline conditions in a floating system.

At the end of storage, the microbial load of wild rocket increased for all the treatments without significant differences between growing media or between packages. It is worth highlighting that the mesophilic counts observed in leaves for peat-cultivated plants that were packaged in PLA exhibited a lower increase compared to the other treatments. This may be because peat-grown, PLA-packaged rocket had the highest weight loss due to dehydration, resulting in an atmosphere less favourable for microbial growth.

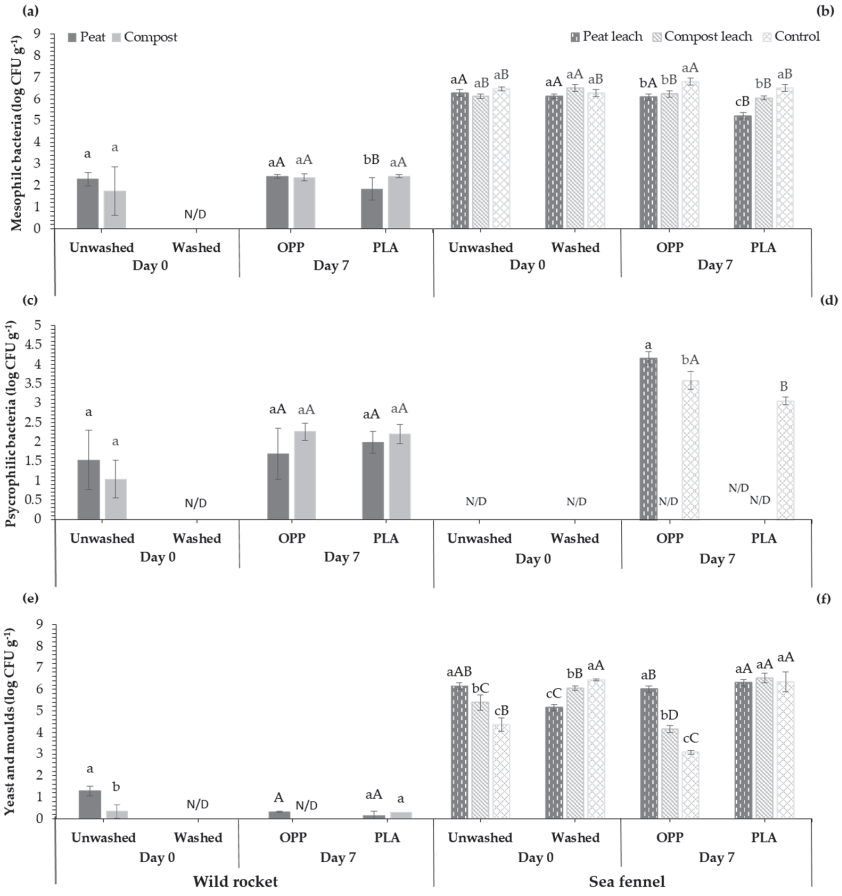

Figure 2. Mesophilic bacteria, psychrophilic bacteria, and yeast and mould counts (log CFU g^{-1}) of fresh-cut wild rocket (**a**,**c**,**e**) and sea fennel (**b**,**d**,**f**) cultivated in different growing media and their leachates, respectively, packaged in OPP or PLA bags, and stored during 7 days at 4 °C. Values at harvest (day 0) and at the end of storage (day 7). Different lowercase letters indicate significant differences among treatments for each vegetable, while different uppercase letters indicate significant differences between packages at $p = 0.05$ according to Tukey's test. N/D: non detected. OPP: oriented polypropylene bags; PLA: polylactic acid bags.

Results for sea fennel indicated a significant interaction among leachates, packaging, and storage for mesophilic and psychrophilic bacteria and yeasts and moulds. Mesophilic counts of sea fennel slightly changed during storage, with the leaves of plants grown in peat leachates stored in PLA showing the lowest values. PLA was also more effective for reducing psychrophiles growth, especially for peat and compost drainages, while yeast and mould were grown at a lower rate in OPP than in PLA. Modified atmosphere packaging is commonly used for decreasing microbial growth on perishable commodities. The atmosphere reached in our experiments for sea fennel was adequate because anaerobic microorganisms were not detected. However, since microbial growth was poorly inhibited, higher CO_2 concentrations could be needed for reaching a bacteriostatic effect. For better control of the microbial safety of fresh-cut sea fennel, the packaging design can be improved. Using different composites to control microbial growth and other aspects of produce metabolism is a critical area of research that can have significant practical applications for the food industry. By combining different composites and techniques, it may be possible to

enhance the shelf-life of food products as has been demonstrated by Singh et al., 2023 [23], in recent research.

It is known that values over 7 log CFU g^{-1} for mesophilic and psychrophilic bacteria can be associated with a shorter shelf-life of fresh-cut vegetables. In our experiments, and independently of the treatments and of the crop, values above those mentioned were not found in any case, as reported by Giménez et al. [38].

3.4. Colour

Non-remarkable differences in colour were observed between leaves of wild rocket cultivated in peat or compost at harvest (Table 2). Additionally, PLA and OPP bags were favourable for keeping colour during storage with any relevant change for any of the colour parameters analysed. It could be related to the atmosphere modification surrounding the plant product that would be appropriate for avoiding the loss of green colour. For sea fennel, the colour was darker when obtained from the 'control' and from 'compost leach' than from 'peat leach'. During storage, only an increase in L* values was detected for all the sea fennel treatments indicating a slight increase in lightness, probably due to the presence of salt crystals on leaves as reported by Amoruso et al. [26]. Likewise, a slight trend towards an increase in L* was observed after 7 days of storage for wild rocket leaves that were grown in compost.

Table 2. Colour parameters for fresh-cut wild rocket and sea fennel cultivated in different growing media and their leachates, respectively, packaged in OPP or PLA bags. Day 0 = at harvest. Day 7 = after storage during 7 days at 4 °C.

	L*			**Hue**		
	Day 0	**Day 7**		**Day 0**	**Day 7**	
		OPP	**PLA**		**OPP**	**PLA**
Wild rocket						
Peat	45.3 ± 4.6 aA	48.3 ± 5.1 aA	47.2 ± 4.3 aA	122.7 ± 1.3 aA	122.3 ± 1.6 aA	122.3 ± 2.0 bA
Compost	44.7 ± 2.1 aB	48.2 ± 3.7 aA	47.7 ± 4.9aA	122.4 ± 0.8 aAB	121.9 ± 1.7 aB	123.3 ± 1.7 aA
Sea fennel						
Peat leach	42.7 ± 4.2 aB	46.9 ± 3.1 aA	46.9 ± 3.2 aA	115.0 ± 4.4 bA	115.8 ± 4.1 cA	115.5 ± 4.5 bA
Compost leach	38.1 ± 4.1 bB	44.2 ± 4.9 aA	42.9 ± 3.5 bA	118.3 ± 9.1 abA	119.3 ± 2.1 bA	123.1 ± 3.1 aA
Control	33.4 ± 4.4 cB	39.2 ± 1.9 bA	39.9 ± 4.6 dA	122.8 ± 2.5 aA	123.5 ± 4.3 aA	125.4 ± 3.5 aA

Different lowercase letters within each column indicate significant differences among treatments for each vegetable on days 0 and 7, while different uppercase letters within each row indicate significant differences between packages at $p = 0.05$ according to Tukey's test. OPP: oriented polypropylene bags; PLA: polylactic acid bags.

The absence of anaerobic condition, which might lead to acidic degradation of chlorophyll and, lastly, to senescence and loss of greenness, avoided relevant colour alterations to the leaves in the products.

Reductions in the hue angle were not drastic. On the contrary, it would indicate a change from green to yellow. Green colour is an important quality parameter of leafy vegetables at the time of purchase as it indicates the freshness of the product [39].

3.5. Sensory Evaluation

Sensory quality at harvest, when colour, aroma, texture, and dehydration were considered, was similar for rocket coming from both growing media (Figure 3a). However, wild rocket grown in peat had a worse overall quality due to a lower flavour punctuation. That aspect was related to an exceptionally strong spicy flavour that was criticised by the panel members as reflected in the tasting notes (data not shown). For sea fennel the overall sensory quality decreased in leaves from plants treated with peat leachate respective to the control and the compost leachate (Figure 3d). After 7 days of storage, rocket quality in biodegradable and plastic packages remained as good and acceptable for consumption, independently of the growing media (Figure 3b,c). In agreement with the data obtained

from the colourimetric measurements, yellowness was not detected in any treatment. The slight loss in texture for PLA-stored wild rocket seemed to have a trend similar to that for dehydration, with treatments from biodegradable bags showing the poorer texture. Sea fennel grown in peat leachate showed a worse overall quality after 7 days of storage in both biodegradable and plastic packages than that from the other treatments (Figure 3e).

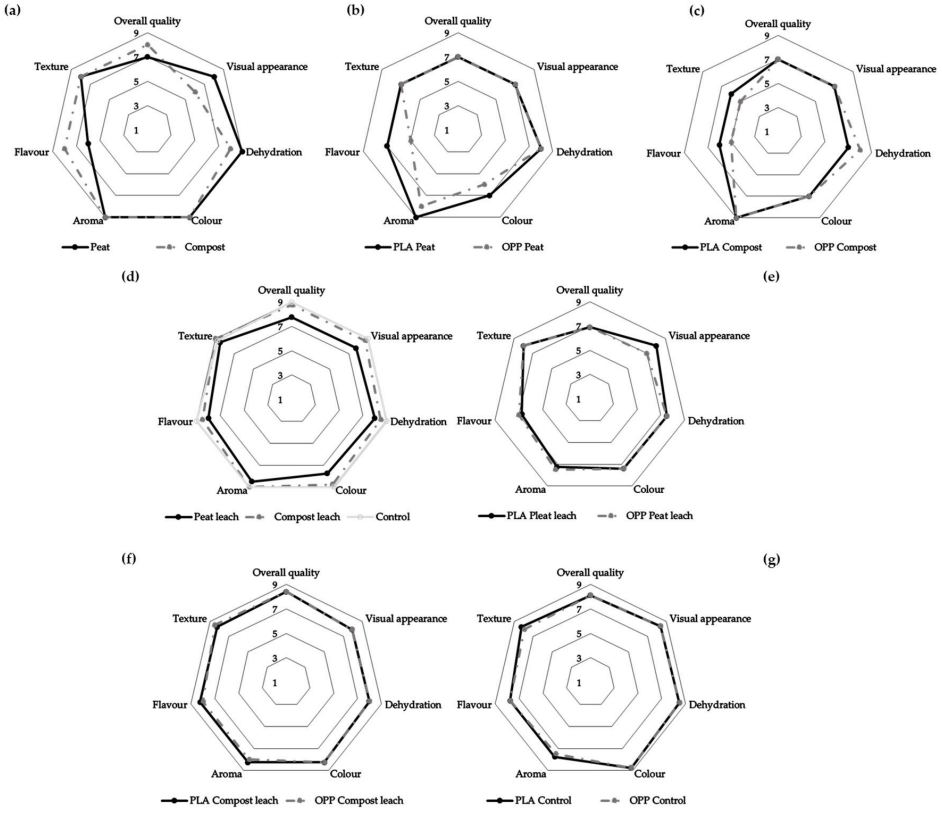

Figure 3. Sensory quality of fresh-cut wild rocket (**a**–**c**) and sea fennel (**d**–**g**) cultivated in different substrates and their leachates, respectively, packaged in OPP or PLA bags and stored during 7 days at 4 °C. (**a**) Rocket leaves from plants grown in peat and compost, at harvest. (**b**) Rocket leaves from plants grown in peat after 7 days in OPP or PLA bags. (**c**) Rocket leaves from plants grown in compost after 7 days in OPP or PLA bags. (**d**) Sea fennel leaves from plants treated with 'peat leach', 'compost leach', and 'control', at harvest. (**e**) Sea fennel leaves from plants treated with 'peat leach' after 7 days in OPP or PLA bags. (**f**) Sea fennel leaves from plants treated with 'compost leach' after 7 days in OPP or PLA bags. (**g**) Sea fennel leaves from 'control' plants after 7 days in OPP or PLA bags. OPP: oriented polypropylene bags; PLA: polylactic acid bags.

The packaging of green leafy vegetables can postpone senescence and yellowing, but a drawback is the risk of anaerobic respiration preceding the development of an olive-brown colour. MAP not correctly designed can lead either to an internal atmosphere composition close to atmospheric air at too high transmission rates or to anaerobic respiration due to too low transmission rates of gases through the package. This would lead to the onset of symptoms of tissue degradation and appearance of off-odours [40]. In contrast, atmospheric air leads to senescence and loss of green colour [38]. The MAP obtained in our experiments was appropriate for keeping flavour in both PLA and OPP bags. The texture is another important quality parameter of green leafy vegetables [41]. The senescence of vegetables is

a degradation process, where the cell walls are broken down. Cell collapse may also be induced by too low O_2 in the intracellular spaces of the living tissues [42], resulting in loss of texture. In our experiments, only a moderated loss of texture was observed for rocket leaves obtained from compost and packaged with PLA, probably related to the higher water loss observed in them when compared to those grown in the same growing medium and packaged with OPP.

Finally, the overall quality, which determined the degree of acceptance, was also influenced by flavour. Guijarro-Real et al. [43] reported that acceptance was mainly related to taste and pungency, with rocket (*Diplotaxis tenuifolia* (L.) in our case) being well accepted only by a cohort of consumers that enjoy spicy flavours. On the other hand, sensory changes in sea fennel were not drastic, indicating that a longer shelf-life could be obtained than that of wild rocket. Sea fennel likely would have the longest postharvest life in terms of sensory quality, which may be attributed to its lower respiration rate compared to wild rocket, as well as to the natural waxy coating on its leaves that reduces moisture loss by retaining it within the plant tissue.

3.6. Nitrate Content

Nitrates are typically accumulated in some green leafy vegetables such as lettuce, spinach, and rocket [44]. Particularly, rocket has been classified as a plant with typically high concentrations of NO_3^- (>2500 mg kg^{-1} FW, [45]) with wild rocket having twice the concentration of cultivated rocket (*Eruca vesicaria*) [46]. The maximum levels set for nitrates according to the European Commission [47] for rocket are 6000 and 7000 mg NO_3^- kg^{-1} FW, if harvested from 1 April to 30 September and from 1 October to 31 March, respectively. However, specific limits for sea fennel are not legally stated yet.

Significant differences in nitrate concentration were found between wild rocket leaves harvested from plants grown in different growing media, with those cultivated in compost having the highest concentrations (Figure 4a). This is not surprising, as the content of total N in compost was four-fold that in peat [25]. The nitrogen in the compost was hence (at least partly) released into the drainage nutrient solution which had, indeed, a nitrate concentration three to four times higher than the drainage obtained from peat (data not shown). This effect, observed for the main crop, was not reflected in the nitrate content in sea fennel, where peat and compost leachates were used (Figure 4b), without any difference between them. However, the nitrate content of sea fennel leaves obtained from the control treatment had higher concentrations. These concentrations were higher than those obtained by other authors [15,26].

Differences were maintained during storage, even when a slightly decreasing trend was observed with time (Figure 4) for both the main and the secondary crop. The type of package did not have any influence on nitrate metabolism during shelf-life. It is known that nitrate might have both health concerns, such as methaemoglobinaemia and cancer, and health benefits, such as positive cardiovascular effects and an increase in human defense against gastroenteritis [44,45]. Although the scientific community has not yet reached a consensus on the issue, the values obtained from the experiments were relatively high. Our findings suggest that this may be attributed to the tendency of wild rocket leaves to accumulate nitrates more efficiently than other leafy vegetables. Wild rocket's short biological cycles, high rate and extent of N recovery, and its capacity to accumulate nitrates all indicate that it could function as a hyperaccumulator of nitrates. However, it is important to note that these values remained below the legally established limits for commercialisation [48]. On the other hand, sea fennel did not accumulate as much nitrate content as wild rocket leaves (Figure 4).

Some strategies can be used to reduce the nitrate content, particularly in hydroponic systems, to avoid its accumulation in rocket leaves, which would hamper the rocket being placed on the market [46,49].

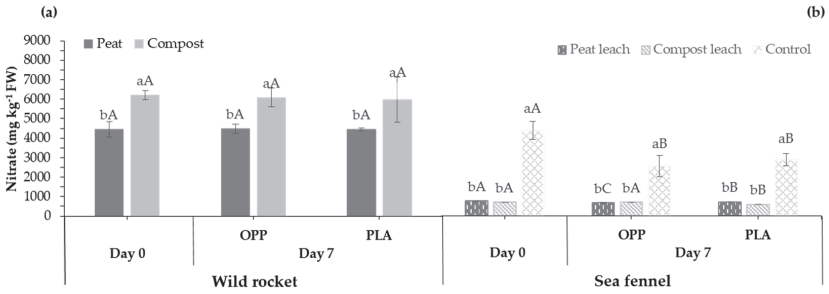

Figure 4. Nitrate content of fresh-cut wild rocket (**a**) and sea fennel (**b**) cultivated in different growing media and their leachates, respectively, packaged in OPP or PLA bags and stored during 7 days at 4 °C. Values at harvest (day 0) and at the end of storage (day 7). Different lowercase letters indicate significant differences among treatments, while different uppercase letters indicate significant differences between packages at $p = 0.05$ according to Tukey's test. OPP: oriented polypropylene bags; PLA: polylactic acid bags.

3.7. Vitamin C

The vitamin C content measured as ascorbic acid (AA) and dehydroascorbic acid (DHA) ranged between 63 and 101 mg kg^{-1} FW for wild rocket (Figure 5a). The highest content of vitamin C at harvest, as well as after storage, was observed for rocket grown in compost. Previous reports indicated that the vitamin C of rocket salad can be positively affected when organic amendments are added [50]. Values found in this study are lower than those previously reported for rocket salad cultivated in soil [51] but similar to those of Duyar and Kiliç [52] for plants grown in a floating system. The vitamin C content in leaves can be influenced by growing conditions, particularly by the NO_3-/NH_4^+ ratio in the nutrient solution [53]. Observations revealed no significant vitamin C degradation after 7 days, which suggests that both the low temperature and the MAP were effective at preventing its degradation. Bonasia et al. [54] stated that vitamin C degradation for wild rocket could occur after 7 days at 5 °C, but would be lower than 21% and would depend on the initial values. Differences due to the package were not detected, indicating that PLA was as suitable as OPP for keeping the initial values of vitamin C during cold storage.

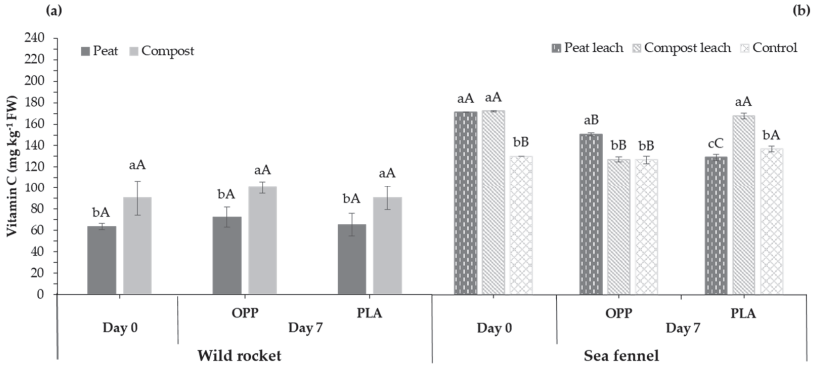

Figure 5. Vitamin C content of fresh-cut wild rocket (**a**) and sea fennel (**b**) cultivated in different growing media and their leachates, respectively, packaged in OPP or PLA bags and stored during 7 days at 4 °C. Values at harvest (day 0) and at the end of storage (day 7). Different lowercase letters indicate significant differences among treatments for each vegetable, while different uppercase letters indicate significant differences between packages at $p = 0.05$ according to Tukey's test. OPP: oriented polypropylene bags; PLA: polylactic acid bags.

The vitamin C content for sea fennel was higher than in wild rocket (Figure 5b). Interestingly, compost and peat leachates significantly increased the vitamin C in leaves compared with the control. A slight decrease was observed during storage, with the highest content in the plants grown in compost leach and stored in PLA. That decrease could be avoided by a MAP with lower oxygen concentrations resembling the atmosphere composition observed for wild rocket. These results confirm that sea fennel is a vegetable rich in vitamin C, quite stable during storage in a biodegradable package, and in agreement with those results previously reported by Renna [55].

3.8. Total Phenolics Content and Total Flavonoids Content

Results obtained for total phenolics content (Figure 6a,c) indicate that the initial values were similar to those previously reported by Gutiérrez et al. [56] for wild rocket and considerably higher than those reported by Amoruso et al. [26] for sea fennel.

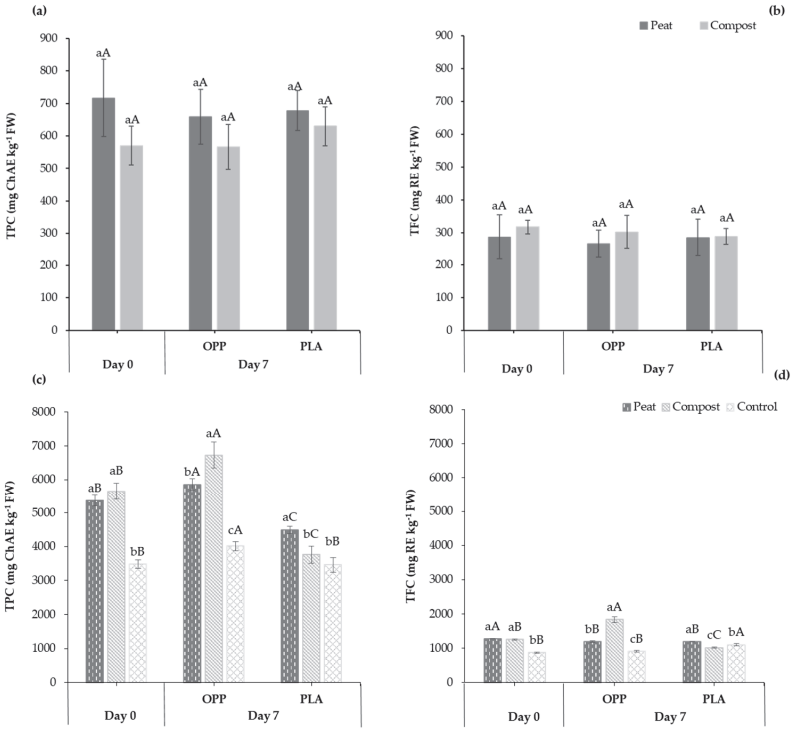

Figure 6. Total phenols (**a**–**c**) and total flavonoids (**b**–**d**) of fresh-cut wild rocket (**a**,**b**) and sea fennel (**c**,**d**), respectively, cultivated in different growing media and their leachates, respectively, packaged in OPP or PLA bags and stored during 7 days at 4 °C. Values at harvest (day 0) and at the end of storage (day 7). Different lowercase letters indicate significant differences among treatments for each vegetable, while different uppercase letters indicate significant differences between packages at $p = 0.05$ according to Tukey's test. OPP: oriented polypropylene bags; PLA: polylactic acid bags.

Any remarkable trend was observed after 7 days of storage for wild rocket. Most of the changes in TPC in leafy vegetables have been reported to be detected after longer storage times and/or higher temperatures than those assessed here [40,56]. In general, the leaves from rocket grown in peat showed a trend of higher values of TPC but still non-significant. For sea fennel, the highest values were observed for plant leaves grown in peat and compost leachates, with the lowest being found in the control. Then, they were quite stable during storage for the OPP bags, with a decrease for the PLA. However,

values were considerably high. Phenolic compounds play a key role in the protection of plant tissues against abiotic stress [56]. Growing plants with leachates of peat and compost would trigger phenolics accumulation.

Similarly, flavonoid concentrations (Figures 6b and 6d, respectively) were similar to those reported by Bell and Wagstaff [12] and Amoruso et al. [26] for wild rocket leaves and sea fennel, respectively. As a part of phenolics compounds, flavonoids were shown to represent more than 50% of them for all the treatments. The storage did not have any influence on flavonoid values with ranges practically constant for all the treatments. Flavanol derivatives of quercetin and kaempferol had been detected in wild rocket with quercetin derivatives being the main compounds in wild rocket, while kaempferol derivatives were the main compounds in salad rocket [51]. For a better understanding of flavonoid pathways, it would be advisable to analyse the flavanols profile, especially for sea fennel, where scarce information is still available. The results indicated that sea fennel is a vegetable rich in flavonoids and that the leachates, especially those based on compost, have a positive influence on flavonoid accumulation.

3.9. Total Antioxidant Capacity

At harvest, the antioxidant capacity in rocket leaves was similar in all the treatments and within the ranges previously reported by other authors [56] and particularly for this species [51], varying from 1.450 to 1.680 mg TE kg^{-1} FW (Figure 7a). As observed for TPC, there were no significant variations in TAC during storage at 4 °C. Furthermore, there were no significant differences between rocket plants cultivated in peat or compost. The same happened for the type of package which did not affect the total antioxidant capacity of the leaves. For short storage periods at low temperatures (<5 °C), TAC has been shown to be constant indicating a high stability of rocket in its antioxidant system. That stability has been previously reported even when the leaves had to cope with strong physical stresses [56]. Sea fennel (Figure 7b) had higher TAC content when plants were cultivated with peat and compost leachates (4.672 and 3.643 mg TE kg^{-1} FW, respectively) when compared to the control (971 mg TE kg^{-1} FW), indicating that antioxidants can be favoured with sea fennel as a secondary crop and wild rocket as the primary. To our knowledge, this is the first time that this beneficial effect of leachates on nutritional quality has been identified. Stressing growing conditions, such as the accumulation of toxic ions in the leaves (i.e., Cl^-, Na^+ 388) and/or the alkaline pH of the NS, can affect sea fennel plants and enhance their phytochemical content [26]. It is well stablished that plants cope with abiotic stress by altering metabolic processes producing reactive oxygen species and stimulating antioxidant activity to scavenge free radicals and ion chelators [56]. TAC decreased during storage, with the highest values for the OPP bags.

For wild rocket, TAC was related to the content of flavonoids and polyphenols showing the same trend, that is, a lack of relevant changes during storage. Previous studies of the antiradical activity in wild rocket leaves have shown that it is correlated to polyphenol content and flavonoids, as well as to vitamin C, these being the major antioxidants of Brassica vegetables [57]. However, in addition to them, other constituents could exhibit antioxidant properties such as vitamin E and carotenoids for wild rocket [51] and sea fennel [26]. In the case of sea fennel, antioxidants such as ascorbic acid, phenolic compounds, and, particularly, flavonoids, as previously indicated, can contribute to these high values.

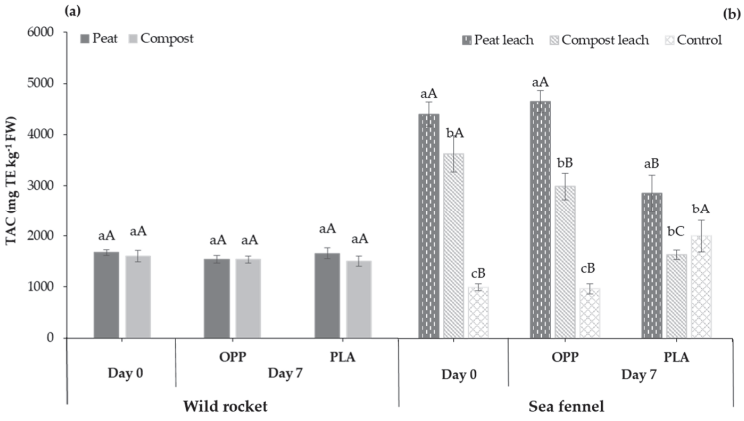

Figure 7. Total antioxidant capacity of fresh-cut wild rocket (**a**) and sea fennel (**b**) cultivated in different growing media and their leachates, respectively. Packaged in OPP or PLA bags and stored during 7 days at 4 °C. Values at harvest (day 0) and at the end of storage (day 7). Different lowercase letters indicate significant differences among treatments for each vegetable, while different uppercase letters indicate significant differences between packages at $p = 0.05$ according to Tukey´s test. OPP: oriented polypropylene bags; PLA: polylactic acid bags.

4. Conclusions

The present study lays the groundwork for future research into evaluating biodegradable packages for the short food supply chain of leafy vegetables grown in a cascade cropping system. Results of this research showed that packaging fresh-cut wild rocket and sea fennel with a PLA-based film is a feasible alternative to common plastic used in the fresh-cut industry, avoiding the waste of polymers in landfills. The biodegradable film has good oxygen barrier properties for packaging wild rocket. For sea fennel, a lower gas exchanging area or a reduced head-space can be recommended to obtain a MAP with higher CO_2 and lower O_2 than observed here. To our knowledge, this is the first report in evaluating a compostable package for the short food supply chain of leafy vegetables. A shelf-life of 7 days at 4 °C, appropriate for the purpose of the ready-to-eat-sector, can be achieved without any relevant detrimental change in quality or microbial safety. Moreover, soilless cultivation with compost as growing media allowed for obtaining wild rocket with lower water loss and respiratory activity when compared to that cultivated in peat. At the same time, sea fennel, as a secondary crop grown using the leachates derived from the wild rocket growing medium, showed a higher nutritional quality than that observed for sea fennel cultivated with standard nutrient solution. The combination of the cascade cropping system using compost as growing medium or its leachate and PLA for packaging was demonstrated to be feasible for wild rocket and sea fennel production, opening a wide range of possibilities for a more environmentally friendly production and commercialisation of fresh-cut vegetables. Our study focused on a specific biodegradable packaging material that yielded satisfactory results. However, there is potential to further improve the shelf-life of food products, reduce food waste, and enhance food safety by exploring different composite materials and production techniques for biodegradable packaging. As the use of less plastic for packaging becomes increasingly important in sustainable agriculture, future research should prioritise the development of eco-friendly alternatives. We hope that our study will contribute to this important field and inspire further investigation into effective methods for food preservation.

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supervision, J.A.F., J.O., P.A.G. and C.E.-G.; funding acquisition, J.A.F. and J.O. All authors have read and agreed to the published version of the manuscript.

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References

1. Orsini, F.; Pennisi, G.; Zulfiqar, F.; Gianquinto, G. Sustainable use of resources in plant factories with artificial lighting (PFALs). *Eur. J. Hortic. Sci.* **2020**, *85*, 297–309. [CrossRef]
2. Mohareb, E.; Heller, M.; Novak, P.; Goldstein, B.; Fonoll, X.; Raskin, L. Considerations for reducing food system energy demand while scaling up urban agriculture. *Environ. Res. Lett.* **2017**, *12*, 125004. [CrossRef]
3. Parkes, M.G.; Azevedo, D.L.; Domingos, T.; Teixeira, R.F.M. Narratives and Benefits of Agricultural Technology in Urban Buildings: A Review. *Atmosphere* **2022**, *13*, 1250. [CrossRef]
4. Floriš, N.; Schwarcz, P.; Schwarczová, L.; Munk, M. Short food supply chains and small farms—Evidence from Slovakia. *Sustainability* **2022**, *14*, 2728. [CrossRef]
5. Buehler, D.; Junge, R. Global Trends and Current Status of Commercial Urban Rooftop Farming. *Sustainability* **2016**, *8*, 1108. [CrossRef]
6. Santos, M.G.; Moreira, G.S.; Pereira, R.; Carvalho, S.M. Assessing the potential use of drainage from open soilless production systems: A case study from an agronomic and ecotoxicological perspective. *Agric. Water Manag.* **2022**, *273*, 107906. [CrossRef]
7. Rufí-Salís, M.; Petit-Boix, A.; Villalba, G.; Sanjuan-Delmás, D.; Parada, F.; Ercilla-Monserrat, M.; Arcas-Pliz, V.; Muñoz-Liesa, J.; Rieradevall, J.; Gabarrell, X. Recirculating water and nutrient in urban agriculture: An opportunity towards environmental sustainability and water use efficiency? *J. Clean. Prod.* **2020**, *261*, 121213. [CrossRef]
8. Kneafsey, M.; Venn, L.; Schmutz, U.; Balázs, B.; Trenchard, L.; Eyden-Wood, T.; Bos, E.; Sutton, G.; Blackett, M. *Short Food Supply Chains and Local Food Systems in the EU. A State of Play of Their Socio—Economic Characteristics*; EUR 25911; Publications Office of the European Union: Luxembourg, 2013; pp. 123–129, JRC80420. [CrossRef]
9. De Corato, U. Agricultural waste recycling in horticultural intensive farming systems by on-farm composting and compost-based tea application improves soil quality and plant health: A review under the perspective of a circular economy. *Sci. Total Environ.* **2020**, *738*, 139840. [CrossRef]
10. Hernández-Lara, A.; Ros, M.; Pérez-Murcia, M.D.; Pascual, J.A. The influence of feedstocks and additives in 23 added-value composts as a growing media component on Pythium irregulare suppressivity. *Waste Manag.* **2021**, *120*, 351–363. [CrossRef]
11. Spadafora, N.; Amaro, A.; Pereira, M.; Müller, C.; Pintado, M.; Rogers, H. Multi-trait analysis of post-harvest storage in rocket salad (*Diplotaxis tenuifolia*) links sensorial, volatile and nutritional data. *Food Chem.* **2016**, *211*, 114–123. [CrossRef]
12. Bell, L.; Wagstaff, C. Rocket science: A review of phytochemical and health-related research in *Eruca* & *Diplotaxis* species. *Food Chem.* **2019**, *1*, 100002. [CrossRef]
13. Alemán, A.; Marín-Peñalver, D.; Fernández de Palencia, P.; Gómez-Guillén, M.C.; Montero, P. Anti-Inflammatory Properties, Bioaccessibility and Intestinal Absorption of Sea Fennel (Crithmum maritimum) Extract Encapsulated in Soy Phosphatidylcholine Liposomes. *Nutrients* **2022**, *14*, 210. [CrossRef] [PubMed]
14. Renna, M.; Gonnella, M.; Caretto, S.; Mita, G.; Serio, F. Sea fennel (*Crithmum maritimum* L.): From underutilized crop to new dried product for food use. *Genet. Resour. Crop Evol.* **2017**, *64*, 205–216. [CrossRef]
15. Labiad, M.H.; Giménez, A.; Varol, H.; Tüzel, Y.; Egea-Gilabert, C.; Fernández, J.A.; Martínez-Ballesta, M.D.C. Effect of exogenously applied methyl jasmonate on yield and quality of salt-stressed hydroponically grown sea fennel (*Crithmum maritimum* L.). *Agronomy* **2021**, *11*, 1083. [CrossRef]
16. Renna, M.; Gonnella, M. The use of the sea fennel as a new spice-colorant in culinary preparations. *Int. J. Gastron. Food Sci.* **2012**, *1*, 111–115. [CrossRef]

17. Battini, D.; Calzavara, M.; Persona, A.; Sgarbossa, F. Sustainable packaging development for fresh food supply chains. *Packag. Technol. Sci.* **2016**, *29*, 25–43. [CrossRef]
18. Sari, N.H.; Suteja, S.; Sapuan, S.M.; Ilyas, R.A. Properties and food packaging application of poly-(lactic) acid. In *Bio-Based Packaging: Material, Environmental and Economic Aspects*, 1st ed.; Sapuan, S.M., Ilyas, R.A., Eds.; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2021. [CrossRef]
19. Diañez, I.; Martínez, I.; Gómez, P.A. Effect of plasticiser on the morphology, mechanical properties and permeability of albumen-based nanobiocomposites. *Food Packag. Shelf Life* **2020**, *24*, 100499. [CrossRef]
20. Jabeen, N.; Majid, I.; Nayik, G.A. Bioplastics and food packaging: A review. *Cogent Food Agric.* **2015**, *1*, 1117749. [CrossRef]
21. Karan, H.; Funk, C.; Grabert, M.; Oey, M.; Hankamer, B. Green bioplastics as part of a circular bioeconomy. *Trends Plant Sci.* **2019**, *24*, 237–249. [CrossRef]
22. Zhao, X.; Cornish, K.; Vodovotz, Y. Narrowing the gap for bioplastic use in food packaging: An update. *Environ. Sci. Technol.* **2020**, *54*, 4712–4732. [CrossRef]
23. Singh, S.; Chunglok, W.; Nwabor, O.F.; Chulrik, W.; Jansakun, C.; Bhoopong, P. Porous biodegradable sodium alginate composite fortified with Hibiscus sabdariffa L. calyx extract for the multifarious biological applications and extension of climacteric fruit shelf-life. *J. Polym. Environ.* **2020**, *31*, 922–938. [CrossRef]
24. Helland, H.; Leufvén, A.; Bengtsson, G.; Pettersen, M.K.; Lea, P.; Wold, A. Storage of fresh-cut swede and turnip: Effect of temperature, including sub-zero temperature, and packaging material on sensory attributes, sugars and glucosinolates. *Postharvest Biol. Technol.* **2016**, *111*, 370–379. [CrossRef]
25. Signore, A.; Amoruso, F.; Gallegos-Cedillo, V.M.; Gómez, P.A.; Ochoa, J.; Egea-Gilabert, C.; Costa-Pérez, A.; Domínguez-Perles, R.; Moreno, D.A.; Pascual, J.A.; et al. Agro-Industrial Compost in Soilless Cultivation Modulates the Vitamin C Content and Phytochemical Markers of Plant Stress in Rocket Salad (*Diplotaxis tenuifolia* (L.) DC.). *Agronomy* **2023**, *13*, 544. [CrossRef]
26. Amoruso, F.; Signore, A.; Gómez, P.A.; Martínez-Ballesta, M.C.; Giménez, A.; Franco, J.A.; Fernández, J.A.; Egea-Gilabert, C. Effect of saline-nutrient solution on yield, quality, and shelf-life of sea fennel (*Crithmum maritimum* L.) plants. *Horticulturae* **2022**, *8*, 127. [CrossRef]
27. Zapata, S.; Dufour, J.P. Ascorbic, dehydroascorbic and isoascorbic and simultaneous determinations by reverse phase ion interaction HPLC. *J. Food Sci.* **1992**, *57*, 506–511. [CrossRef]
28. Martínez-Zamora, L.; Castillejo, N.; Artés-Hernández, F. Postharvest UV-B and photoperiod with blue + red LEDs as strategies to stimulate carotenogenesis in bell peppers. *Appl. Sci.* **2021**, *11*, 3736. [CrossRef]
29. Castillejo, N.; Martínez-Zamora, L.; Gómez, P.A.; Pennisi, G.; Crepaldi, A.; Fernández, J.A.; Orsini, F.; Artés–Hernández, F. Postharvest LED Lighting: Effect of red, blue, and far red on quality of minimally processed broccoli sprouts. *J. Sci. Food Agric.* **2021**, *101*, 44–53. [CrossRef]
30. Løkke, M.; Seefeldt, H.; Skov, T.; Edelenbos, M. Freshness and sensory quality of packaged wild rocket. *Postharvest Biol. Technol.* **2012**, *73*, 99–106. [CrossRef]
31. Martínez-Sánchez, A.; Marín, A.; Llorach, R.; Ferreres, F.; Gil, M.I. Controlled atmosphere preserves quality and phytonutrients in wild rocket (*Diplotaxis tenuifolia*). *Postharvest Biol. Technol.* **2006**, *40*, 26–33. [CrossRef]
32. Holcroft, D. Water Relations in Harvested Fresh Produce. PEF White Paper No. 15-01. The Postharvest Education Foundation (PEF). 2015. Available online: http://www.postharvest.org/Water%20relations%20PEF%20white%20paper%20FINAL%20MAY%202015.pdf (accessed on 24 February 2023).
33. González-Buesa, J.; Page, N.; Kaminski, C.; Ryser, E.T.; Beaudry, R.; Almenar, E. Effect of non-conventional atmospheres and bio-based packaging on the quality and safety of *Listeria monocytogenes*-inoculated fresh-cut celery (*Apium graveolens* L.) during storage. *Postharvest Biol. Technol.* **2014**, *93*, 29–37. [CrossRef]
34. Proikakis, C.S.; Mamouzelos, N.J.; Tarantili, P.A.; Andreopoulos, A.G. Swelling and hydrolytic degradation of poly (D, L–lactic acid) in aqueous solutions. *Polym. Degrad. Stab.* **2006**, *91*, 614–619. [CrossRef]
35. Mangin, R.; Vahabi, H.; Sonnier, R.; ChivasJoly, C.; López-Cuesta, J.M.; Cochez, M. Improving the resistance to hydrothermal ageing of flame-retarded PLA by incorporating miscible PMMA. *Polym. Degrad. Stab.* **2018**, *155*, 52–66. [CrossRef]
36. Scarfato, P.; Di Maio, L.; Incarnato, L. Recent advances and migration issues in biodegradable polymers from renewable sources for food packaging. *J. Appl. Polym. Sci.* **2015**, *132*, 48–55. [CrossRef]
37. Gutiérrez, D.; Rodríguez, S. Combined Effect of UV-C and Ozone on Bioactive Compounds and Microbiological Quality of Fresh-Cut Rocket Leaves. *Am. J. Food Sci. Technol.* **2019**, *7*, 71–78. [CrossRef]
38. Giménez, A.; Gómez, P.A.; Bustamante, M.Á.; Pérez-Murcia, M.D.; Martínez-Sabater, E.; Ros, M.; Pascual, J.A.; Egea-Gilabert, C.; Fernández, J.A. Effect of Compost Extract Addition to Different Types of Fertilizers on Quality at Harvest and Shelf Life of Spinach. *Agronomy* **2021**, *11*, 632. [CrossRef]
39. Løkke, M.; Seefeldt, H.; Skov, T.; Edelenbos, M. Color and textural quality of packaged wild rocket measured by multispectral imaging. *Postharvest Biol. Technol.* **2013**, *75*, 86–95. [CrossRef]
40. Mastrandrea, L.; Amodio, M.L.; de Chiara, M.V.; Pati, S.; Colelli, G. Effect of temperature abuse and improper atmosphere packaging on volatile profile and quality of rocket leaves. *Food Packag. Shelf Life* **2017**, *14 Pt A*, 59–65. [CrossRef]
41. Martínez-Sánchez, A.; Luna, M.; Selma, M.V.; Tudela, J.A.; Abad, J.; Gil, M.I. Baby-leaf and multi-leaf of green and red lettuces are suitable raw materials for the fresh-cut industry. *Postharvest Biol Technol.* **2012**, *63*, 1–10. [CrossRef]
42. Saltveit, M.E. A summary of CA requirements and recommendations for vegetables. *Acta Hortic.* **2003**, *600*, 723–727. [CrossRef]

43. Guijarro-Real, C.; Prohens, J.; Rodríguez-Burruezo, A.; Fita, A. Consumers acceptance and volatile profile of wall rocket (*Diplotaxis erucoides*). *Food Res. Int.* **2020**, *132*, 109008. [CrossRef]
44. Cavaiuolo, M.; Ferrante, A. Nitrates and glucosinolates as strong determinants of the nutritional quality in rocket leafy salads. *Nutrients* **2014**, *6*, 1519–1538. [CrossRef] [PubMed]
45. Santamaria, P. Nitrate in vegetables: Toxicity, content, intake and EC regulation. *J. Food Agri.* **2006**, *86*, 10–17. [CrossRef]
46. Santamaria, P.; Elia, A.; Serio, F. Effect of solution nitrogen concentration on yield, leaf element content, and water and nitrogen use efficiency of three hydroponically-grown rocket salad genotypes. *J. Plant Nutr.* **2002**, *25*, 245–258. [CrossRef]
47. European Commission. *Commission Regulation (EU) N1258/2011 of 2 December 2011 Amending Regulation (EC) N1881/2006 as Regards Maximum Levels for Nitrates in Foodstuffs*; European Commission: Brussels, Belgium, 2011.
48. Santamaria, P.; Elia, A.; Papa, G.; Serio, F. Nitrate and ammonium nutrition in chicory and rocket salad plants. *J. Plant Nutr.* **1998**, *21*, 1779–1789. [CrossRef]
49. Signore, A.; Bell, L.; Santamaria, P.; Wagstaff, C.; Van Labeke, M.C. Red light is effective in reducing nitrate concentration in rocket by increasing nitrate reductase activity, and contributes to increased total glucosinolates content. *Front. Plant Sci.* **2020**, *11*, 604. [CrossRef]
50. Selma, M.V.; Martínez-Sánchez, A.; Allende, A.; Ros, M.; Hernández, M.T.; Gil, M.I. Impact of Organic Soil Amendments on Phytochemicals and Microbial Quality of Rocket Leaves (Eruca sativa). *J. Agric. Food Chem.* **2010**, *58*, 8331–8337. [CrossRef]
51. Martínez-Sánchez, A.; Gil-Izquierdo, A.; Gil, M.I.; Ferreres, F. A comparative study of flavonoid compounds, vitamin C, and antioxidant properties of baby leaf *Brassicaceae* species. *J. Agric. Food Chem.* **2008**, *56*, 2330–2340. [CrossRef] [PubMed]
52. Duyar, H.; Kiliç, C.C. A research on production of rocket and parsley in floating system. *J. Agric. Sci.* **2016**, *8*, 54–60. [CrossRef]
53. Conesa, E.; Niñirola, D.; Vicente, M.J.; Ochoa, J.; Bañón, S.; Fernández, J.A. The influence of nitrate/ammonium ratio on yield quality and nitrate, oxalate and vitamin c content of baby leaf spinach and bladder campion plants grown in a floating system. *Acta Hortic.* **2009**, *843*, 269–274. [CrossRef]
54. Bonasia, A.; Conversa, G.; Lazzizera, C.; Elia, A. Post-harvest performance of ready-to-eat wild rocket salad as affected by growing period, soilless cultivation system and genotype. *Postharvest Biol. Technol.* **2019**, *156*, 110909. [CrossRef]
55. Renna, M. Reviewing the prospects of sea fennel (*Crithmum maritimum* L.) as emerging vegetable crop. *Plants* **2018**, *7*, 92. [CrossRef] [PubMed]
56. Gutiérrez, D.; Chaves, A.; Rodríguez, S. UV-C and ozone treatment influences on the antioxidant capacity and antioxidant system of minimally processed rocket (*Eruca sativa* Mill.). *Postharvest Biol. Technol.* **2018**, *138*, 107–113. [CrossRef]
57. Podsedek, A. Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *LWT Food Sci. Technol.* **2007**, *40*, 111. [CrossRef]

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Testing the Greenhouse Emission Model (GEM) for Pesticides Applied via Drip Irrigation to Stone Wool Mats Growing Sweet Pepper in a Recirculation System

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Abstract: Pesticide emissions to surface water from greenhouses with crops grown on substrates in open or closed systems may be significant. It is important, therefore, to test models such as the Greenhouse Emission Model (GEM), which was developed to assess these emissions as part of the Dutch authorization procedure for use of plant protection products in greenhouses. GEM was tested using an experiment in which imidacloprid and pymetrozine were applied via drip irrigation to stone wool mats growing sweet pepper. The irrigation system in such greenhouses consists of a mixing tank to prepare the nutrient solution and a series of tanks to treat and recirculate the drain water back to the mixing tank. Emissions may occur because (part of) this recirculation water may be discharged or leached to the surface water. GEM assumes that all tanks are perfectly mixed. GEM further assumes that the water in these mats is perfectly mixed and that the pesticide behavior can be simulated by assuming one perfectly mixed reservoir. The model predicted breakthrough of both pesticides out of the mats earlier than measured, and the measured maximum concentrations were approximately two times lower than predicted. We considered a series of possible causes, including a smaller water volume in the mats, a higher plant uptake factor, and sorption to the stone wool. The model performance improved by representing the mats as a sequence of two equally large tanks with plant uptake restricted to the first tank. We recommend to study the solute transport process and the distribution of plant roots in the mats in more detail to further underpin the hypothesis used and improve the model. After this first validation, the GEM model might also be used in other countries to forecast emissions of PPPs to surface water.

Keywords: pesticide emission to surface water; greenhouse emission model; model testing; drip irrigation; soilless cultivation; pymetrozine; imidacloprid

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1. Introduction

In the Netherlands, the area of greenhouses grown with vegetables or flowers is currently approximately 10,000 ha, of which approximately 8500 ha are soilless growing systems with substrates such as stone wool, peat, perlite, and coir [1,2]. The surface area of greenhouses is only a small fraction of the total Dutch agricultural area (nearly 2 million ha [3]). However, pesticide monitoring data for Dutch surface water have shown that pesticide use in greenhouses led to more exceedances of the acceptable concentrations in surface water than any of the agricultural land uses [4]. This may be partly caused by the higher pesticide use in terms of kg per ha for crops grown in greenhouses than for field crops [5]. Another cause may be that for the same application rate (dose), pesticide emissions from greenhouses to surface water are higher than from agricultural fields. Although excess irrigation water from soilless growing systems is reused (i.e., recirculated), part of this recirculation water may be emitted to the surface water to warrant good quality of irrigation water and prevent, for example, the sodium concentration from becoming too high [6]. Therefore, the assessment of pesticide emissions from soilless growing systems

in greenhouses is an important aspect of the Dutch pesticide registration procedure. The Greenhouse Emission Model (GEM) was developed to assess such emissions and calculate pesticide concentrations in the water that is discharged to nearby surface water [7]. The EU Water Framework Directive (EU, 2000) aimed to have ecologically and chemically sound surface water in 2015, with an additional 12 years if goals could not be achieved. The year 2027 is the target year for Dutch growers to have achieved the sound water quality goal as indicated in the WFD, and GEM is a tool to support the achievement of that goal. Although rural situations in other parts of Europe are often different, there is great interest in applying GEM in other regions with a high density of greenhouses or large areas of surface water.

For model acceptance in the pesticide registration procedure, it is important that the model has been tested against experimental data. The GEM model consists of a sub-model for the greenhouse water flows (the Waterstreams model, earlier described and tested [8]) and a sub-model for simulating the pesticide behavior in the greenhouse (called SEM: Substance Emission Model). The concentration in the water course to which excess water is discharged is calculated thereafter with a surface water model that simulates the solute transport, degradation, and adsorption processes in the water course. This work aims to test the SEM sub-model. An earlier study showed that an adequate test of SEM requires that the water flows in the experiment are measured in detail [9]. This work provides a test of the SEM model based on an experiment in which water flows were measured frequently and at various locations within the greenhouse [10].

There is a variety of growing systems (vegetables, pot plants, and flowers) and substrate types (stone wool, perlite, coir, and lava) used in greenhouses. The combination vegetables–stone wool, which covers approximately 4000 from the 8500 ha soilless growing systems in the Netherlands[2], was selected for the test. Here, a full nutrient solution is given to the plants, with the surplus (20–30%) being collected and reused after disinfection. Due to unbalanced nutrient compositions and accumulation of non-absorbed ions (sodium and chloride), part of the solution (1–10% annually) was discharged to the surface water [6]. Pesticides can be applied via spray application, low volume misting, or drip irrigation. We selected the application via drip irrigation. Drip irrigation leads to high emission concentrations because the complete dose is applied to the recirculating water, whereas for spray or low volume mister applications (LVM), only a fraction of the dose ends up in the recirculating water and, consequently, in the surface water. Two pesticides were studied, which are applied in commercial practice via the irrigation water, i.e., imidacloprid and pymetrozine. In 2016, these substances were among two of the most frequently used substances in greenhouses applied via drip irrigation. Monitoring data on surface water concentrations show that both substances are found in water courses near greenhouses and that imidacloprid is found at concentrations above the water quality threshold [4]. Note that both substances are no longer approved for use as plant protection products in open and closed cultivations in the Netherlands.

2. Materials and Methods

2.1. Experimental Procedures

The experiment was executed in a greenhouse of Wageningen University and Research in Bleiswijk (the Netherlands). The greenhouse was climate controlled based on incoming radiation and other weather parameters. The experimental compartment had a surface area of 144 m^2 and contained 12 rows of plants. In each row, 25 plants were grown at a distance of 40 cm in stone wool mats (Grodan Grotop Expert; 100 × 12 × 7.5 cm) which were surrounded by plastic foil. This was realized by growing three plants on each mat at a distance of 10, 50, and 90 cm from the start of a mat and with a distance of 20 cm between the mats (Figure 1).

Figure 1. Blocks containing sweet pepper plants placed on top of the stone wool mats in holes in the plastic foil; photo taken before the start of the experiment. Each plant receives irrigation water, pesticide, and nutrients via drip irrigation. Water is collected in drain troughs below the stone wool mats.

Sweet pepper plants (cultivar Marinello) were raised at another location in stone wool blocks with sides also surrounded by plastic foil (Grodan Plantop Delta; 10 × 10 × 7.5 cm). On 7 January 2016, blocks containing seven-week-old plants were transferred to the experimental compartment and placed on top of the stone wool mats in holes in the plastic foil (Figure 1). The experiment began (and pesticides were applied) on 31 May 2016, when the sweet pepper was full grown. The last harvesting date of sweet pepper was early November 2016, so only one growth cycle was considered.

The plants received water via drip irrigation (each plant had one pressure compensated dripper of 3 L/h). The irrigation water was prepared in a mixing reservoir (Figure 2). This included addition of nutrients and adjustment of the pH to 6.2. The water was added to the stone wool mats through PVC pipes (driplines) with an inner diameter of 40 mm and PE (poly-ethylene) pipes with an inner diameter of 16 mm. These drippers were connected to the 16 mm PE pipes by flexible PE tubes with an inner diameter of approximately 4 mm. The day before application of the two substances, i.e., on 30 May 2016, the irrigation was stopped at 16.00 h to obtain a relatively low water content of the mats at the time of application. This is common practice in Dutch greenhouses to obtain a more efficient uptake of the applied pesticide. On the first day, all irrigations were carried out manually, facilitating easy sampling. The volume of the first irrigation was 76 L (5 min) for the entire greenhouse compartment; it took place immediately after application, and it was followed by irrigation volumes of approximately 30 L (2 min) each hour. On the second day, the irrigation scheduling was automatically driven by global radiation; each dripper supplied 100 mL per 200 J/cm^2 radiation.

The base of irrigation water was a mixture of rain water that was collected from the roof of the greenhouse, reverse osmosis water, and drain water from the stone wool mats. Drain water from the mats was collected via coated metal troughs and flowed through PVC pipes to a sequence of reservoirs, as shown in Figure 2. The first reservoir was the filtration unit in which the drain water was filtered through a 3 µm fiber filter. The water was pumped from this unit to the so-called used-water reservoir in batches of approx. 35 L. The water was pumped out of the used-water reservoir in batches of approx. 38 L, i.e., the treatment volume of the subsequent ozone treatment unit. The ozone unit from Agrozone works as a batch reactor in which the water is treated with a redox value of 800 mV. As a consequence, the water level in the used-water reservoir changed in discrete

steps. It appeared that the ozone treatment degraded both pymetrozine and imidacloprid completely; the measured concentrations in treated water were always below the detection limit. After the treatment, the water was collected in a reservoir for cleaned water and then made available for reuse (application of nutrients up to a certain electrical conductivity).

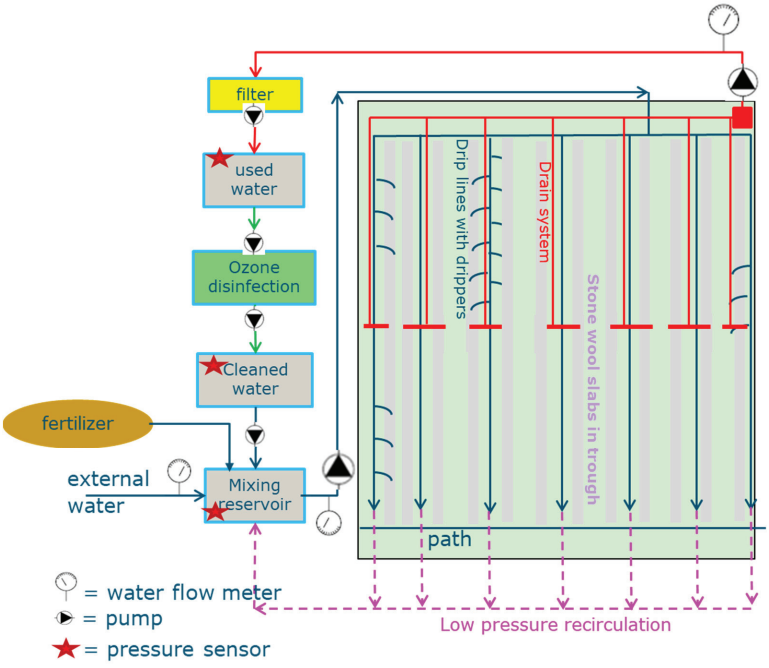

Figure 2. Schematization of the water flows in experimental compartment (surface area 144 m^2). External water (rainwater and osmosis water) is added to the mixing reservoir, in which it is mixed with nutrient solution by a fertilizer system. From the mixing reservoir, it is pumped to the dripping system via drip lines to the plants. The drain water is collected via troughs in the drain system and added to the recirculation system. Before reuse, the water is filtrated and disinfected. The pesticides are added to the mixing reservoir. The low-pressure recirculation is installed to make sure that all plants receive water with the same mixture of nutrients and with the same concentration of pesticides.

Floaters determined whether renewed filling of the mixing reservoir was required. After each renewed filling of the mixing reservoir, the solution was circulated under low pressure (below 0.8 bar) through the pipe lines, flowing back to the mixing reservoir in order to achieve a constant mixture of nutrient solution over the dripline system. During this low-pressure circulation (1–5 min), the so-called pressure-compensated drippers were closed. The advantage of using a drip irrigation system with low-pressure circulation is that all plants receive water with the same mixture of nutrients and with the same concentration of pesticides.

As shown in Figure 2, water volumes were measured in the mixing reservoir, the used-water reservoir, and the cleaned-water reservoir every 5 min with automatic pressure sensors. The volume of water in the cultivation compartment was based on measurements of the water content of the stone wool, which was measured every 3 min in duplicate with Grodan frequency–domain water sensors (based on measurement of the dielectric constant in the stone wool. The volumetric water content is highly correlated with the dielectric constant). These sensors consisted of three metal pins with lengths of 6 cm, which were horizontally placed in the stone wool in the middle between two plants. It resulted in 5–7 irrigations per day and a drain percentage of the surplus of 30%. The cumulative

water flow between the mixing reservoir and the cultivation compartment and between the cultivation compartment and the filtration unit were measured every 5 min with water volume counters. In addition, the volume of added rainwater was measured. During the experiment, there was no discharge of recirculation water to the surface water. Air temperature in the greenhouse was measured every 5 min.

The pesticides were applied to the mixing reservoir at approximately 10 h on 31 May as water-dispersible granulates, which contained 204 L of water. This included 41 L of water in the pipes used for the circulation of water under low pressure. Granulates were dissolved in 1 L water before being added to the mixing tank. A mass of 2.25 g of pymetrozine was applied as the formulated product Plenum (containing 50% pymetrozine), and a mass of 2.94 g imidacloprid was applied as the formulated product Admire (containing 70% imidacloprid). Imidacloprid is a neonicotinoid with the molecular formula $C_9H_{10}C_1N_5O_2$. Pymetrozine is a neuroactive insecticide and a member of the class of 1,2,4-triazines. It has the molecular formula $C_{10}H_{11}N_5O$. These application amounts were in line with the recommended doses on the label. Initial concentrations in the mixing reservoir, which were measured in duplicate, were 13,204 µg/L and 16,002 µg/L for imidacloprid and 8579 µg/L and 10,741 µg/L for pymetrozine. Samples for analysis of pesticide concentrations were taken in duplicate from the mixing reservoir and the used-water reservoir every two hours during working hours on the first two days. In addition, samples from the clean water reservoir were taken. Concentrations in these samples were all below the detection limit. The first sampling of the mixing reservoir took place immediately after application, i.e., before the circulation under low pressure (see above) took place. After two days, samples were taken only once a day. Samples were transferred to the lab and stored both prior and after analysis in a refrigerator at 4 °C (range: 2 to 8 °C). All samples were analyzed by reversed-phase liquid chromatography–tandem mass spectrometry (LC-MS/MS) after dilution with methanol: ultrapure water (15/85, *v/v*). The analyses were performed on an Agilent 1260 Infinity liquid chromatograph coupled with a 6460 Triple quad mass spectrometer (LC-MSMS) and equipped with Agilent jet stream electrospray ionization source (AJS-ESI) (Agilent Technologies, Santa Clara, California, USA).

Separations were carried out on an Agilent Eclipse XDB C18 column (4.6 × 150 mm, 5 µm) at 40°. The injection volume of the samples was set to 40 µL. The mobile phase used was Milli-Q water with 0.1% Formic acid (C) and MeOH with 0.1% Formic acid (D), with the following gradient: 0–5 min: 70/30 (C/D, *v:v*); 5.00–5.20 min: from 70/30 (C/D, *v:v*) to 10/90 (C/D, *v:v*); 5.20–8.20 min: hold on 10/90 (C/D, *v:v*); 8.20–8.30 min: from 10/90 (C/D, *v:v*) to 70/30 (C/D, *v:v*); and 8.30–11.00 min: hold on 70/30 (C/D, *v:v*) at flow rate of 0.5 mL/min. The mass spectrometer was operated using AJS-ESI in the positive mode. Nitrogen was used both as nebulizer and collision gas, the capillary voltage was 3500 V, and the temperature of the ion source was set to 300 °C.

The compounds were detected in the multiple reaction monitoring (MRM) using two transition per compound: imidacloprid 256.1/209 m/z and 256.1/175.1 m/z and pymetrozine 218.1/105 m/z and 218.1/78.1 m/z. Retention time was 3.6 min. for pymetrozine and 9.5 min. for imidacloprid.

Injected samples were quantified by peak area using a linear and forced-through-the-origin (x-axis zero; y-axis zero) calibration curve constructed from external standards included in the same sample sequence. Agilent Masshunter software was used for instrument control and data acquisition.

The detection limits (LOD) of imidacloprid and pymetrozine were 0.04 µg/L and 0.03 µg/L, respectively. The limit of quantifications (LOQ) were 0.12 µg/L and 0.10 µg/L, respectively. All data were collected in a free accessible experimental dataset [11].

2.2. Model Description

The SEM sub-model conceives of a greenhouse as a number of interconnected reservoirs that exchange water and solutes and in which each reservoir the water is perfectly mixed. This seems a priori defensible for the mixing reservoir, the filtration unit, and the

used-water reservoir, as these are water tanks. However, this can be called into question for the cultivation compartment (i.e., the stone wool mats), as the water flow through the mats is driven by gravity and by suction from the plant roots, which is likely to result in a solute movement process that differs from complete mixing. Nevertheless, complete mixing was assumed (as a starting point), this being the simplest approach possible and because no further information on flow processes in drip-irrigated rooted stone wool mats was available. Plant uptake was assumed to be proportional to the transpiration rate of the plants and the pesticide concentration in the water using the concept of the so-called transformation stream concentration factor (TSCF) [12]. TSCF indicates the efficiency of the translocation of a chemical in a root. The conservation equation for the mass of pesticide in each tank with number i with upstream tanks j and downstream tanks k is then given by

$$\frac{d\,m_i}{dt} = +\sum_{j=1}^{\nu} Q_{fl,j,i}\, c_{w,j} - \sum_{k=1}^{\lambda} Q_{fl,i,k}\, c_{w,i} - V_{w,i}\, k_{t,i}\, c_{w,i} - Q_{up,i}\, TSCF\, c_{w,i} \quad (1)$$

where m_i is the mass of pesticide in tank i (kg), ν is the number of incoming water fluxes, $Q_{fl,j,i}$ is the volume rate of water flow (m^3/d) from tank j to tank i, $Q_{fl,i,k}$ is the volume rate of water flow (m^3/d) from tank i to tank k, $c_{w,j}$ is the mass concentration of pesticide in the water of tank j (kg/ m^3), λ is the number of outgoing water fluxes, $c_{w,i}$ is the mass concentration of pesticide in the water of tank i (kg/m^3), $V_{w,i}$ is the volume of water in tank i (m^3), $k_{t,i}$ is the rate coefficient of transformation of the pesticide in tank i (d^{-1}) assuming first-order kinetics, where $k_{t,i} = \mathrm{Ln}(2)/\mathrm{DT50}$ and DT50 (d) is the transformation half-life of the pesticide, $Q_{up,i}$ is the volume rate of uptake of water by plant roots (m^3/d) which is zero for all tanks except the cultivation tank, and $TSCF$ is the transpiration stream concentration factor of the pesticide (-).

Figure 3 shows the model configuration as it was tested against the experimental data. Each reservoir had only a single outgoing flux to another tank so no summation of the outgoing mass fluxes in Equation (1) was needed in this model test.

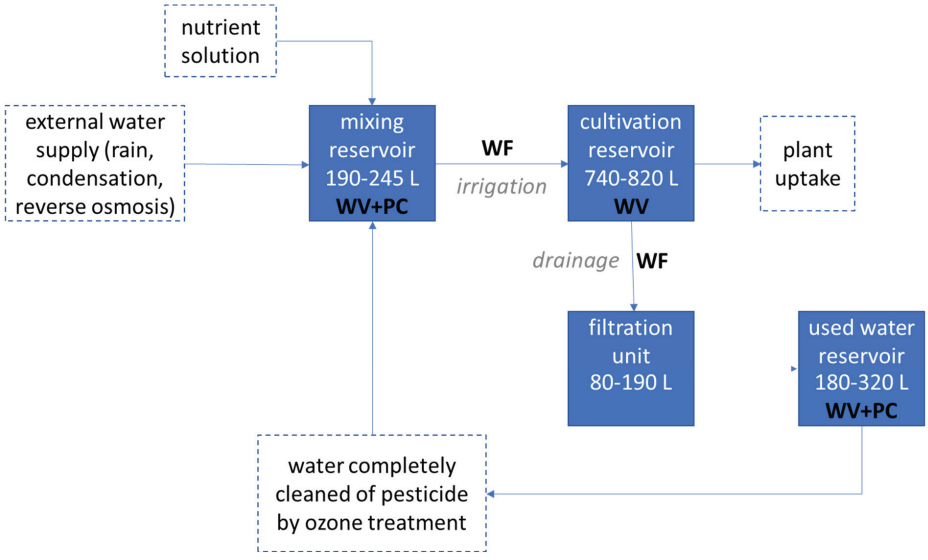

Figure 3. Schematic representation of the greenhouse system. The numbers indicate the range of the volumes of water in the reservoirs, WV indicates that the water volume in the reservoir was measured, WF indicates that the water flow rate was measured between the reservoirs in total volume per 5 min, and PC indicates that the pesticide concentration was measured in the reservoir. The blue boxes are considered in the model testing.

The mass of pesticide in each tank m_i equaled $V_{w,i} * c_{w,i}$, so adsorption to the stone wool or any other material was not considered. The rate coefficient of transformation $k_{t,i}$ was assumed to increase with temperature following the Arrhenius equation (see [7]).

2.3. Model Parameterization

As described before, water flows and volumes were measured. However, some processing and interpretation of the measurements was needed to transfer them into a complete set of water volumes and water flow rates required for the model test.

The time course of the water volume in the cultivation reservoir was derived from duplicate measurements of the water content in the stone wool mats using the average of these measurements. As described before, the stone wool growing system consisted of mats (height 7.5 cm and volume 9 L each) on top of which three blocks were placed (height 7.5 cm and volume of 0.75 L each, so 2.25 L in total). Measurements of pF curves of stone wool [13] show that stone wool loses most of its water when the suction pressure of the water increases from zero (i.e., saturated) to 20 hPa: the volume fraction of water at saturation is approximately 0.98, whereas it is only approximately 0.20 at a suction pressure of 20 hPa. During most of the time, the bottoms of the stone wool mats were saturated (i.e., at zero suction, leading to drainage flow), whereas the top of the blocks (15 cm higher than this bottom) may have had a suction pressure close to 15 hPa (1 hPa corresponds to a pressure of a water layer of 1 cm). It is likely, therefore, that the volume fraction of water in the blocks is considerably lower than that in the mats. Thus, it was assumed (as a best guess) that the volume fraction of the water in the blocks was half that of the mats. So, the measured volume of water was based on a combined mat plus blocks volume of 10.12 L instead of the total rock wool volume of 11.25 L. This estimation procedure indicates that the estimated volume of water in the cultivation reservoir is somewhat uncertain (the possible effect of this uncertainty on pesticide behavior will be addressed later).

As indicated in Figure 2, the inflow of water into the mixing reservoir was not measured. However, it could be derived from the time courses of the water volume in and the water outflow from this reservoir. The water uptake rate of the crop was derived from the water balance of the cultivation reservoir (by combining the difference between inflow and outflow rates with the change in water volume in the mats). In addition, the water volume and the water outflow of the filtration unit were not measured. This water outflow could be derived from the stepwise increases in the water volume of the used-water reservoir. The water volume of the filtration unit could be derived from the difference between the measured inflow and the estimated water outflow (after deriving the initial volume via a measurement of the water height). The outflow of the used-water reservoir could be derived from the stepwise decreases in the water volume in this reservoir. Thus, a complete set of time courses of flow rates and water volumes (changing every 5 min) could be derived. The test of the model was limited to these four days.

The temperature in the cultivation reservoir was assumed to be equal to the air temperature in the greenhouse.

The dosages of the pesticides were based on the masses added to the mixing tank as described in the experimental procedures. The TSCF depends on the lipophilicity of a compound as shown by Briggs et al. (1982) [12]. It was estimated from the octanol–water partition coefficient using the equation:

$$TSCF = 0.784e^{-[(\log K_{ow} - 1.78)^2/2.44]} \quad (2)$$

This returned 0.43 for imidacloprid on the basis of its octanol water coefficient (K_{ow}) of 3.7 [14] and 0.16 for pymetrozine on the basis of its K_{ow} of 0.646 [15]. The half-life for transformation in the water was based on available hydrolysis half-lives. The hydrolysis half-life of imidacloprid was set at 1000 d at 25 °C because imidacloprid is reported to be stable [12]. The hydrolysis half-life of pymetrozine is 5–12 d at 25 °C and pH = 5, and it is reported to be stable at pH = 7. The half-life was assumed to be the average of 5 and 12 d, so

8.5 d at 25 °C. This may overestimate the hydrolysis transformation rate somewhat because the pH of the mixing tank was kept at 6.2. The molar enthalpy of the transformation rate (input to the Arrhenius equation) was assumed to be 65 kJ/mol (based on that for transformation in soil in the absence of better information [16]).

3. Results

The air temperature in the greenhouse showed a diurnal pattern with daily minima of 18–20 °C and daily maxima of 23–28 °C; daily average temperatures were 21–23 °C. Figure 4 shows that irrigation was restricted to the daytime (driven by the requirement of 100 mL irrigation for each dripper per 200 J/cm^2 radiation, as described before). The average daily irrigation volume was approximately 300–500 L, which corresponds to a water layer of approximately 2–4 mm for the 140 m^2 surface area of the compartment. The figure shows also that the drainage amount was, on average, approximately 30% of the irrigation amount, which is according to grower practices. The time of the start of the drainage outflow was closely linked to the time of irrigation inflow: detailed inspection showed that drainage started typically at approximately 1 h after the start of irrigation (please note that this does not mean that the residence time of a droplet of irrigation water in the cultivation unit is approximately 1 h: the irrigation induces a downward water flow which likely leads to drainage of water that was already present at the bottom of the mat). Detailed inspection revealed also that the first drainage occurred approximately 2 h after the first irrigation event and approximately 1 h after the second event. This 1-h delay after the first event was the result of the relatively low water content of the mats at the start.

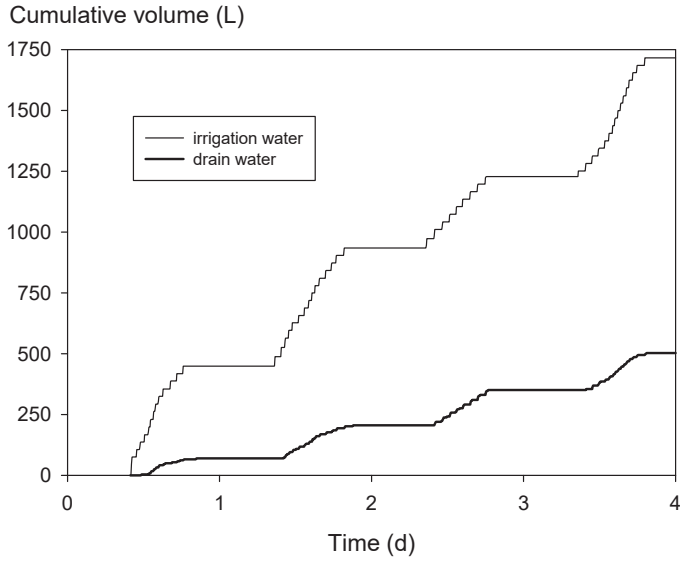

Figure 4. Cumulative irrigation and drain water volumes as a function of time as measured in the experiment. Irrigation is the flow from the mixing reservoir to the cultivation reservoir, and drainage is the flow from the cultivation reservoir to the filter reservoir. Time zero is 00.00 h 31 May.

Figure 5 shows that the time courses of the water content in the mats as measured with the two sensors were very similar. These time courses were strongly linked to the irrigation pattern: during the daytime, the water contents increased stepwise due to irrigation events followed by decreases until the next irrigation event; during the nighttime, there was a slow decrease. Using the average of the two water contents to estimate the time course of the water volume in the mats (as described before) resulted in a water volume in the cultivation reservoir ranging between 740 and 820 L. Figure 5 shows also that the two water contents

differed from each other by approximately 15%. In combination with the uncertainty in the water content of the blocks (see Model parameterization), we estimate that the uncertainty in the water volume of the cultivation reservoir (i.e., the 95% confidence interval) to be approximately ±25%. This uncertainty will be considered in the test of the model.

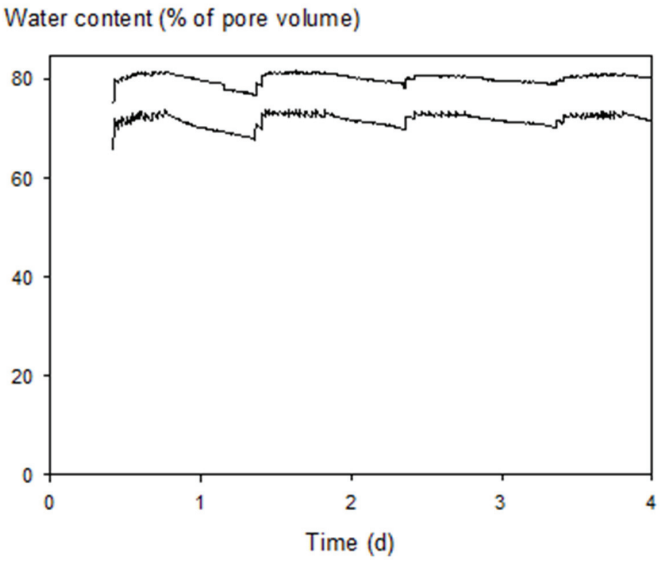

Figure 5. Water content (as % of pore volume) in the stone wool as a function of time as measured with duplicate sensors in two stone wool slabs. Time zero is 00.00 h 31 May.

On the basis of the added pesticide masses and the measured initial volume of the mixing tank, initial concentrations of imidacloprid and pymetrozine were expected to be 14.4 and 11.0 mg/L, respectively. Initial concentrations in the mixing tank were measured in duplicate within 6 min after application (before the first, manual started irrigation event) and were found to be 13.2 and 16.0 mg/L (average 14.6 mg/L) for imidacloprid and 8.6 and 10.7 mg/L (average 9.7 mg/L) for pymetrozine. So, for imidacloprid, the measured concentration was a few percentage points higher than expected, and for pymetrozine, it was approximately 10% lower. In view of the approximate 20% difference between the duplicate samples, it is likely that the mixing was not yet complete at the first sampling despite the thorough mixing of the water in the mixing tank. Later sampling times could not be used to check the dose because these took place after the first irrigation event.

Figure 6 shows that measured and simulated concentrations in the mixing tank corresponded quite well. Note that the horizontal axis does not denote time but rather the cumulative water volume that flowed out of the tank. This is chosen because this cumulative volume is the driving force for the decrease. This good correspondence was to be expected, as the uncertainty resulting from the model assumptions and the parameter values is quite small for this tank, i.e., the only relevant processes are perfect mixing and degradation. For imidacloprid, no degradation was assumed by using a half-life of 1000 d; for pymetrozine, a half-life of 8.5 d was assumed at 25 °C. In Figure 6, a cumulative volume of irrigation water of 1000 L corresponds to a time period of approximately 1 day (see also Figure 4), so degradation of pymetrozine hardly influenced these simulated concentrations. The possible incomplete mixing during the first sampling did not lead to an increased difference between measured and simulated concentrations.

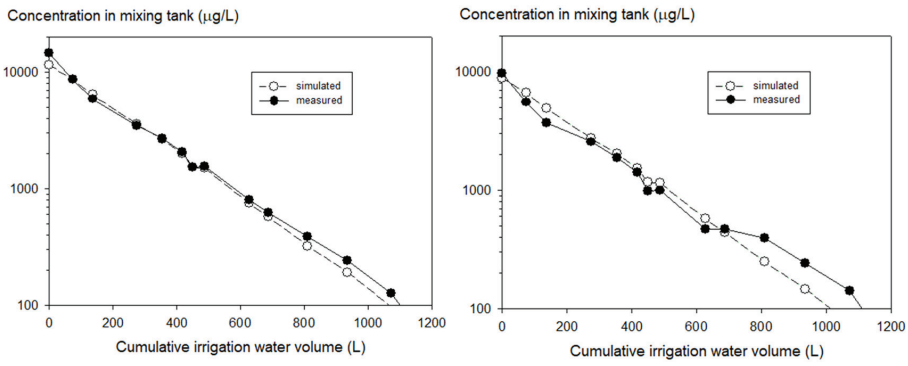

Figure 6. Measured and simulated concentrations of imidacloprid (**left**) and pymetrozine (**right**) in the mixing tank as a function of the cumulative volume of irrigation water, i.e., the water that was pumped out of the mixing tank. Irrigation occurred in batches every 2–3 h, and the mixing tank was refilled. Concentrations were measured in duplicate, and the average value is shown in the graphs.

Figure 7 shows that simulated breakthrough of both pesticides in the used-water reservoir was faster than measured and that simulated concentrations at the end of the model test were approximately two times higher than measured (we plotted here on the horizontal axis the cumulative volume of water that was discharged into this reservoir, as concentration changes in this reservoir are driven by this inflow). This factor of two is rather high for a model, especially when used in regulatory practice.

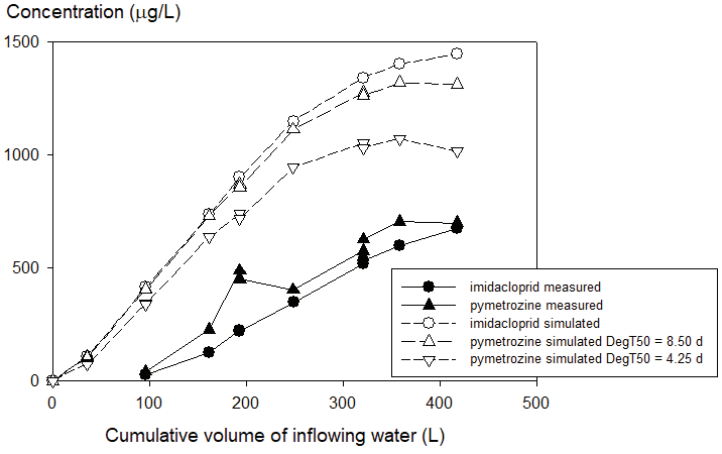

Figure 7. Measured and simulated concentrations of imidacloprid and pymetrozine in the used-water tank as a function of the cumulative water volume flowing into this tank. Simulations were based on first estimates for all parameters except the run with the shorter half-life for pymetrozine. Both measured and simulated concentrations of pymetrozine were multiplied by 2.94/2.25 to account for the difference in dosage between imidacloprid and pymetrozine.

Possible causes for the too-high simulated concentrations are: (i) more dilution in the cultivation reservoir than simulated due to an estimated too-low water volume in the mats, (ii) more plant uptake than simulated due to a too-low TSCF or partitioning into the plant roots (not included in the model, which considers only uptake due to transpiration), (iii) faster degradation in the rooted stone wool than simulated, (iv) significant sorption to the stone wool mats or transport pipes (sorption is not included in the model).The faster simulated breakthrough may also have been due to incomplete mixing in the cul-

tivation reservoir. Hereafter, we will consider these possibilities one by one and discuss their plausibility.

As described before, we consider the uncertainty in the water volume in the cultivation reservoir to be approximately 25%. Thus, we made indicative calculations assuming a 25% higher volume, as a higher volume will lead to lower calculated concentrations. Results in Figure 8 show that increasing the volume indeed led to a lower simulated concentration. However, the figure also shows that the uncertainty in the water volume of the cultivation reservoir is unlikely to be responsible for the poor performance of the model, as the effect is relatively small.

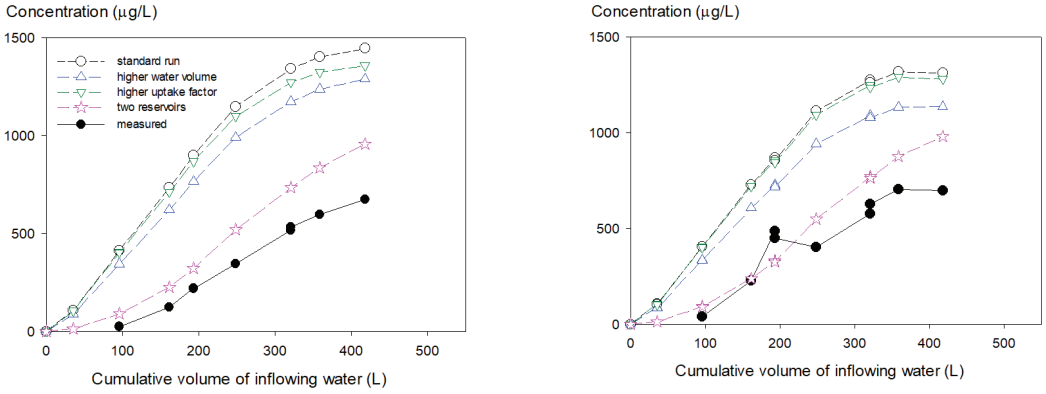

Figure 8. Measured and simulated concentrations of imidacloprid (**left**) and pymetrozine (**right**) in the used-water tank as a function of the cumulative water volume flowing into this tank. The standard run was based on first estimates of the parameters (also shown in Figure 7); the run with the higher water volume assumed a 25% increase in the volume of water in the cultivation reservoir; the run with higher uptake factor assumed a 25% increase in the TSCF; for the run with the two reservoirs, the cultivation reservoir was divided into two reservoirs of equal size with plant uptake only from the first reservoir. Both measured and simulated concentrations of pymetrozine were multiplied by 2.94/2.25 to account for the difference in dosage between imidacloprid and pymetrozine.

The possible effect of increased plant uptake was checked by performing calculations with a 25% higher TSCF (i.e., 0.54 instead of 0.43 for imidacloprid and 0.20 instead of 0.16 for pymetrozine). Figure 8 shows that this decreased the simulated concentrations only to a small extent. It was a priori already somewhat unlikely that the TSCF could be responsible for the discrepancies in view of the large difference between the two TSCFs (0.43 versus 0.16), while the discrepancies were similar for the two pesticides. Furthermore, increasing the TSCF did not lead to a slower breakthrough. Thus, it is unlikely that uncertainty in the TSCF was responsible for the difference between simulated and measured concentrations.

The model considers only plant uptake that is proportional to the transpiration rate using the TSCF concept. However, additionally plant uptake by partitioning into the roots will take place (this was not yet included in the model because only very limited information on the fresh root mass in stone wool mats was available). This partitioning can be described by the concept of the so-called root concentration factor (RCF [10]). This RCF is defined as the concentration in the roots (i.e., mass of pesticide in roots per mass of wet roots) divided by the concentration in the nutrient solution. Briggs et al. (1982) [12] established a relationship between the RCF and the K_{ow}, showing that the RCF increases with increasing K_{ow}. This relationship gives an RCF of 0.84 L/kg for pymetrozine and of 0.90 L/kg for imidacloprid. Assuming equilibrium between the roots and the solution,

Boesten and Matser (2017) [17] showed that the fraction of the total pesticide mass in the cultivation reservoir present in the roots (f_r) is given by

$$f_r = \frac{M\,RCF}{V + M\,RCF} \tag{3}$$

where M is mass of wet roots (kg), and V is volume of water (L) in the system. From measurements for a full-grown sweet pepper crop, they estimated that M equals approximately 0.1 kg if V is approximately 1 L. This gives an f_r of 0.08 (i.e., 8%) for both pymetrozine and imidacloprid. Thus, it is unlikely that partitioning into the plant roots explains the difference between modelled and measured concentrations. For pesticides with a much larger K_{ow}, the partitioning into plant roots may have a considerable effect on simulated concentrations. However, such pesticides are unlikely to be applied with the irrigation water because their translocation to the above ground parts of the plants is very limited, as such pesticides have low TSCF values [12].

Boesten et al. (2018) [18] reviewed available information on degradation half-lives of pesticides in rooted stone wool growing systems and compared these with hydrolysis studies. They found reliable information for metalaxyl, oxamyl, dimethomorph, fluopyram, and imidacloprid. All these pesticides were stable in hydrolysis studies in the relevant pH range. For metalaxyl, half-lives of 5 and 6 d were found. For the other pesticides, only lower limits of the half-lives could be derived: much larger than 22 d for oxamyl and much larger than 6 d for the other three pesticides (including imidacloprid). So, it is unlikely that a faster degradation of imidacloprid (than assumed on the basis of hydrolysis) could explain the difference between simulated and measured drainage concentrations. However, in view of the short half-lives of metalaxyl, it is possible that the half-life of pymetrozine was considerably shorter than derived from the hydrolysis rates. So, we made a calculation assuming a half-life of 4.25 d (i.e., half the value used before and close to the half-live of metalaxyl). Figure 7 shows that this did not lead to a significant improvement of the description of the measurements for pymetrozine. So, both for imidacloprid and pymetrozine, it is unlikely that faster degradation in the rock wool can explain the differences between measured and simulated drainage concentrations.

Given that the efficacy of a pesticide depends on its availability, it is very unlikely that the overestimation of the concentration is due to sorption. To explore the potential impact of sorption on the calculated concentrations, we found that Boesten and Matser (2017) [15] measured the sorption of pymetrozine (with a K_{ow} of 0.65) to Grotop stone wool and found a linear sorption coefficient of 0.2 L/kg. They estimated that this sorption would lead to a decrease in the concentration in the water in the stone wool mats of approximately 2%. Using sorption measurements from the literature of two pesticides (K_{ow} 1000–10,000) and including their sorption measurement of dimethomorph (K_{ow} 479) showed a positive correlation between the octanol water coefficient (K_{ow}) and sorption to stone wool. These three pesticides showed sorption coefficients between 1 and 2 L/kg. For dimethomorph, they estimated a decrease in the concentration in liquid phase due to sorption of 9%. The K_{ow} of imidacloprid (3.7) is much closer to that of pymetrozine (0.65) than to those of these three pesticides (479–10,000). As a result, its sorption coefficient to stone wool is likely much closer to 0.2 L/kg than to 1 L/kg. Therefore, sorption of imidacloprid to the stone wool will likely lead to a concentration decrease that is only slightly higher than the 2% found for pymetrozine. Boesten and Matser (2017) [15] found that sorption of pymetrozine to the PVC transport pipes was unmeasurably small. It can be expected that this sorption is also related to the K_{ow}. As the K_{ow} values of pymetrozine and imidacloprid are quite close, sorption of imidacloprid to the pipes is expected to be small as well. So, sorption to the stone wool or pipe materials is unlikely to be responsible for the poor performance of the model.

Assuming that the cultivation reservoir behaves as a perfectly mixed reservoir is the simplest approach possible, which was taken. Ideally the model would simulate the water flow and transport in each slab separately while considering the root distribution

in the slabs. The water and pesticide mass would then be collected in the troughs and the transport in the troughs simulated over time. Because, as yet, no studies are available on solute flow processes in stone wool mats grown with crops, we used a simplified model approach, assuming that the entire system of slabs, plants, tubes, and troughs could be simulated as a perfectly mixed reservoir. As a next step, we assumed that solute behaviour in the cultivation reservoir can be described with two sequential perfectly mixed reservoirs of equal size (50%–50%) with plant uptake from both reservoirs (i.e., the next most simple model. The final step in this series would then be to have an infinite number of interconnected reservoirs, each representing a part of the system). This decelerated the breakthrough and lowered the concentrations in the first 200 L of water flowing into the used-water tank but increased even the concentrations after approximately 250 L of water inflow, so this did not improve the correspondence between the measurements and the simulations. We then checked the influence of the size of the reservoirs, assuming that the first and second reservoirs had volumes of 83% and 17%, respectively, of the total cultivation reservoir with plant uptake rates proportional to the volume of the reservoir. This produced almost exactly the same result as the 50%–50% assumption. So, the seize of the two reservoirs had no significant effect on the breakthrough curve.

As a next step, it was tested whether the measurements can be described by inhomogeneous root uptake from the cultivation reservoir. Again, the cultivation reservoir was subdivided into two sequential perfectly mixed reservoirs of equal size, but now the plant uptake took place from only the first reservoir. The rationale behind this step is that the water with solutes first enters the part of the slabs with a higher abundance of roots, and in a next step, it leaches to the troughs and is transported to the next reservoir. So, in the second reservoir, there are no or limited roots to enable the plant uptake. Simulated concentrations in the first reservoir will be higher than those in the second reservoir. Improvement of the model description of the measured concentrations can be obtained only by higher plant uptake, so by assuming plant uptake from the first reservoir only. Simulations with plant uptake only from the second reservoir confirmed that this increased concentrations in the drainage water. Figure 8 shows that assuming plant uptake from only the first reservoir improved the correspondence between simulations and measurements considerably. However, the simulated concentrations are still approximately 25% too high. We checked whether a smaller size of the first reservoir could result in a better description. We did so for imidacloprid because its TSCF is much larger than that of pymetrozine (0.43 versus 0.16), so the effect of a change in plant uptake is expected to be higher for imidacloprid. Decreasing the volume of the first tank from 50% (i.e., equal size) to only 20% of the total cultivation reservoir volume decreased the concentration after approximately 400 L of water inflow from approximately 960 μg/L (Figure 8) to approximately 880 μg/L, so it was still much larger than the measured concentration of approximately 700 μg/L (see Figure 8).

4. Discussion and Conclusions

Testing of the SEM model against presented experimental data showed that although the concentration in the mixing reservoir were relatively well predicted, the concentrations in the waste-water reservoir were poorly predicted. The simulation of the concentrations draining from the stone wool mats could be considerably improved by assuming that the behaviour in the mats can be represented by two sequential perfectly mixed reservoirs with plant uptake only from the first reservoir. One mechanism behind this assumption may be that plant roots are more abundant in the regions where the irrigation water enters the mats because nutrient concentrations will be highest in these regions. Another mechanism may be that there are preferential flow paths of the water in the mats and that roots are concentrated in these flow paths because these paths contain the highest nutrient concentrations. The irrigation water dripped into the three planting blocks (7.5 cm high) whose centres were 40 cm apart, whereas the height of the mats below is only 7.5 cm. It seems likely that, at this point, irrigation leads to downward water flow rates in the mats

(driven by gravity) that are faster in the region below the drippers than in the region in the middle between the drippers. So, this may be a driver for the occurrence of preferential flow.

The irrigation volume was typically 30 L per unit, whereas the total water volume in the mats was 720–840 L. So, each individual irrigation resulted in increases in the water content of the mats of only a few percentage points (as also illustrated by Figure 5), whereas these small increases, nevertheless, resulted in drainage of approximately 30% of the irrigation volume. It seems probable that such small increases led to a solute flow pattern that can be described better by assuming a convection–dispersion model (i.e., a water-displacement model) than by assuming perfect mixing. The numerical solution of a convection–dispersion model is commonly obtained by a series of numerical layers that are each perfectly mixed. So, assuming two sequential reservoirs instead of one is a step in the direction of a convection–dispersion model.

Incomplete mixing of the water in the mats in combination with inhomogeneous water uptake by roots was likely the main cause of the differences between measured and simulated concentrations. However, this conclusion is based on indirect evidence: after elimination of other likely causes of the discrepancies, this combination led to a significant improvement of the description of the measurements. The new version of GEM now represents the cultivation part of the greenhouse by two sequential perfectly mixed reservoirs. It may be tempting to develop a more sophisticated solute flow model for such rooted stone wool mats. However, given the limited data available, we recommend doing so hand in hand with experiments aimed at giving direct evidence and data that quantify and underpin the parameters used in the solute flow model. These experiments could include, for example, slicing the mats into layers and measuring the inhomogeneity of the pesticide concentrations and the plant roots and visualising the water flow paths by adding a coloured tracer to the irrigation water. In addition, measuring the concentrations in the individual drippers and at various location in the troughs would then be advised.

As described before, adding equilibrium partitioning into plant roots using the concept of the root concentration factor RCF (based on a single measurement of the fresh root mass) would have decreased simulated concentrations in the homogeneous cultivation reservoir by approximately 8%. Splitting this reservoir into two equal parts with plant uptake restricted to the first part will likely increase the effect of root partitioning on simulated concentrations to levels above 10% for these two pesticides. It seems advisable, therefore, to include partitioning into plant roots in the model. This will require collection of data on fresh root masses in stone wool mats, as these are hardly available. It is recommended to include this process in addition to the above-described options to account for the inhomogeneous water uptake by the plants.

Since the GEM model is used in the regulatory risk assessment for pesticides, confidence in the model is a prerequisite. This test made clear that testing GEM against experimental data is needed to increase the confidence in the model and to assess and understand which processes are driving the concentration of the recirculation water with the final aim of improving the model concepts. As a first step, we recommend the more detailed experiment as suggested above. In a next step, other application methods, e.g., spraying or low volume misting (LVM), should be considered. For these application methods, additional processes play a role; for example, they determine the entry of the substances in recirculation water, such as deposition on various surfaces in the greenhouse and volatilization. For extending the GEM model application to greenhouse systems in other countries, e.g., Spain or Sweden, it will be worthwhile to assess variants with an open system without recirculation, as emission to surface water will be much higher in such systems.

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Data Availability Statement: The experimental dataset used in this manuscript is available via [11].

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References

1. CBS Website. 2021. Available online: https://opendata.cbs.nl/statline/#/CBS/nl/dataset/81302ned/table?fromstatweb (accessed on 6 April 2023).
2. Raaphorst, M. *Quantitative Information for Greenhouse Horticulture*; Report GTB-5154; Wageningen University & Research: Wageningen, The Netherlands, 2017; 334p.
3. CBS. 2022. Available online: https://www.cbs.nl/en-gb/news/2022/02/less-pesticide-used-in-agriculture (accessed on 6 April 2023).
4. Bestrijdingsmiddelenatlas. 2021. Available online: www.bestrijdingsmiddelenatlas.nl (accessed on 21 March 2023). (In Dutch)
5. Compendium Voor de Leefomgeving. Gebruik van Gewasbeschermingsmiddelen in de Landbouw per Gewas, 2012–2016 | Compendium voor de Leefomgeving (clo.nl). 2022. Available online: www.clo.nl/indicatoren (accessed on 21 March 2023). (In Dutch).
6. Van der Salm, C.; Voogt, W.; Beerling, E.; van Ruijven, J.; van Os, E. Minimising emissions to water bodies from NW European greenhouses; with focus on Dutch vegetable cultivation. *Agric. Water Manag.* **2020**, *242*, 106398. [CrossRef]
7. Van der Linden, A.M.A.; van Os, E.A.; Wipfler, E.L.; Cornelese, A.A.; Ludeking, D.J.W.; Vermeulen, T. Scenarios for Exposure of Aquatic Organisms to Plant Protection Products in the Netherlands Soilless Cultivations in Greenhouses. RIVM Report 2015-0128. 2015. Available online: https://www.rivm.nl/bibliotheek/rapporten/2015-0128.pdf (accessed on 6 April 2023).
8. Voogt, W.; Swinkels, G.J.A.M.; van Os, E.A. Waterstreams: A model for estimation of crop water demand, water supply, salt accumulation and discharge for soilless crops. *Acta Hortic.* **2012**, *957*, 123–130. [CrossRef]
9. Van der Linden, A.M.A.; Hoogsteen, M.J.J.; Boesten, J.J.T.I.; van Os, E.A.; Wipfler, E.L. Fate of Plant Protection Products in Soilless Cultivations after Drip Irrigation: Measured vs. Modelled Concentrations. Interpretation of the 2014 Experiment with the Substance Emission Model. RIVM Report 2016-0063. 2017. Available online: https://www.rivm.nl/bibliotheek/rapporten/2016-0063.pdf (accessed on 6 April 2023).
10. Wipfler, E.L.; Beltman, W.H.J.; Boesten, J.J.T.I.; Hoogsteen, M.J.J.; van der Linden, A.M.A.; van Os, E.A.; van der Staaij, M.; Swinkels, G.L.A.M. Testing of the Greenhouse Emission Model for Application of Plant Protection Products via Drip Irrigation in Soilless Cultivation. Wageningen Environmental Research Report 3004. Available online: https://edepot.wur.nl/522831 (accessed on 6 April 2023).
11. Van Os, E.A.; Wipfler, E.L.; Beltman, W.H.J.; Boesten, J.J.T.I.; Hoogsteen, M.J.J.; van der Linden, A.; van der Staaij, M.; Swinkels, G.L.A.M. *Greenhouse Data Experiment Drip Irrigation 2016*; 4TU.ResearchData; Wageningen University & Research: Wageningen, The Netherlands, 2020. [CrossRef]
12. Briggs, G.G.; Bromilow, R.H.; Evans, A.A. Relationship between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. *Pest. Sci.* **1982**, *13*, 495–504. [CrossRef]
13. Bougoul, S.; Ruy, S.; de Groot, F.; Boulard, T. Hydraulic and physical properties of stonewool substrates in horticulture. *Sci. Hortic.* **2005**, *104*, 391–405. [CrossRef]
14. Lewis, K.A.; Tzilivakis, J.; Warner, D.; Green, A. An international database for pesticide risk assessments and management. *Hum. Ecol. Risk Assess. Int. J.* **2016**, *22*, 1050–1064. [CrossRef]
15. EFSA. Conclusion on the peer review of the pesticide risk assessment of the active substance pymetrozine. *EFSA J.* **2014**, *12*, 3817. [CrossRef]
16. EFSA. Opinion on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil. *EFSA J.* **2007**, *622*, 1–32.
17. Boesten, J.J.T.I.; Matser, A.M. *Sorption of Pymetrozine and Dimethomorph to Substrate Materials*; Wageningen Environmental Research Report 2841; Wageningen University & Research: Wageningen, The Netherlands, 2017; 48p. Available online: https://edepot.wur.nl/425040 (accessed on 6 April 2023).
18. Boesten, J.J.T.I.; Wipfler, E.L.; van Os, E.A.; Hoogsteen, M. *GEM Model for Soilless Cultures: Review of Processes and Suggestions for Improvement*; Wageningen Environmental Research Report 2950; Wageningen University & Research: Wageningen, The Netherlands, 2018; 68p. Available online: https://edepot.wur.nl/495570 (accessed on 6 April 2023).

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Impact of Sodium Hypochlorite Applied as Nutrient Solution Disinfectant on Growth, Nutritional Status, Yield, and Consumer Safety of Tomato (*Solanum lycopersicum* L.) Fruit Produced in a Soilless Cultivation

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Abstract: Soilless crop production is spread worldwide. It is a cultivating technique that enhances yield quality and quantity, thus contributing to both food safety and food security. However, in closed-loop soilless crops, the risk of spreading soil-borne pathogens through the recycled nutrient solution makes the establishment of a disinfection strategy necessary. In the current study, sodium hypochlorite was applied to the recycled nutrient solution as a chemical disinfectant to assess its impact on plant growth, leaf gas exchange, fruit yield, tissue mineral composition, and possible accumulation of chlorate and perchlorate residues in tomato fruits. The application of 2.5, 5, and 7.5 mg L^{-1} of chlorine three times at fortnightly intervals during the cropping period had no impact on plant growth or gas exchange parameters. Furthermore, the application of 2.5 mg L^{-1} of chlorine led to a significant increase in the total production of marketable fruits (total fruit weight per plant). No consistent differences in nutrient concentrations were recorded between the treatments. Moreover, neither chlorate nor perchlorate residues were detected in tomato fruits, even though chlorate residues were present in the nutrient solution. Therefore, the obtained tomatoes were safe for consumption. Further research is needed to test the application of chlorine in combination with crop inoculation with pathogens to test the efficiency of chlorine as a disinfectant in soilless nutrient solutions.

Keywords: soilless; disinfection of nutrient solution; chlorates; perchlorates; tomato classes

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1. Introduction

Independence from the soil as a means of rooting allows the optimization of both physical and chemical characteristics in the root environment, as well as a more effective control of the phytopathogenic microorganisms [1]. These characteristics result in higher crop yields with usually lower production costs, combined with reduced pesticide use and high product quality [2]. Given these shortcomings of soil-based production systems, crop production worldwide has shifted to soilless culture in greenhouses, and one of the main reasons for this development is the more efficient control of soil-borne pathogens [3]. Nevertheless, soilless cultivation provides a free start from pathogens but cannot exclude the incidence of a pathogen infection during the cropping period. Especially in closed soilless systems, there are reports on the spread of phytopathogens through the recycled nutrient solution (NS), leading to complete crop failure [4,5]. In closed-loop soilless cultivation systems, the spread of plant diseases associated with the recycling of the NS has been

suggested to be among the most important problems for growers [6]. Hence, disinfection of the NS by applying chlorine is a common method of NS disinfection in closed-loop soilless crops. However, in the Mediterranean greenhouses, periodic application of chlorine as a NS disinfectant is a common practice also in open soilless crops, which constitute the standard type of soilless systems in the region. Nevertheless, the safety of chlorine application is still an open question, as there are limited data about the possible accumulation of chlorate/perchlorate residues in harvested vegetables originating from soilless cultivations treated with chlorine [7].

Soilless culture is a cultivation technique that can increase the quantity and quality of production while reducing water use [8], thus contributing to the requirements for food security and sustainability [9]. Moreover, many governmental and non-governmental organizations highlight its benefits for food security [10–13]. In fact, the imperative need to meet the increasing nutritional needs of the growing population has upgraded the importance of soilless cultivation as an efficient crop production system [14].

In addition, soilless cultivation is a controlled environment system of agricultural production that could enhance food safety in a crop, thanks to the lower need for application of agrochemicals against soil-borne plant diseases [1] and the much lower risk of contamination with heavy metals originating from polluted soils [13,15]. Nevertheless, it has been pointed out in the literature that there are safety risks also in soilless grown vegetables [16–18], although there are approaches towards resolving such issues [19]. Nonetheless, although soilless culture provides a free start from soil-borne pathogens [8], various soil pathogens have been detected in soilless crops, e.g., fungi, bacteria, viruses, and nematodes [4]. Hence, the need to install disinfection systems in soilless cultivation systems is imperative. The chemical methods of NS disinfection in a soilless culture include the use of chlorine (Cl), chlorine dioxide, bromine, ozone, hydrogen peroxide, etc. [4]. Regarding chlorine, it can be applied in liquid, solid, or gaseous form [20]. The most common method, especially in the Mediterranean basin [21], is the application of Cl in its liquid form as sodium hypochlorite, namely bleach [4,7]. In general, the recommended Cl concentration after the addition of sodium hypochlorite is between 2.5 and 5 mg L^{-1} for the treatment of various fungi and oomycetes, such as species of the genus *Pythium* sp. [22,23], *Phytophthora* sp. [24], *Fusarium oxysporum* f. sp. *dianthii* [25], bacteria such as *Agrobacterium tumefaciens* [26], *CLSV* (cucumber leaf spot virus) [27] and root-knot nematodes (*Meloidogyne javanica*) [28], so that no phytotoxicity is expected. Nevertheless, further research is necessary about the effect of bleach on cultivated plants when used as an NS disinfectant, as there are concerns for sodium (Na^+) and Cl^- accumulation [21] as well as the presence of residues and byproducts toxic to humans in the edible products [4].

Regarding disinfection with sodium hypochlorite, it has been reported that it is likely to lead to the formation of derivatives such as chloramines, organic halogens, and trihalomethanes (chloroform, dichlorobromomethane, bichromobromomethane, and bromoform) [4]. In addition, Bull et al. [29] highlighted that the use of the hypochlorite anion (ClO^-) in the disinfection of NS may be associated with the production of chlorates that can cause acute toxicity in humans [29]. Nevertheless, this risk is associated with the dosage and the frequency of application, as well as the plant part consumed as an edible product.

Exposure of humans to chlorates (ClO_3^-) and perchlorates (ClO_4^-) is expected from residues occurring as by-products of the use of chlorinated disinfectants in food processing and water treatment [30]. From monitoring data collected between 2014 and 2018 [31], residues at quantitative levels were found in various commodities, leading to the establishment of maximum residue levels (MRLs) for chlorates, which were currently set to 0.05–0.7 mg kg^{-1} depending on the commodity [32]. These MRLs are tentative, and the discussion at EU level is still ongoing [33–35]. For tomatoes, the current MRL is set at 0.1 mg kg^{-1}. In a previous investigation [7], the use of potassium hypochlorite (KClO) for disinfection of the NS supplied to a soilless tomato cultivation led to the accumulation of chlorate residues in fruits.

Globally, the tomato is one of the most important vegetables [36]. Additionally, it serves as an important model-organism in plant research [37]. In 2019, tomato was cultivated on 6,117,242.00 hectares with a production of 243,635,433.00 tons worldwide, and the countries with the highest production were China, India, and Turkey [38]. Within this context, the scope of the current work was to investigate the effect of sodium hypochlorite application in the NS supplied to an open soilless cultivation system of tomato on plant growth and total yield and detect possible safety risks for the consumer. Therefore, in addition to the agronomic parameters, residue analysis of chlorates and perchlorates in tomato fruits was performed.

2. Results and Discussion

2.1. Chloride Concentrations in the Drainage Solution

As shown in Table 1, the chloride concentration in the drainage solution was significantly higher in the treatment group of 7.5 mg L^{-1} compared to the control group and the 2.5 mg L^{-1} treatment group at 30 days after the first application (DAFA). This is due to the addition of sodium hypochlorite, which was not accompanied by a commensurate increase in its uptake by the plants after its application. This hypothesis is supported by measurements of the chloride concentration in leaf and root samples as well as fruit samples (results in Section 2.3), which were similar before and after sodium hypochlorite application to the NS.

Table 1. Chloride concentration in the drainage solution samples collected at 30, 37, and 48 DAFA of sodium hypochlorite in a soilless cultivation of tomato. Sodium hypochlorite was applied at concentrations of 2.5, 5.0, and 7.5 mg L^{-1} of chlorine, while in the control treatment no sodium hypochlorite was added.

Time Point	Chlorine (mg L^{-1})	Cloride (mg L^{-1})
30 DAFA (11 July 2019)	Control	6.30 ± 0.25 b
	2.5	7.29 ± 0.87 b
	5.0	7.49 ± 0.53 ab
	7.5	9.05 ± 0.25 a
	Significance	*
37 DAFA (18 July 2019)	Control	8.45 ± 0.55
	2.5	7.93 ± 0.77
	5.0	8.27 ± 0.19
	7.5	7.64 ± 0.51
	Significance	NS
48 DAFA (29 July 2019)	Control	9.42 ± 1.24
	2.5	9.28 ± 0.72
	5.0	9.04 ± 0.28
	7.5	8.12 ± 0.09
	Significance	NS

Values (means of 4 replications), followed by different letter in each column indicate significant differences according to Duncan's multiple range test ($p < 0.05$). NS = Not Significant, * = Significant ($p \leq 0.05$).

Although chlorination is a popular method of disinfecting NS in soilless cultures, it is not considered to be used to its full potential, mainly due to technical issues related to the monitoring of free available chlorine [39]. Most phytopathogens are controlled by chlorine concentrations of 1–3 mg L^{-1}, while higher initial concentrations (5–10 mg L^{-1}) are required as chlorine reacts with various other substances in NS [20,39]. Other important factors affecting the success of chlorination are pH, temperature, and organic matter content in the NS, as well as pathogen type and pathogen load [4].

As shown in Table 1, the highest chloride concentration in the drainage (9.05 mg L^{-1}) was detected in the treatment with a 7.5 mg L^{-1} chlorine application one day after the third application of sodium hypochlorite (30 DAFA). However, the difference was significant only in comparison with the control treatment and the treatment with the addition of sodium hypochlorite at 2.5 mg L^{-1} of chlorine.

2.2. Growth of Plant and Gas Exchange

The disinfection of the NS by applying sodium hypochlorite did not significantly affect the growth of the plants, as indicated by the absence of any statistical differences in the leaf biomass and total leaf area (Table 2). Furthermore, no phytotoxicity symptoms were observed in any of the treatments.

Table 2. Estimation of fresh weight (fw), dry weight (dw), dry matter content (DMC, %), specific leaf area (SLA) and leaf area (cm^2) in the collected leaf samples.

Time Point	Treatments	fw (g)	dw (g)	DMC (%)	SLA (m^2 kg^{-1} dw)	Leaf Area (cm^2)
(11-6-2019) 0 DAFA	control	61.84 ± 9.62	7.07 ± 0.59	11.80 ± 0.97	13.15 ± 0.88	935.75 ± 126.45
	2.5	53.40 ± 2.81	5.80 ± 0.27	10.88 ± 0.19	14.62 ± 0.58	846.00 ± 41.45
	5.0	51.75 ± 3.86	5.89 ± 0.66	11.28 ± 0.55	14.90 ± 1.19	854.25 ± 38.60
	7.5	56.42 ± 5.02	5.93 ± 0.46	10.56 ± 0.37	10.96 ± 3.14	678.00 ± 204.32
	significance	NS	NS	NS	NS	NS
(12-7-2019) 31 DAFA	control	28.46 ± 2.62	3.90 ± 0.28	13.77 ± 0.29	8.60 ± 0.50	330.99 ± 10.49
	2.5	30.32 ± 2.89	4.13 ± 0.63	13.40 ± 0.79	8.22 ± 1.09	321.74 ± 20.43
	5.0	32.30 ± 4.13	4.32 ± 0.42	13.52 ± 0.59	8.485 ± 0.35	368.51 ± 47.08
	7.5	36.78 ± 7.69	4.67 ± 1.09	12.57 ± 0.48	10.48 ± 1.24	452.68 ± 59.25
	significance	NS	NS	NS	NS	NS
(24-7-2019) 43 DAFA	control	13.95 ± 0.99	2.27 ± 0.22	16.15 ± 0.44	10.43 ± 0.54	233.11 ± 12.03
	2.5	17.01 ± 2.64	2.65 ± 0.54	15.20 ± 0.82	11.32 ± 1.25	281.17 ± 35.27
	5.0	19.39 ± 7.26	3.03 ± 1.21	15.27 ± 0.48	9.14 ± 1.71	230.91 ± 50.83
	7.5	23.53 ± 4.76	3.73 ± 0.79	15.62 ± 0.81	8.06 ± 3.49	221.26 ± 75.65
	significance	NS	NS	NS	NS	NS

The numbers represent mean values of 4 replications. NS = not significant based on one-way ANOVA ($p < 0.05$).

Generally, many studies have reported on the effect of chlorine on various plant species by applying different forms of chlorine (gas, chlorine dioxide, etc.) through different disinfection protocols [6,25,26,40–44]. Therefore, a direct comparison of their results is not possible.

In general, chlorine at concentrations higher than 5 mg L^{-1} can cause phytotoxicity in many plants, while some plants are sensitive even at much lower concentrations (0.05 mg L^{-1}) [43]. In this study, disinfection with sodium hypochlorite was selected because it is economically affordable for producers, effective against important plant pathogens, and very common in agricultural practice. Unlike other chemical methods of disinfection, sodium hypochlorite does not degrade quickly, thereby resulting in a longer disinfection capacity. The results of the present study concerning the disinfection effect on tomato growth coincide with the 5 mg L^{-1} rule [43]. Nevertheless, chlorine application following the current disinfection protocol with sodium hypochlorite did not affect the growth of tomato plants cv. 'ELPIDA' even at 7.5 mg L^{-1} chlorine.

Gas exchange was not affected by the application of chlorine up to a concentration of 7.5 mg L^{-1}, as indicated by the absence of any significant differences in the net photosynthetic rates, transpiration rate, stomatal conductance, intercellular CO_2, and water use efficiency (Table 3). Nevertheless, there are examples in which chlorine, applied in gaseous form, affected the photosynthesis of *Pinus* plants by reducing their photosynthetic capacity [40]. Chlorine, as an anion, can be beneficial to plants by substituting for nitrates in vacuoles and positively impacting photosynthesis [44]. Therefore, some researchers used

chlorine to replace part of the nitrates in the NS in tomato [45] and tobacco [46] and found that the stomatal conductivity was affected. However, the concentrations of chlorine tested in this experiment were lower than those in the above-mentioned studies and did not result in any significant differences.

Table 3. Net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate and water use efficiency in leaves (3rd or 4th fully developed leaf from the top) of tomato plants at 0, 31, and 43 DAFA of sodium hypochlorite at three different concentrations of chlorine in the supplied nutrient solution.

Time Point	Treatments	Net Photosynthetic Rate	Stomatal Conductance	Intercellular CO_2 Concentration	Transpiration Rate	Water Use Efficiency
		(μmol CO_2 m^{-2} s^{-1})	(mmol H_2O m^{-2} s^{-1})	(μmol CO_2 m^{-2} s^{-1})	(mmol H_2O m^{-2} s^{-1})	(μmol CO_2 m^{-2} s^{-1}/ mmol H_2O m^{-2} s^{-1})
(11-6-2019) 0 DAFA	control	20.94 ± 1.33	0.54 ± 0.12	286.70 ± 11.84	5.30 ± 0.54	4.04 ± 0.39
	2.5	23.64 ± 1.05	0.69 ± 0.08	295.79 ± 6.73	5.84 ± 0.41	4.14 ± 0.36
	5.0	20.08 ± 1.64	0.49 ± 0.16	263.06 ± 27.22	4.35 ± 0.97	5.23 ± 0.94
	7.5	18.22 ± 4.35	0.52 ± 0.16	257.63 ± 43.74	4.69 ± 1.36	4.95 ± 1.24
	Significance	NS	NS	NS	NS	NS
(12-7-2019) 31 DAFA	control	11.19 ± 1.41	0.13 ± 0.00	223.06 ± 19.71	1.74 ± 0.04	6.44 ± 0.81
	2.5	9.10 ± 2.96	0.10 ± 0.01	228.59 ± 33.64	1.34 ± 0.09	6.49 ± 1.67
	5.0	6.62 ± 0.18	0.07 ± 0.02	168.89 ± 62.38	1.06 ± 0.31	8.28 ± 2.37
	7.5	8.39 ± 1.20	0.15 ± 0.03	273.95 ± 8.09	1.92 ± 0.30	4.40 ± 0.26
	Significance	NS	NS	NS	NS	NS
(24-7-2019) 43 DAFA	control	6.45 ± 2.63	0.18 ± 0.13	279.08 ± 12.12	2.07 ± 0.95	3.25 ± 0.24
	2.5	8.11 ± 2.18	0.22 ± 0.09	296.99 ± 20.00	2.72 ± 0.73	3.03 ± 0.47
	5.0	6.25 ± 1.35	0.13 ± 0.02	298.64 ± 12.62	2.26 ± 0.25	2.75 ± 0.45
	7.5	6.51 ± 2.42	0.23 ± 0.07	324.69 ± 6.79	3.06 ± 0.80	2.03 ± 0.43
	Significance	NS	NS	NS	NS	NS

The numbers represent mean values of 4 replications ± standard error. NS = Not Significant based on one-way ANOVA ($p < 0.05$).

2.3. Fruit Yield and Fruit Mineral Composition

Analysis of variance showed that the disinfection of NS using sodium hypochlorite at a concentration of 2.5 mg L^{-1} of chlorine significantly boosted the total production of marketable fruits when considering total fruit weight per plant (Figure 1A). Nonetheless, it should be noted that the total production for the control treatment was relatively low due to a lower average weight per fruit than the typical weight for this variety. This is possible due to the small scale of this experiment compared to a commercial production.

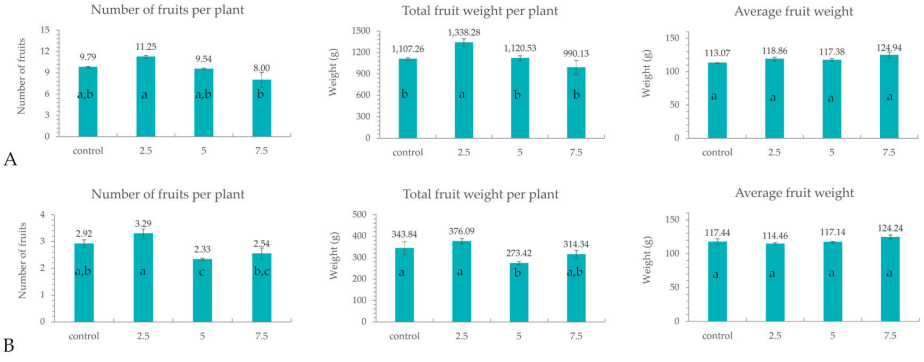

Figure 1. (**A**) Total and (**B**) Extra class production of tomato fruit after disinfection of the nutrient solution using sodium hypochlorite in an open soilless system applying chlorine concentrations of 2.5, 5 and 7.5 mg L^{-1}. Values (means of four replications, bars = SE), followed by different letter in each column indicate significant differences according to Duncan's multiple range test ($p < 0.05$).

To the best of our knowledge, this is the first study in the relevant scientific literature indicating an enhancement of tomato yield after the addition of chlorine in the NS. The increased yield in the treatment of 2.5 mg L^{-1} of chlorine was not accompanied by statistical differences between the treatments regarding gas exchange. Hence, it cannot be ascribed to an enhancement of anabolic functions. However, considering that the dose of chlorine that led to this yield increase was low, it could be attributed to hormetic effects [47,48]. An alternative explanation is that the presence of chlorine in the NS protected plants from low-impact infections of the root system, which did not cause symptoms detectable by visual observation, thus improving their performance compared to plants not treated with chlorine.

The mineral analysis of tomato fruits revealed a significantly higher Mg concentration when chlorine was applied at a concentration of 2.5 mg L^{-1}, which correlates well with the higher yield in this treatment (Table 4). The chloride content in the fruit was increased by chlorine application only in the second harvest (43 DAFA) and only in the 7.5 mg L^{-1} treatment, and the difference was significant compared not only with the control but also with the other two chlorine treatments. In general, the concentration of nutrients in the fruits ranged between 2.4–4.1 mg g^{-1} for phosphorus, 41.5–98.0 mg g^{-1} for potassium, 2.5–3.5 mg g^{-1} for chlorine, 0.16–0.21 mg g^{-1} for calcium, and 1.5–1.8 mg g^{-1} for magnesium.

Table 4. Mineral nutrient analysis in tomato fruit (mg g^{-1} dw) harvested 31 and 43 DAFA of sodium hypochlorite in an open soilless system at chlorine concentrations of 2.5, 5.0 and 7.5 mg L^{-1}.

Time Point	Treatments	P	K	Cl	Ca	Mg
31 DAFA (12 July 2019)	control	3.98 ± 0.18	87.25 ± 3.30	2.90 ± 0.10	0.18 ± 0.03	1.45 ± 0.02 b
	2.5	3.55 ± 0.73	98.00 ± 4.14	3.51 ± 0.12	0.19 ± 0.01	1.80 ± 0.03 a
	5.0	4.10 ± 0.32	95.25 ± 5.15	3.21 ± 0.39	0.21 ± 0.01	1.65 ± 0.11 ab
	7.5	3.74 ± 0.21	86.50 ± 4.66	2.88 ± 0.25	0.19 ± 0.01	1.52 ± 0.05 b
	Significance	NS	NS	NS	NS	*
43 DAFA (24 July 2019)	control	3.16 ± 0.04	43.50 ± 3.28	2.53 ± 0.07 b	0.19 ± 0.02	1.54 ± 0.11
	2.5	3.30 ± 0.47	46.00 ± 4.69	2.49 ± 0.05 b	0.16 ± 0.00	1.66 ± 0.05
	5.0	3.65 ± 0.37	47.25 ± 2.56	2.72 ± 0.08 b	0.17 ± 0.01	1.59 ± 0.13
	7.5	2.43 ± 0.18	41.50 ± 2.53	3.11 ± 0.13 a	0.16 ± 0.00	1.53 ± 0.03
	Significance	NS	NS	**	NS	NS

Values (means of four replications), followed by the same letter in each column indicate significant differences according to Duncan's multiple range test ($p < 0.05$). NS = Not Significant, * significant ($p \leq 0.05$), ** = significant ($p \leq 0.001$).

Chlorine is usually reported for its phytotoxic action when absorbed by the roots at excessively high rates in the form of chloride ions [49]. However, chlorine is an essential trace element in chloroplasts, where it participates in the oxygen evolution process in photosystem II [50] and possibly controls the function of some enzymes involved in cell division [51]. Chlorine is absorbed by plants as the chloride ion at concentrations ranging from low [0.1–0.2 mg g^{-1} [44]] to high [2–20 mg g^{-1} [51]]. At higher concentrations than those required for its essential functions, chloride may have beneficial functions such as osmoregulation [52,53]. However, high concentrations of chloride in the plant tissues are considered to limit the growth of plants [54,55]. The reasons behind this phenomenon are not clear [52] and probably differ in the various susceptible plants such as *Lotus corniculatus* [56], citrus [57] and grapevine [54], which show phytotoxicity at concentrations of 4–7 mg g^{-1} dw [58]. Tomatoes have a high tolerance to chloride concentration compared to other vegetables grown in greenhouses [45]. More specifically, in a closed soilless system, when part of the nitrogen was replaced by chloride, no effect on total yield was reported [45]. Nonetheless, reduction of physiological abnormalities in tomato fruits (white and green spots, blossom end rot) has been indicated when chloride levels are high (8–10 mM) [59,60]. Finally, it should be noted that high chloride concentrations under stress (NaCl) could increase soluble solids and dry matter content and also affect fruit firmness [61].

2.4. Chlorates and Perchlorates in the Nutrient Solution

2.4.1. Stability of Chlorate and Perchlorate Residues in the Nutrient Solution

Perchlorate residues were not detected in any of the fruit samples. Regarding chlorate, which was formed at the beginning (0 day), residues were found at 0.45 and 1.45 mg L^{-1} in the solutions with 2.5 and 5% ammonia, respectively. On the first day, a recovery of 62% compared to day 0 was observed in the 5% ammonia solution, but in the next sample points, recoveries were all above 70%, indicating that no degradation above 30% or formation of perchlorate was observed during the 28-day period of the study (Table 5).

Table 5. Summary results of the 28-day storage stability study of chlorate residues in the nutrient solutions with 2.5 and 5% ammonia concentration. The nutrient solution was composed to support the fruiting of an open soilless system cultivation of tomato.

Storage Period (Days)	Chlorate Residue Levels (mg L^{-1}) in Samples Stored under Freezer Conditions		% Mean Recovery of Chlorate Residues Compared to Day 0 [3]	
Level (% Ammonia)	2.5	5	2.5	5
	Mean ± SD [2]	Mean ± SD [2]		
0 [1]	0.45 ± 0.07	1.45 ± 0.07	-	-
1	0.3 ± 0.00	0.9 ± 0.00	75.3	62.4
4	0.55 ± 0.07	1.65 ± 0.07	115.2	114
6	0.7 ± 0.14	2.05 ± 0.21	123.1	130.9
8	0.35 ± 0.07	1.45 ± 0.07	61.8	87.9
11	0.55 ± 0.07	1.4 ± 0.14	103.7	89.2
21	0.55 ± 0.07	1.4 ± 0.00	108.3	95.2
22	0.35 ± 0.07	1.1 ± 0.14	71	71.4
28	0.55 ± 0.07	1.5 ± 0.14	120.3	101.9

[1] initial concentration of chlorate residues detected in each NS. [2] SD: Standard deviation. [3] Recoveries above 100% indicate that a degradation did on occur, but the higher concentration compared to the initial are observed due to measurement uncertainty which in our case is 50% [62].

2.4.2. Detection of Chlorate Residues in the Nutrient Solution

Chlorate residues were detected in the NS of all treatments (Figures 2 and S1), whilst no perchlorate residues were found in any treatment. Furthermore, the concentration of chlorate residues was increasing in the different treatments following the increased sodium hypochlorite addition in the NS. More specifically, the highest concentration of chlorates determined was 0.325 mg L^{-1} in the treatment of 7.5 mg L^{-1} of chlorine.

Industry quality controls and official food safety controls have detected chlorate residues in various vegetables. Relatively high concentrations have been found in tomato (0.2 mg kg^{-1}) and carrot (0.3 mg kg^{-1}) samples, which exceeded the MRLs set by Reg. 2020/749 (0.1 mg kg^{-1} and 0.15 mg kg^{-1}, respectively) [30]. Nonetheless, it has been suggested that these high levels derive from post-harvest handling where bleach is used as a disinfectant [63–65].

2.5. Chlorates and Perchlorates in Tomato Fruit

After the fortification and analysis of QC samples in the LC/MS/MS, recovery values and relative standard deviations for each substance and charge level were calculated. The accuracy of the method was expressed as a percent recovery. Chlorate recovery values ranged from 111 to 119%, while for perchlorates the range was from 116 to 119%. Repeatability was expressed as % RSD, which ranged from 7–19% for chlorates and from 2–7% for perchlorates. The results are considered acceptable and prove that the method was reliable.

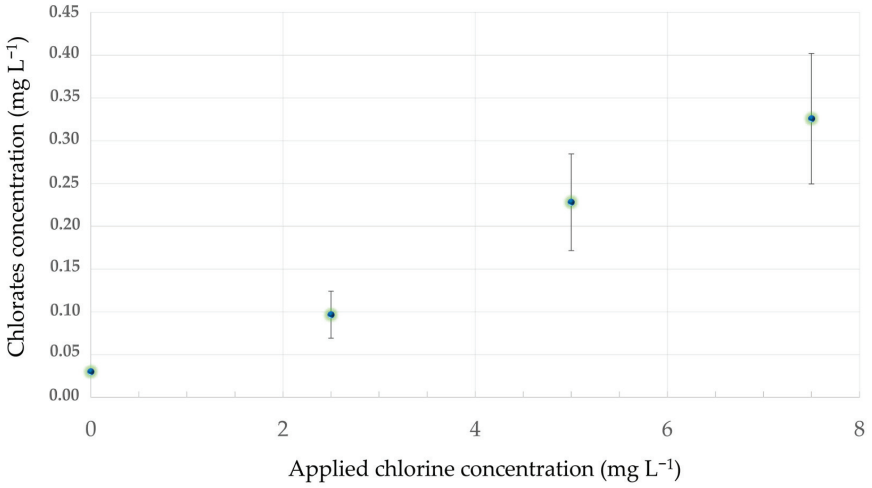

Figure 2. Chlorate residues detected in the nutrient solution of an open soilless cultivation of tomato after application of chlorine at concentrations of 2.5, 5 and 7.5 mg L^{-1}. The bars represent standard deviations.

Chlorate and perchlorate residues were not detected in tomato fruit samples of the extra class grade (Figure S2). These results indicate that the fruits were safe for consumption. Chlorates are generally an important inorganic derivative of chlorine used for disinfection, and the key question is if they can be absorbed and accumulated in edible parts during plant production [66]. Here, in all analyzed fruit samples, the residues were lower than the limit of quantification (LOQ = 0.01 mg kg^{-1}). In contrast, Dannehl et al. (2016) found that chlorate residues were detected after disinfection of the NS with KClO in a closed soilless tomato growing system [7]. Furthermore, chlorate residues have been recorded in "baby" lettuce and spinach samples when the crops were irrigated with chlorinated water [67,68]. However, chlorates and perchlorates do not coexist [7]. Finally, Lonigro et al. (2017) suggested that organochlorine compounds in soil, roots, and leaves are linked to the chlorine concentration in the irrigation water or nutrient solution [42].

Disinfection of vegetables using chlorine has many applications, e.g., production, harvest, and post-harvest handling of fresh fruits and vegetables for many decades [69–71]. In the past, high concentrations of chlorine were applied since there was no awareness about possible toxic residues in the final products [72,73]. Nevertheless, it is now clear that chlorine can react with organic matter, leading to derivatives such as chloroform ($CHCl_3$) or trihalomethanes that are carcinogenic at high doses [74]. Therefore, low doses of chlorine, when using bleach, are recommended for disinfection of NSs in soilless crops to produce safe products free from chlorine byproducts, in addition to avoiding possible symptoms of phytotoxicity [66].

3. Conclusions

Disinfection of the nutrient solution in open soilless cropping systems by applying chlorine up to a dose of 7.5 mg L^{-1} three times at fortnightly intervals did not lead to phytotoxicity in tomato. However, the application of chlorine at a concentration of 2.5 mg L^{-1} increased the total fruit production, due mainly to an increase in the number of fruits per plant. Additionally, no residues of chlorates or perchlorates were detected in tomato fruits. Nonetheless, further research is necessary to address the gap in the literature regarding the disinfection methods applied and their efficiency in controlling economically important pathogens.

4. Materials and Methods

4.1. Experimental Design, Biological Material and Cropping Conditions

4.1.1. Greenhouse Cultivation

An experiment with tomato (*Solanum lycopersicum* cv. "ELPIDA") cultivated in bags containing perlite was carried out in a glasshouse at the Agricultural University of Athens in Greece (37°59′10″ N, 23°42′29″ E, altitude 24 m) from May to July 2019.

The soilless cultivation system comprised 10 channels, eight of which were used to apply the experimental treatments (2 channels per treatment), while the other two were used as border lines (Figure 3). A completely randomized design was followed. Six bags of perlite (33 L, Perlite Hellas, Athens, Greece) were placed in each channel, and two tomato plants were planted in each bag. Prior to transplanting, the perlite bags were irrigated with NS up to saturation, and subsequently their bottoms were slit to allow for free drainage of the NS from the root zone. The composition of the NS was computed using the algorithm developed by Savvas and Adamidis (1999) [75] after setting as target nutrient values those suggested by Savvas et al. (2013) [76] for open soilless cultivations of tomato. The pH of the supplied NS was adjusted to 5.6 using nitric acid. The nutrient solution compositions applied at different cropping stages are shown in Table S1. One day after wetting the substrate (10 May 2019), the tomato seedlings, which were at the 8-leaf stage, were transplanted. The channels had a slope of 1–2% to facilitate drainage, and every two days the values of pH and electrical conductivity (EC) were recorded. The NS collected at the lower end of each channel was discharged. To aid fruit setting, a bumblebee hive (*Bombus terrestris*) (Bio Insecta, Thessaloniki, Greece) was placed inside the experimental greenhouse compartment, and the usual pruning [2,77] and plant protection practices were followed [78,79]. More specifically regarding plant protection, integrated pest management (IPM) principles were applied. Protective nets were put on the greenhouse openings as well as adhesive traps to prevent infestation by insects. In addition, seven days after transplanting the plants, the beneficial insect *Macrolophus pygmaeus* was released as a plant protection measure against *Tuta absoluta*.

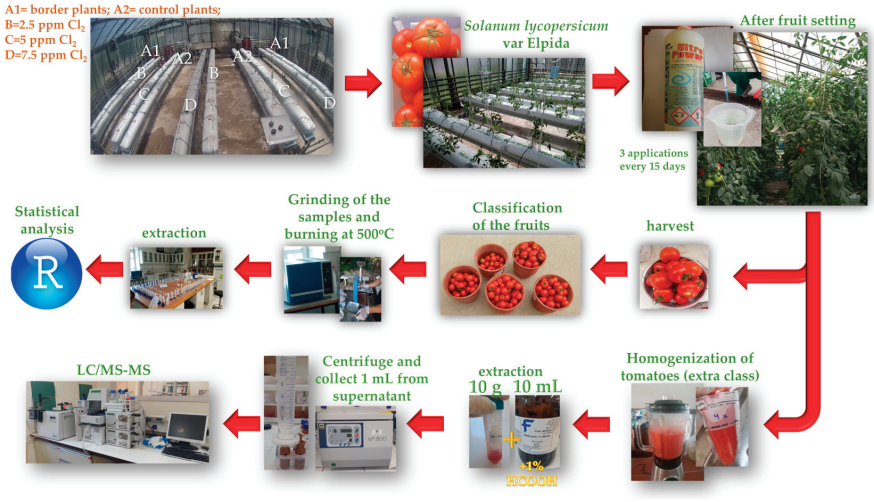

Figure 3. Experimental pipeline for the study of the impact of sodium hypochlorite applied as a nutrient solution disinfectant on growth, nutritional status, yield, and consumer safety of tomato fruit produced in a soilless cultivation.

Corrective recipes were also used during the cropping period whenever they were necessary. The irrigation frequency was automatically adjusted using a heliometer to

measure the solar radiation intensity and an irrigation controller, aiming to achieve a drainage percentage of about 30%.

4.1.2. Disinfection Methodology

Sodium hypochlorite was applied through the last irrigation cycle of the day, while no irrigation was applied during the night. During the cropping period, sodium hypochlorite was applied to the tomato crop three times at fortnightly intervals. Common bleach containing 4.5% *w*/*v* sodium hypochlorite (Ostria Ultra Power, Lamia, Greece) was used. More specifically, the amounts of bleach needed to apply three different chlorine concentrations, namely 2.5, 5, and 7.5 mg L^{-1}, were calculated and added to the NS supplied. These chlorine concentrations were selected to range around the average concentration of chlorine that is not supposed to negatively affect the plants according to the EPA (5 mg L^{-1}) [43]. The final concentration of chloride was not tested in the NS after the application of sodium hypochlorite, but it was measured at the drainage the following day (Table 1). Furthermore, the pH was tested on site in the NS after the addition of sodium hypochlorite and in the drainage solutions. The first application took place on 11 June 2019, i.e., about one month after the beginning of fruit set (16 May 2019). All applications of sodium hypochlorite are shown in detail in Table S2.

4.1.3. Sample Collection

In order to estimate the impact of the treatments on plant growth, nutrient concentrations and the possible formation of chlorates and/or perchlorates in plant tissues, including leaves, fruits, and roots, were sampled. Leaf samples (3rd and 4th fully developed leaves from the top) were collected 0, 31, and 43 days after the first application of sodium hypochlorite (DAFA—days after first application). Fruit samples were collected at DAFA 31 and 43. The time points of sampling were chosen in an effort to discover the effect of sodium hypochlorite on tomato plants after the third chlorine application (31 DAFA) and almost ten days later, namely slightly before the end of the culture. At the end of the cultivation (48 DAFA), the whole root system was also selected, separated from perlite grains, and used as root samples. A total of two plant tissue samples were collected from two different plants in each channel, thereby obtaining four replicate samples per treatment. Prior to the nutrient analysis, the fresh weight of each sample was recorded, and then all samples were placed in an oven at 65°C for drying until their weight was stabilized. Finally, the dry matter content (DMC) and specific leaf area (SLA, on fresh leaves) were calculated [80].

In addition, samples of NS were collected from the drainage solution before the first harvest (30 DAFA), 7 days later (37 DAFA), and at the end of the cultivation (48 DAFA) to determine the level of chloride and nutrients. Two drainage solution samples were collected from each channel, thereby obtaining four replicates per treatment.

4.2. Gas Exchange Measurements

The treatment impact on net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate were determined using a Li-6400 instrument (Li-Cor, Inc., Lincoln, NE, USA) [81]. The measurements were conducted in leaves of the same physiological stage (the most recent fully expanded leaf) at the same time of the day (between 09:00 am and 12:00 pm) prior to their sampling, as described in Section 4.1.3. All measurements were conducted under natural light conditions and ambient CO_2 atmospheric concentration on sunny days to exclude light intensity effects. Furthermore, water use efficiency was estimated as previously described [82].

4.3. Plant Mineral Status

Initially, the dried plant samples were ground using a ball mill and sieved (40-mesh) until they obtained the texture of powder. The procedure was followed by dry burning at 500 °C for 8 h and the extraction of minerals using 10 mL of 1 M HCl to determine tissue P, K, Ca, and Mg concentrations. The extracts were filtered and stored at 4 °C

until further processing. Phosphorus was estimated photometrically (Anthos Zenyth 200, Biochrom, Holliston, MA, USA) by applying the ammonium phosphomolybdate method [83]. Potassium was estimated through flame photometry using a Sherwood Model 410 (Sherwood Scientific Ltd., Cambridge, UK). Ca and Mg were measured by employing atom absorption spectrophotometry using a Shimadzu AA-7000 instrument (Shimadzu Europa GmbH, Duisburg, Germany). Chloride was measured using a chloridometer (Thermo Scientific Orion Star A214, Thermo Fisher Scientific, Waltham, MA, USA) in aqueous extracts of the powdered leaf samples after filtering.

4.4. Estimation of Total Fruit Production

Fruit was harvested twice, on 12 July 2019 and 24 July 2019, i.e., 2 and 14 days after the last application of sodium hypochlorite, respectively. At each harvest date, all ripe fruits were collected and graded into extra class, class I, class II, and non-marketable produce in accordance with the EU Regulation (543/2011) [84]. The fruit yield was estimated by recording the total number of fruits per plant (for both time points together and for each tomato class separately) and the total fruit weight per plant, while calculating the average fruit weight.

4.5. Residue Determinations

In order to investigate the possible contamination of harvested tomato fruits with chlorates (ClO_3^-) and perchlorates (ClO_4^-) due to disinfection of the NS with sodium hypochlorite, residue analysis was performed as described below:

4.5.1. Chemicals and Reagents

Chlorate, perchlorate, and internal standards (IS) (perchlorate $^{18}O_4$ and chlorate $^{18}O_3$) stock solutions at 80 mg L^{-1} in MeOH were obtained from the EU-Reference Laboratory for pesticides requiring single residue methods. Methanol (HPLC gradient grade) and water (LC-MS grade) were bought from Fischer chemicals. Acetic acid (99% for analysis) and formic acid (99%, for analysis) were bought from Carlo-Erba reagents.

4.5.2. Investigation of the Stability of Chlorates and Perchlorates in the NS Samples

During the final application of sodium hypochlorite in the different treatments, two 0.5 L samples of NS were collected from the drippers, and their chlorate and perchlorate concentrations were determined.

The nutrient solution, which had previously been analyzed to confirm that no detectable residues of chlorates and perchlorates were present, served as a control to investigate the stability of chlorate and perchlorate residues in NS under freezing conditions. Two solutions with 2.5 and 5% ammonia concentrations were prepared and stored at 4 °C. During this time, successive sampling of the fortified NS was performed at days 0, 1, 2, 4, 6, 8, 11, 21, 22, and 28 and was followed by injection into the LC/MS/MS chromatographic system.

4.5.3. Extraction Procedure of Tomato Fruit Samples

A laboratory sample of 2 kg of the extra class tomato fruits was collected and homogenized 8 h after harvest, after removal of the calyx. The homogenized samples were stored at −20 °C until further processing.

Residues analysis was performed following the in-house laboratory method M17, which is based on the QuPPe protocol [85]. A brief description of the procedures is described below:

An aliquot of 10 ± 1 g of homogenized sample was weighted, and 10 mL of the extraction solvent methanol (acidified 1% HCOOH) was added. The mixture was allowed to soak for 30 min before the addition of the extraction solvent. The mixture was shaken by hand for 1 min and centrifuged at 4000 rpm (1792× *g*) for 5 min. An aliquot of the extract was transferred into a screw cup-top storage vial. Before injecting it into the

chromatographic system, the final solution was filtered through a 0.45 μm disposable cellulose syringe filter.

4.5.4. Chromatographic Analysis of Fruit Samples—Instrumentation

For the chromatographic analysis of the samples, a Varian liquid chromatography LC-MS/MS system consisting of two Varian Prostar 210 pumps and a Prostar 420 autosampler using a 100 mL syringe was used, combined with a triple quadrupole mass spectrometer (Varian model 1200 L) and equipped with an electrospray ionization (ESI) interface, operating in the negative mode. Separation was performed on a Hypercarb 2.1 × 100 mm 5 μm at a flow rate of 0.4 mL min^{-1}. The column was at room temperature. Eluent A consisted of an aqueous solution of 1% CH3COOH, while eluent B was MeOH with 1% CH3COOH. The LC gradient started at 100% A and was linearly decreased to 70% B, over 10 min. Finally, the gradient was instantly switched to 100% A and equilibrated for 5 min before the next injection took place. The injection volume was 10 mL.

The following instrumental settings were used: the source temperature was set at 50 °C and the drying gas temperature at 300 °C. Drying gas and nebulizing gas were nitrogen generated from a high purity generator at 18 psi and synthetic air (purity > = 99.99%) at 40 psi, respectively. For the operation in MS/MS mode, Argon 99.999% was used as a collision gas with a pressure of 1.8 mTorr. The scanning of the transitions was conducted with a dwell time of 50 ms per transition. The number of data points across the peaks was at least ten. Capillary voltage (CV) and collision energy (CE) varied depending on the precursor ion and product ion and are presented in Table S3.

4.5.5. Method Performance

The analytical method applied to determine chlorates and perchlorates in tomato fruit samples of extra class has been proposed by the EURLs (European Reference Laboratories for Single Residue Methods), hence method validation data are available. In order to ensure the performance of the present analysis and verify the obtained results, a brief validation of the method was performed. Considering that an internal standard was used to quantify the results, calibration standards were applied to the solvent. Specifically, quality control samples containing 0.01 mg kg^{-1} (in 2 replicates, n = 3), 0.1 mg kg^{-1} (in 2 replicates, n = 3), and 1 mg kg^{-1} (in 2 replicates, n = 2) in chlorates—perchlorates were prepared by fortifying the corresponding concentration in control tomato samples to determine the accuracy of the method. The samples used as controls had previously been analyzed, and it was confirmed that they had no residues from or interferences with the substances under investigation. Accuracy was checked by assessing the recovery for the selected concentrations, and repeatability was verified by measuring the relative standard deviation (RSD).

4.6. Statistical Analysis

The experiment was set up as a completely randomized design examining one factor (sodium hypochlorite concentration) with four levels and four replicates per treatment. The statistical analysis was performed using the Rstudio program (Version: 1.3.1093, Boston, MA, USA) and the Agricolae package [86]. First, box plots were made to ensure the lack of outliers in the various parameters studied for each treatment separately. The normality of the data was checked in terms of skewness and kurtosis [87], and Levene's test was employed to test the equality of the variances. When an ANOVA analysis rendered a significant difference at the significance level of 95%, post-hoc comparisons using the Duncan's Multiple Range Test with $p < 0.05$ were performed [88].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9030352/s1, Figure S1: Chlorate residue analysis in a nutrient solution sample (red) of an open soilless system cultivation of tomato and in HPLC water (green). The nutrient solution sample corresponds to the 7.5 mg L^{-1} of chlorine treatment after the application of sodium hypochlorite in which chlorates residues were detected; Figure S2: LC-MS/MS scan data of chlorate (red) and perchlorate (green) residue analysis in tomato fruit samples

collected from the 5 mg L^{-1} chlorine treatment; Table S1: Recipes of nutrient solutions used in tomato cultivated in an open soilless system; Table S2: Details about the three disinfection applications using sodium hypochlorite in the nutrient solution supplied to a tomato cultivated in an open soilless system; Table S3: Precursor ion, product ion, Capillary Voltage (CV) and Collision Energy (CE) for the chlorates and perchlorates analytes examined with LC/MS/MS for the determination of their residues in tomato fruits and nutrient solution samples of an open soilless system cultivation.

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References

1. Jan, S.; Rashid, Z.; Ahngar, T.A.; Iqbal, S.; Naikoo, M.A.; Majeed, S.; Bhat, T.A.; Gull, R.; Nazir, I. Hydroponics–A Review. *Int. J. Curr. Microbiol. App. Sci.* **2020**, *9*, 1779–1787. [CrossRef]
2. Gruda, N.; Savvas, D.; Colla, G.; Rouphael, Y. Impacts of genetic material and current technologies on product quality of selected greenhouse vegetables–A review. *Eur. J. Hortic. Sci.* **2018**, *83*, 319–328. [CrossRef]
3. Critten, D.L.; Bailey, B.J. A review of greenhouse engineering developments during the 1990s. *Agric. For. Meteorol.* **2002**, *112*, 1–22. [CrossRef]
4. Stewart-Wade, S.M. Plant pathogens in recycled irrigation water in commercial plant nurseries and greenhouses: Their detection and management. *Irrig. Sci.* **2011**, *29*, 267–297. [CrossRef]
5. Martínez, F.; Castillo, S.; Carmona, E.; Avilés, M. Dissemination of *Phytophthora cactorum*, cause of crown rot in strawberry, in open and closed soilless growing systems and the potential for control using slow sand filtration. *Sci. Hortic.* **2010**, *125*, 756–760. [CrossRef]
6. Zheng, Y.; Cayanan, D.F.; Dixon, M. Control of pathogens in irrigation water using chlorine without injury to plants. *Comb. Proc. Int. Plant Propag. Soc.* **2008**, *58*, 248–258. Available online: http://admin.ipps.org/uploads/58_051.pdf (accessed on 20 December 2022).
7. Dannehl, D.; Schuch, I.; Gao, Y.; Cordiner, S.; Schmidt, U. Effects of hypochlorite as a disinfectant for hydroponic systems on accumulations of chlorate and phytochemical compounds in tomatoes. *Eur. Food Res. Technol.* **2016**, *242*, 345–353. [CrossRef]
8. Savvas, D.; Gruda, N. Application of soilless culture technologies in the modern greenhouse industry—A review. *Eur. J. Hortic. Sci.* **2018**, *83*, 280–293. [CrossRef]
9. FAO; IFAD; UNICEF; WFP; WHO. *The State of Food Security and Nutrition in the World 2022. Repurposing Food and Agricultural Policies to Make Healthy Diets More Affordable*; FAO: Rome, Italy, 2022. [CrossRef]
10. MIT. Mission 2014: Feeding the World. Available online: http://12.000.scripts.mit.edu/mission2014/solutions/hydroponics (accessed on 21 November 2022).
11. Bank, W. The Road to a Greener Future. Available online: https://blogs.worldbank.org/climatechange/road-greener-future (accessed on 21 November 2022).
12. World Food Programme. How2grow. Available online: https://innovation.wfp.org/project/h2grow-hydroponics (accessed on 21 November 2022).
13. Giro, A.; Ciappellano, S.; Ferrante, A. Vegetable production using a simplified hydroponics system inside city of Dead (Cairo). In *Advances in Horticultural Science*; Mancuso, S., Ed.; Department of Agri-Food Production and Environmental Sciences, University of Florence: Florence, Italy, 2016; pp. 23–29.
14. Mordor Intelligence. Hydroponic Market–Growth, Trends, COVID-19 Impact and Forecasts (2022–2027). Available online: https://www.mordorintelligence.com/industry-reports/hydroponics-market (accessed on 30 October 2022).

15. Eden Green Technology. How Hydroponics & Vertical Farming Can Improve Food Safety and Protect Our Food Supply. Available online: https://www.edengreen.com/blog-collection/how-hydroponics-and-vertical-farming-can-improve-food-safety (accessed on 30 October 2022).
16. Riggio, G.M.; Wang, Q.; Kniel, K.E.; Gibson, K.E. Microgreens—A review of food safety considerations along the farm to fork continuum. *Int. J. Food Microbiol.* **2019**, *290*, 76–85. [CrossRef]
17. Sawyer, T. Food safety and E. coli in aquaponic and hydroponic systems. *Horticulturae* **2021**, *7*, 36. [CrossRef]
18. Wang, Y.-J.; Deering, A.J.; Kim, H.-J. The occurrence of shiga toxin-producing *E. coli* in aquaponic and hydroponic systems. *Horticulturae* **2020**, *6*, 1. [CrossRef]
19. Ilic, S.; LeJeune, J.; Lewis Ivey, M.L.; Miller, S. Delphi expert elicitation to prioritize food safety management practices in greenhouse production of tomatoes in the United States. *Food Control* **2017**, *78*, 108–115. [CrossRef]
20. Clark, G.A.; Smajstrla, A.G. Treating irrigation systems with chlorine. *Foliage Dig.* **1992**, *15*, 3–5.
21. Postma, J.; Van Os, E.; Bonants, P.J.M. 10-Pathogen detection and management strategies in soilless plant growing systems. In *Soilless Culture*; Raviv, M., Lieth, J.H., Eds.; Elsevier: Amsterdam, The Netherlands, 2008; pp. 425–457. [CrossRef]
22. Cayanan, D.F.; Zheng, Y.; Zhang, P.; Graham, T.; Dixon, M.; Chong, C.; Llewellyn, J. Sensitivity of five container-grown nursery species to chlorine in overhead irrigation water. *HortScience* **2008**, *43*, 1882–1887. [CrossRef]
23. Cayanan, D.F.; Dixon, M.; Zheng, Y.; Llewellyn, J. Response of container-grown nursery plants to chlorine used to disin-fest irrigation water. *HortScience* **2009**, *44*, 164–167. [CrossRef]
24. Hong, C.X.; Richardson, P.A.; Kong, P.; Bush, E.A. Efficacy of chlorine on multiple species of Phytophthora in recycled nursery irrigation water. *Plant Dis.* **2003**, *87*, 1183–1189. [CrossRef]
25. Price TV, F.P. Behaviour of fungicides in recirculating nutrient film hydroponic systems. In Proceedings of the 6th in-Ternational Congress on Soilless Culture, Lunteren, The Netherlands, 28 April–5 May 1984; pp. 511–522.
26. Poncet, C.; Offroy, M.; Bonnet, G.; Brun, R. Disinfection of recycling water in rose cultures. *Acta Hortic.* **2001**, *547*, 121–127. [CrossRef]
27. Rosner, A.; Lachman, O.; Pearlsman, M.; Feigelson, L.; Maslenin, L.; Antignus, Y. Characterisation of cucumber leaf spot virus isolated from recycled irrigation water of soil-less cucumber cultures. *Ann. Appl. Biol.* **2006**, *149*, 313–316. [CrossRef]
28. Stanton, J.M.; O'Donnell, W.E. Hatching, motility, and infectivity of root-knot nematode (Meloidogyne javanica) following exposure to sodium hypochlorite. *Aust. J. Exp. Agric.* **1994**, *34*, 105–108. [CrossRef]
29. Bull, R.J.; Gerba, C.; Trussell, R.R. Evaluation of the health risks associated with disinfection. *Crit. Rev. Environ. Control* **1990**, *20*, 77–113. [CrossRef]
30. Kaufmann-Horlacher, I. Chlorat-Rüchstände in Pflanzlichen Lebensmitteln-Ein Update. CVUA, Stuttgart. 2014. Available online: https://www.ua-bw.de/pub/beitrag.asp?subid=1&Thema_ID=5&ID=2008 (accessed on 1 December 2022).
31. European Commission. Chlorate. Available online: https://ec.europa.eu/food/plants/pesticides/maximum-residue-levels/chlorate_en (accessed on 30 October 2022).
32. Regulation 2020/749. Commission Regulation (EU) 2020/749 of 4 June 2020 Amending Annex III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as Regards Maximum Residue Levels for Chlorate in or on Certain Products. Available online: http://data.europa.eu/eli/reg/2020/749/oj (accessed on 30 October 2022).
33. *BfR Recommendations for the Health Assessment of Chlorate Residues in Food, BfR Opinion No. 028/2014*; The German Federal Institute for Risk Assessment: Berlin, Germany, 2014.
34. EFSA. *Trilateral Meeting on Perchlorate Risk Assessment*; Report of the Meeting on 12 February 2014; European Food Safety Authority: Parma, Italy, 2014.
35. World Health Organization. Joint FAO/WHO Expert Committee on Food Additives. Meeting (67th: 2006: Geneva, Switzerland) & International Programme on Chemical Safety. Safety Evaluation of Certain Food Additives and Contaminants/Prepared by the Sixty-Eighth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JEFCA). 2008. Available online: https://apps.who.int/iris/handle/10665/43823 (accessed on 30 October 2022).
36. Rodríguez-Ortega, W.M.; Martínez, V.; Nieves, M.; Simón, I.; Lidón, V.; Fernandez-Zapata, J.C.; Martinez-Nicolas, J.J.; Cámara-Zapata, J.M.; García-Sánchez, F. Agricultural and physiological responses of tomato plants grown in different soilless culture systems with saline water under greenhouse conditions. *Sci. Rep.* **2019**, *9*, 6733. [CrossRef]
37. Razifard, H.; Ramos, A.; Della Valle, A.L.; Bodary, C.; Goetz, E.; Manser, E.J.; Li, X.; Zhang, L.; Visa, S.; Tieman, D.; et al. Genomic evidence for complex domestication history of the cultivated tomato in latin america. *Mol. Biol. Evol.* **2020**, *37*, 1118–1132. [CrossRef]
38. FAOSTAT. Crops and Livestock Products. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 30 October 2022).
39. De Hayr, R.; Bodman, K.; Forsberg, L. Bromine and chlorine disinfestation of nursery water supplies. *Comb. Proc. Int. Plant Propag. Soc.* **1994**, *44*, 60–66.
40. Schreuder, M.D.J.; Brewer, C.A. Persistent effects of short-term, high exposure to chlorine gas on physiology and growth of *Pinus ponderosa* and *Pseudotsuga menziesii*. *Ann. Bot.* **2001**, *88*, 197–206. [CrossRef]
41. Ehret, D.; Alsanius, B.; Wohanka, W.; Menzies, J.; Utkhede, R. Disinfestation of recirculating nutrient solutions in greenhouse horticulture. *Agron. EDP Sci.* **2001**, *21*, 323–339. [CrossRef]

42. Lonigro, A.; Montemurro, N.; Laera, G. Effects of residual disinfectant on soil and lettuce crop irrigated with chlorinated water. *Sci. Total Environ.* **2017**, *584–585*, 595–602. [CrossRef] [PubMed]
43. Environmental Protection Agency (USA). Guidelines for Water Reuse. 2012. Available online: https://www.epa.gov/sites/default/files/2019-08/documents/2012-guidelines-water-reuse.pdf (accessed on 17 December 2022).
44. Wege, S.; Gilliham, M.; Henderson, S.W. Chloride: Not simply a 'cheap osmoticum', but a beneficial plant macronutrient. *J. Exp. Bot.* **2017**, *68*, 3057–3069. [CrossRef]
45. Neocleous, D.; Nikolaou, G.; Ntatsi, G.; Savvas, D. Nitrate supply limitations in tomato crops grown in a chloride-amended recirculating nutrient solution. *Agric. Water Manag.* **2021**, *258*, 107163. [CrossRef]
46. Franco-Navarro, J.D.; Brumós, J.; Rosales, M.A.; Cubero-Font, P.; Talón, M.; Colmenero-Flores, J.M. Chloride regulates leaf cell size and water relations in tobacco plants. *J. Exp. Bot.* **2015**, *67*, 873–891. [CrossRef] [PubMed]
47. Agathokleous, E.; Kitao, M.; Calabrese, E.J. Hormesis: A compelling platform for sophisticated plant science. *Trends Plant Sci.* **2019**, *24*, 318–327. [CrossRef] [PubMed]
48. Erofeeva, E.A. Hormesis in plants: Its common occurrence across stresses. *Curr. Opin. Toxicol.* **2022**, *30*, 100333. [CrossRef]
49. Geilfus, C.-M. Chloride: From Nutrient to Toxicant. *Plant Cell Physiol.* **2018**, *59*, 877–886. [CrossRef]
50. Bové, J.M.; Bové, C.; Whatley, F.R.; Arnon, D.I. Chloride requirement for oxygen evolution in photosynthesis. *Z. Für Nat.-B* **1963**, *18*, 683–688. [CrossRef]
51. Colmenero-Flores, J.M.; Franco-Navarro, J.D.; Cubero-Font, P.; Peinado-Torrubia, P.; Rosales, M.A. Chloride as a bene-ficial macronutrient in higher plants: New roles and regulation. *Int. J. Mol.* **2019**, *20*, 4686. [CrossRef]
52. Geilfus, C.-M. Review on the significance of chlorine for crop yield and quality. *Plant Sci.* **2018**, *270*, 114–122. [CrossRef] [PubMed]
53. Raven, J.A. Chloride: Essential micronutrient and multifunctional beneficial ion. *J. Exp. Bot.* **2017**, *68*, 359–367. [CrossRef]
54. Henderson, S.W.; Baumann, U.; Blackmore, D.H.; Walker, A.R.; Walker, R.R.; Gilliham, M. Shoot chloride exclusion and salt tolerance in grapevine is associated with differential ion transporter expression in roots. *BMC Plant Biol.* **2014**, *14*, 273. [CrossRef] [PubMed]
55. Teakle, N.L.; Tyerman, S.D. Mechanisms of Cl^- transport contributing to salt tolerance. *Plant Cell Environ.* **2010**, *33*, 566–589. [CrossRef] [PubMed]
56. Sanchez, D.H.; Pieckenstain, F.L.; Escaray, F.; Erban, A.; Kraemer, U.T.E.; Udvardi, M.K.; Kopka, J. Comparative ionomics and metabolomics in extremophile and glycophytic *Lotus* species under salt stress challenge the metabolic pre-adaptation hypothesis. *Plant Cell Environ.* **2011**, *34*, 605–617. [CrossRef]
57. Brumos, J.; Talon, M.; Bouhlai, R.; Colmenero-Flores, J.M. Cl^- homeostasis in includer and excluder citrus rootstocks: Transport mechanisms and identification of candidate genes. *Plant Cell Environ.* **2010**, *33*, 2012–2027. [CrossRef]
58. Xu, G.; Magen, H.; Tarchitzky, J.; Kafkafi, U. Advances in chloride nutrition of plants. In *Advances in Agronomy, Sparks, D.L., Ed.*; Academic Press: Cambridge, MA, USA, 1999; Volume 68, pp. 97–150. [CrossRef]
59. Gruda, N. Do Soilless Culture Systems Have an Influence on Product Quality of Vegetables? Available online: https://edoc.hu-berlin.de/handle/18452/10085 (accessed on 30 October 2022).
60. Schnitzler, W.H.; Gruda, N. Hydroponics and product quality. In *Hydroponic Production of Vegetables and Ornamentals*; Savvas, D., Passam, H.C., Eds.; Embryo Publications: Athens, Greece, 2002; pp. 373–414.
61. Leonardi, C.; Martorana, M.; Giuffrida, F.; Fogliano, V.; Pernice, R. Tomato fruit quality in relation to the content of sodium chloride in the nutrient solution. *Acta Hortic.* **2004**, *659*, 769–774. [CrossRef]
62. European Commission. *Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues and Analysis in Food and Feed*; European Commission: Brussels, Belgium, 2019; SANTE/12682/2019.
63. Goodburn, C.; Wallace, C.A. The microbiological efficacy of decontamination methodologies for fresh produce: A review. *Food Control* **2013**, *32*, 418–427. [CrossRef]
64. Ramos, B.; Miller, F.A.; Brandão, T.R.S.; Teixeira, P.; Silva, C.L.M. Fresh fruits and vegetables—An overview on applied methodologies to improve its quality and safety. *Innov. Food Sci. Emerg.* **2013**, *20*, 1–15. [CrossRef]
65. Joshi, K.; Mahendran, R.; Alagusundaram, K.; Norton, T.; Tiwari, B.K. Novel disinfectants for fresh produce. *Trends Food Sci. Technol.* **2013**, *34*, 54–61. [CrossRef]
66. Leonel, L.P.; Tonetti, A.L. Wastewater reuse for crop irrigation: Crop yield, soil and human health implications based on giardiasis epidemiology. *Sci. Total Environ.* **2021**, *775*, 145833. [CrossRef]
67. Garrido, Y.; Marín, A.; Tudela, J.A.; Truchado, P.; Allende, A.; Gil, M.I. Chlorate accumulation in commercial lettuce cultivated in open field and irrigated with reclaimed water. *Food Control* **2020**, *114*, 107283. [CrossRef]
68. López-Gálvez, F.; Gil, M.I.; Meireles, A.; Truchado, P.; Allende, A. Demonstration tests of irrigation water disinfection with chlorine dioxide in open field cultivation of baby spinach. *J. Sci. Food Agric.* **2018**, *98*, 2973–2980. [CrossRef]
69. Bartz, J.A. Potential for postharvest disease in tomato fruit infiltrated with chlorinated water and chlorination of asparagus hydrocooling water for the control of post-harvest decay organisms. *Plant Dis.* **1988**, *72*, 9–13. [CrossRef]
70. Goodin, P.L. Chlorine for sick tomatoes (Erwinia carotovora). *Agric. Res* **1977**, *26*, 8–10.
71. Winston, J.R.; Johnson, H.B.; Harvey, E.M. Using chemicals to stop spoilage. In *Yearbook of Agriculture*; USDA Printing Office: Washington, DC, USA, 1953; pp. 842–843.
72. Combrink, J.C.; Visagie, T.R. Chlorination of dump tank water to reduce postharvest rot in apples. *Deciduous Fruit Grow.* **1982**, *32*, 61–63.

73. Rabin, J. Pack tomatoes for higher profits-chlorinating packing shed wash water improves quality. *Am. Veg. Grow.* **1986**, *34*, 12.
74. Suslow, T. Chlorination in the production and postharvest handling of fresh fruits and vegetables. In *Use of Chlorine-Based Sanitizers and Disinfectants in the Food Manufacturing Industry*; University of California-Davis: Davis, CA, USA, 2000; Available online: https://www.siphidaho.org/env/pdf/Chlorination_of_fruits_and_veggies.PDF (accessed on 20 December 2022).
75. Savvas, D.; Adamidis, K. Automated management of nutrient solutions based on target electrical conductivity, pH, and nutrient concentration ratios. *J. Plant Nut.* **1999**, *22*, 1415–1432. [CrossRef]
76. Savvas, D.; Gianquinto, G.P.; Tüzel, Y.; Gruda, N. Soilless Culture. In *Good Agricultural Practices for Greenhouse Vegetable Crops. Principles for Mediterranean Climate Areas*; Food and Agriculture Organization of the United Nations, Plant Production and Protection Paper 217: Rome, Italy, 2013; pp. 303–354. Available online: http://www.fao.org/3/a-i3284e.pdf (accessed on 20 December 2022).
77. Navarrete, M.; Jeannequin, B. Effect of frequency of axillary bud pruning on vegetative growth and fruit yield in greenhouse tomato crops. *Sci. Hortic.* **2000**, *86*, 197–210. [CrossRef]
78. Kalozoumis, P.; Vourdas, C.; Ntatsi, G.; Savvas, D. Can Biostimulants Increase Resilience of Hydroponically-Grown Tomato to Combined Water and Nutrient Stress? *Horticulturae* **2021**, *7*, 297. [CrossRef]
79. Lykogianni, M.; Bempelou, E.; Ntatsi, G.; Karavidas, I.; Ropokis, A.; Aliferis, K.A.; Savvas, D. Spinosad residues in hydroponically grown tomato fruits. *Acta Hortic.* **2021**, *1320*, 197–204. [CrossRef]
80. Garnier, E.; Shipley, B.; Roumet, C.; Laurent, G. A standardized protocol for the determination of specific leaf area and leaf dry matter content. *Funct. Ecol.* **2001**, *15*, 688–695. [CrossRef]
81. He, Y.; Zhu, Z.; Yang, J.; Ni, X.; Zhu, B. Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity. *Environ. Experim. Bot.* **2009**, *66*, 270–278. [CrossRef]
82. Medrano, H.; Tomás, M.; Martorell, S.; Flexas, J.; Hernández, E.; Rosselló, J.; Pou, A.; Escalona, J.M.; Bota, J. From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target. *Crop J.* **2015**, *3*, 220–228. [CrossRef]
83. Murphy, J.; Riley, J.P. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* **1962**, *27*, 31–36. [CrossRef]
84. Regulation 543/2011. EN 15.6.2011 Official Journal of the European Union L 157/109. Brussels: European Union. Available online: https://eur-lex.europa.eu/legal-content/GA/TXT/?uri=CELEX%3A32011R0543 (accessed on 30 October 2022).
85. Anastassiades, M.; Kolberg, D.I.; Eichhorn, E.; Benkenstein, A.; Lukačević, S.; Mack, D.; Wildgrube, C.; Sigalov, I.; Dörk, D.; Barth, A. *Quick Method for the Analysis of Numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS Involving Simultaneous Extraction with Methanol (QuPPe-Method)*; Version 9.2.; CVUA: Stuttgart, Germany, 2015.
86. de Mendiburu, F. Agricolae: Statistical Procedures for Agricultural Research. R Package Version 1.3-3. Available online: https://CRAN.R-project.org/package=agricolae (accessed on 15 August 2022).
87. Kim, H.Y. Statistical notes for clinical researchers: Assessing normal distribution (2) using skewness and kurtosis. *Restor. Dent. Endod.* **2013**, *38*, 52–54. [CrossRef]
88. Bewick, V.; Cheek, L.; Ball, J. Statistics review 9: One-way analysis of variance. *Crit. Care* **2004**, *8*, 130. [CrossRef] [PubMed]

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Bio-Fertilizers Reduced the Need for Mineral Fertilizers in Soilless-Grown Capia Pepper

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Abstract: Soilless cultivation is extensively used in the greenhouse industry. Recently, hydroponic cultivation of capia pepper has become popular among growers. Capia pepper is harvested at the red maturity stage, and intensive mineral fertilizers are usually used for soilless cultivation. This study was performed in a greenhouse during spring under Mediterranean climatic conditions. The effects of bacteria and mycorrhiza on capia pepper plant growth, yield, fruit quality, and nutrition were investigated. Furthermore, the synergistic effects of these two bio-fertilizers were investigated. Our objective was to replace 20% of mineral fertilizers with bio-fertilizers in a soilless culture system. The use of 80% mineral fertilizers, in combination with mycorrhiza and bacteria, provided a 32.4% higher yield than the control (100% mineral fertilizer without bio-fertilizers). Moreover, the concentrations of N, P, K, Ca, Mg, Fe, Mn, Zn, and Cu in the leaves of pepper plants fed with the reduced mineral fertilizers combined with bio-fertilizers were higher than that of the control. In addition, fruit parameters, such as fruit weight, diameter, volume, the electric conductivity of the fruit juice, and total soluble solids, were significantly higher in this treatment compared to the control. Using 80% mineral fertilizer with only bacteria provided a 24.2% higher yield than the control. In conclusion, mineral fertilizers were successfully reduced by 20% using bacteria and mycorrhiza. These results provide an eco-friendly approach to a sustainable environment.

Keywords: bacteria; *Capsicum annuum* L.; coco pith; mycorrhiza; synergistic effects; yield

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1. Introduction

Intense monoculture vegetable growing in conventional soil greenhouses increases the risk of disease and pest outbreaks. Consequently, high amounts of pesticides and herbicides are needed, increasing environmental pollution. On the other hand, this cultivation method can reduce certain nutrients' availability, leading to soil exhaustion. Our endeavors to compensate for the situation could, in turn, increase the level of unbalanced nutrients and negatively affects soil fertility. For instance, long-term chili monoculture generated significant changes in soil nutrients, aggregates and enzymes [1]. All these factors severely limit crop productivity over the years.

In recent years, the gradual decrease in agricultural lands, the effects of climate change, soil-borne problems, food security, and environmental issues have increased the tendency towards soilless cultivation as controlled agriculture [2,3]. A soilless culture system (SCS) is considered one of the most promising approaches, combining increased production without damaging its supporting ecosystem [3]. Likewise, soilless culture systems have been adopted in many countries for a long time. Plant nutrition management is a crucial factor in the success of soilless cultivation systems. The right nutrient solution will increase the complete fulfillment of plants' requirements for optimal growth and development. However, although soilless cultivation in the modern greenhouse business is the favorite

choice among growers, it brings intensive nutrition to the plants through mineral fertilizers. Coco pith is an organic substrate produced from the wastes of coco fruits. It has a high water-holding capacity, cation-exchange capacity, aeration, a light bulk density, an ideal pH and EC, and is free of pathogens. These properties have made it one of the most preferred soilless growing media in the greenhouse industry in recent years [3]. Therefore, coco pith was chosen for capia pepper cultivation in this study.

In recent years, improvements in beneficial microorganisms have increased the tendency to use bio-fertilizers as valuable tools in sustainable agriculture. Unlike in soil, in hydroponic growing soilless systems there are no beneficial microorganisms in the root environment, and plants cannot benefit from these microorganisms. Therefore, bio-fertilizers such as mycorrhiza fungi, bacteria, and microalgae are commonly adapted to soilless growing systems [4–7]. Bio-fertilizers are effective strains of microorganisms that help crop plants' nutrition by enhancing growth, yield, and crop quality in soilless culture systems [8]. At the same time, they provide environmentally friendly agriculture by reducing the use of mineral fertilizers. Inoculation of arbuscular mycorrhizal fungi (AMF) and plant-growth-promoting rhizobacteria (PGPR) become an alternative solution to increase the efficiency of nutrient usage as well as absorption of nutrients by plants [5,9]. The use of bio-fertilizers in the soilless growing of several vegetable species was previously reported in related literature [10–13].

Similarly, studies also acknowledged the beneficial effects of bio-fertilizers on pH and electrical conductivity (EC) of the nutrient solution regulations, chelator secretion in the rootzone, increased uptake of nutrients, plant growth, yield, and crop quality [7,14]. In an SCS, the nutrient solution that consists of mineral fertilizers is used in every irrigation. An open system means the nutrient solution is not recyclable, and the excess drainage solution (about 20–25% of the applied nutrient solution) can pollute the environment and groundwater resources. In the greenhouse sector, it is noteworthy that a significant majority of SCSs produce crops in an open system. For this reason, less mineral fertilizer means environmental protection. Bio-fertilizers improve, in addition, the pH and EC of the nutrient solution in the root zone [7]. Therefore, they provide a high intake of nutrients from plants. In addition, biofertilizers decrease nitrate and increase the antioxidants and minerals of vegetables [13,15]. Hence, biofertilizers provide positive contributions to human nutrition. However, studies on bio-fertilizers used in reducing mineral fertilizers are quite limited. On the other hand, farmers' and consumers' awareness of environmental and ecological concepts has recently increased. As a result, some farmers who want to produce according to a sustainable model prefer to use organic fertilizers and/or bio-fertilizers in soilless cultivation systems [16].

Capia (*Capsicum annuum* L.) is a type of pepper with a long conical shape, red color in maturity, and a sweet taste; it is rich in vitamins C and A, folic acid, potassium, mineral substances, phenolic compounds, carotene, and antioxidant compounds [17,18]. It has a dense juicy pulp with a rich aroma in the red maturity stage. Capia pepper fruit at the red ripeness stage has the following morphological properties: 80–125 g weight, 15–20 cm length, 40–55 mm width, 150–250 cm^3 volume, and 3.5–4.0 mm flesh thickness. Capia pepper seeds germinate in 7–10 days. The seedlings grow in around 30–40 days. The plant grows best between 22–25 °C during the day and 15–18 °C at night. An increase in daytime temperature to 28 °C accelerates red ripening. Red ripe capia fruits are harvested in approximately 90 days from seedling planting. Capia pepper, very popular in the Balkan countries and Turkey, is commonly used for pepper paste, pepper juice, pickles, frozen products, frying, and peppery sauce in summer field production [18].

On the contrary, greenhouse capia is used for table consumption during the cold winter season. The capia pepper's soilless cultivation in the greenhouse has recently become popular among growers. Approximately 3,018,775 tons of pepper are produced in Turkey, and 49% of this is capia pepper [19]. The consumer preference in Turkey is primarily capia pepper. Recently, capia cultivation has increased with soilless techniques in

the greenhouse. Greenhouse producers search for environmentally friendly fertilizers that increase product yield and quality.

To our knowledge, reducing mineral fertilizers and substituting them with bio-fertilizers has not been previously investigated in SCSs for capia pepper. Therefore, this study aimed to reduce the intensive use of mineral fertilizers by inoculating with beneficial microorganisms such as AMF and PGPR. Moreover, bio-fertilizers' effects on plant growth, yield and fruit quality of soilless-grown capia pepper were investigated in this study.

2. Materials and Methods

2.1. Plant and Bio-Fertilizer Materials and Experimental Conditions

This study was conducted in a glasshouse at 36°59′ N, 35°18′ E, and 23 m above the Mediterranean Sea level in the early spring growing season (February-July). Climatic conditions inside the glasshouse were 23–25 °C during the day and 15–20 °C at night, with 50–60% relative humidity and natural sunlight conditions. Trademarked Lale F_1 capia pepper of the Istanbul Tarim company was used. We used coco pith slabs as a soilless cultivation medium, cultivating four plants in every slab, with four slabs in each replication. The size of the coco pith slab was 100 cm long, 20 cm wide, and 10 cm deep. For the randomized complete block experimental design with seven treatments and four replicates, 16 plants were used in each replicate. Pepper seedlings 35 days old were transplanted into the coco pith slabs 25 cm above the row and 80 cm between the rows Figure S1.

The mycorrhiza bio-fertilizer Endo Roots Soluble (ERS), a cocktail from nine different mycorrhiza species: Glomus intraradices, Glomus aggregatum, Glomus mosseae, Glomus clarum, Glomus monosporus, Glomus deserticola, Glomus brasilianum, Glomus etunicatum, and Gigaspora margarita, was used. The liquid bacteria Medbio bio-fertilizer used in the experiment contained four different bacteria species: Bacillus subtilis (1×10^9), Bacillus licheniformis (2×10^6), Bacillus megaterium (1×10^9) and Pseudomonas putita (1×10^{10}). Pepper seedlings were inoculated only once during transplanting, with approximately 2000 mycorrhizae spores per plant; with the growth of the root system, mycorrhiza spores multiply in a symbiosis relationship. Meanwhile, bacteria were applied every ten days to the roots during growing. PGPR was applied at 50 mL per plant from the 1 mL Metbio in 1 L nutrient solution. Repeated use of PGPR can stabilize the number of bacteria in the rootzone. Preliminary trials were carried out to determine the bacteria application method. Very successful results were obtained from these preliminary trials. The method used did not ever lead to uneven application or other causes of error. Soilless pepper plants, provided with a nutrient solution with 100% mineral fertilizers, served as a control (Table 1) [20]. Moreover, we substituted 20% and 40% of the mineral fertilizers with mycorrhiza, bacteria, and their combination. In the study, seven treatments were applied.

Table 1. The nutrient solution used in the control treatment (100% mineral fertilization) (mg L^{-1}).

N	P	K	Ca	Mg	Fe	Mn	Zn	B	Cu	Mo
100–239	40–81	96–370	150–250	50–92	5–10	1.97	0.25	0.7	0.07	0.05

1. Common nutrient solution, 100% mineral fertilization (as control) (Table 1),
2. 60% mineral fertilization (MF) + PGPR,
3. 60% MF + AMF
4. 60% MF + PGPR + AMF
5. 80% MF + PGPR
6. 80% MF + AMF
7. 80% MF + PGPR + AMF

2.2. Nutrient Solution and Irrigation

The amount of nutrient solution applied to the plants was determined based on the daily drainage ratio (DR) from the base of the coco pith slabs. The drainage ratio was approximately 20% ± 5 [21]. The pH and EC of the nutrient solution during the cultivation period were maintained within the range of 6.0–6.5 and 2.0–2.8 dS m^{-1}, respectively.

$$DR = \text{drainage solution (mL)} \div \text{applied nutrient solution (mL)} \times 100$$

2.3. Parameters Examined in the Experiment

Plant growth parameters, such as plant height, stem diameter and the number of branches, were measured 80 days after seedling transplanting (DAT) (Table 2). In addition, shoot and leaf fresh weights and leaf area per plant were recorded at 164 DAT at the end of the experiment. The leaf area was determined by a leaf area meter (Li-3100, LICOR, Lincoln, NE, USA) and indicated as cm^2 $plant^{-1}$. Ten plants per plot were used for the measurements.

Table 2. Effects of the bio-fertilizers on plant height, diameter and branches, 80 DAT.

Treatments	Plant Height (cm)	Stem Diameter (mm)	Number of Branches
100%MF	81.08 b	10.66	6.42
60%MF + PGPR	78.75 b	9.61	5.75
60%MF + AMF	66.50 c	9.00	5.67
60%MF + PGPR + AMF	83.25 b	9.75	5.33
80%MF + PGPR	90.83 a	10.04	5.20
80%MF + AMF	94.92 a	10.03	5.33
80%MF+ PGPR + AMF	98.13 a	10.09	4.92
$LSD_{0.05}$	7.398	NS	NS
P	<0.0001	0.1620	0.5873

DAT: days after transplanting; MF: mineral fertilizer; PGPR: plant-growth-promoting rhizobacteria; AMF: arbuscular mycorrhizal fungi; NS: not significant. Different letters within a column indicate significant differences.

Pepper fruits were harvested weekly when they reached the red maturity stage (Figure 1). The cumulative yield of pepper fruit is expressed as kg m^{-2} for the total harvest. Red ripe pepper fruit sampling, 15 fruits per replication, was used for fruit quality measurements. The pH, EC, total soluble solids (TSS), and titratable acidity were measured in the capia pepper fruit.

2.3.1. Determination of Leaf Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Zinc (Zn), Manganese (Mn), and Copper (Cu) by Atomic Absorption Spectrophotometry

Leaf samples, 20 fully mature leaves of 10 plants per replicate, were collected at 80 DAT for mineral nutrient analysis. Leaves were dried in a forced-air oven at 65 °C for 48 h and ground through a 40-mesh sieve for elemental analysis [21]. The samples were dry-ashed in a muffle furnace at 550 °C for six hours. The ash was then dissolved in 0.1 M hydrochloric acid (HCl). K, Ca, Mg, Fe, Mn, Zn, and Cu concentrations were determined using an atomic absorption spectrophotometer [22].

Figure 1. Soilless-grown capia peppers were harvested weekly when they reached the red maturity stage. MF: mineral fertilizer.

2.3.2. Determination of Leaf Total Nitrogen (N) by the Kjeldahl Method

Dry-ground leaf samples weighing 1 gm were weighed out; 5 mL of concentrated H_2SO_4 and a selenium tablet were placed on them; there were burned in the combustion unit of a Kjeldahl apparatus at 400 °C for 1 h until the color turned pale. Then, distillation was performed with 28% NaOH in a Kjeldahl tube distillation apparatus. Boric acid and the indicator solution were added to the ammonia released during distillation, and then, titration was performed with 0.01 N HCl. The total nitrogen of the leaf was calculated with the amount of HCl consumed in the titration (modified from [22]).

2.3.3. Determination of Leaf Phosphorus (P) by the Barton Method

The dry-ashed, furnaced and dissolved leaf samples (as mentioned above) were reacted with Barton's solution. The phosphorus concentration was determined at a wavelength of 430 nm in the spectrophotometer (modified from [22]).

2.4. Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) with the SAS-JMP/7 statistical program. The averages of the treatments were compared with the least significant difference (LSD) test at $p \leq 0.05$ level.

3. Results

3.1. Effects of Bio-Fertilizers on Plant Growth

The synergistic effect of mycorrhiza and bacteria with 80% mineral fertilizer increased pepper plant height in our experiment. They were approximately 21% taller than the control. The tallest pepper plants were grown by using both bio-fertilizers (Table 2). Pepper plants grown in 60% MF + PGPR + AMF were as tall as the control plants. There was no statistically significant difference between treatments for plant stem diameter and number of branches (Table 2). Lucas et al. [23] reported a positive enhancing effect of the beneficial bacteria *Bacillus licheniformis* on tomato and pepper plant height and stem thickness. Moreover, *Bacillus licheniformis* increased auxins and gibberellins in the pepper and tomato plants.

AMF are beneficial to plants by mobilizing nutrients in the root zone, production of siderophores, improving nutrient and water use efficiencies, promoting nutrient uptake of roots, protecting plants from pathogens, and increasing plants' tolerance for abiotic stresses [24]. PGPR are also beneficial to plants by increasing photosynthesis and stimulating the production of phytohormones (indole acetic acid-IAA, cytokinin, gibberellin), secondary metabolic products such as vitamins, and amino acids [25,26]. At the end of the experiment, the heaviest shoot and leaf weights after the control were 545.0 and 262.3 g plant^{-1}, using bacteria and mycorrhiza with 80% mineral fertilizer (Table 3). Similarly, a positive effect of bacteria on tomato and pepper leaf area was found [23]. However, the combination of bacteria and mycorrhiza induced a synergistic effect in our experiment. The photosynthetic performance of the plants largely determines biomass. Kandiannan et al. [27] investigated two bacteria and one mycorrhiza, applied in single, double, and triple combinations to the black pepper plant (*Piper nigrum*) grown in containers. The double and triple combinations significantly increased plant height, leaf area, and biomass production compared to the control plants.

Table 3. Effects of the bio-fertilizers on shoot and leaf weights 164 DAT at the end of the cultivation.

Treatments	Shoot Fresh Weight (g Plant^{-1})	Leaf Fresh Weight (g Plant^{-1})
100%MF	635.2 a	297.5 a
60%MF + PGPR	377.4 cd	185.3 de
60%MF + AMF	308.2 d	157.7 e
60%MF + PGPR + AMF	412.8 bcd	206.3 cd
80%MF + PGPR	530.6 abc	258.0 b
80%MF + AMF	516.8 abc	243.3 bc
80%MF+ PGPR + AMF	545.0 ab	262.3 ab
$LSD_{0.05}$	156.484	34.008
P	0.0053	<0.0001

DAT: days after transplanting; MF: mineral fertilizer; PGPR: plant growth promoting rhizobacteria, AMF: arbuscular mycorrhizal fungi. Different letters within a column indicate significant differences.

The leaf area of pepper plants increased by using biofertilizers. The leaf is the major photosynthetic apparatus of plants. A synergistic effect of microorganisms was observed for this parameter. AMF, PGPR and 80% mineral fertilizer resulted in a leaf area close to the control (Figure 2). The increase in leaf area might be due to increased nutrient availability due to the production of phytohormones. This, in turn, caused an enhancement in plant growth and fruit yield. As an efficient photosynthetic organ, the leaf area most likely induced the building of more plant carbohydrates [13].

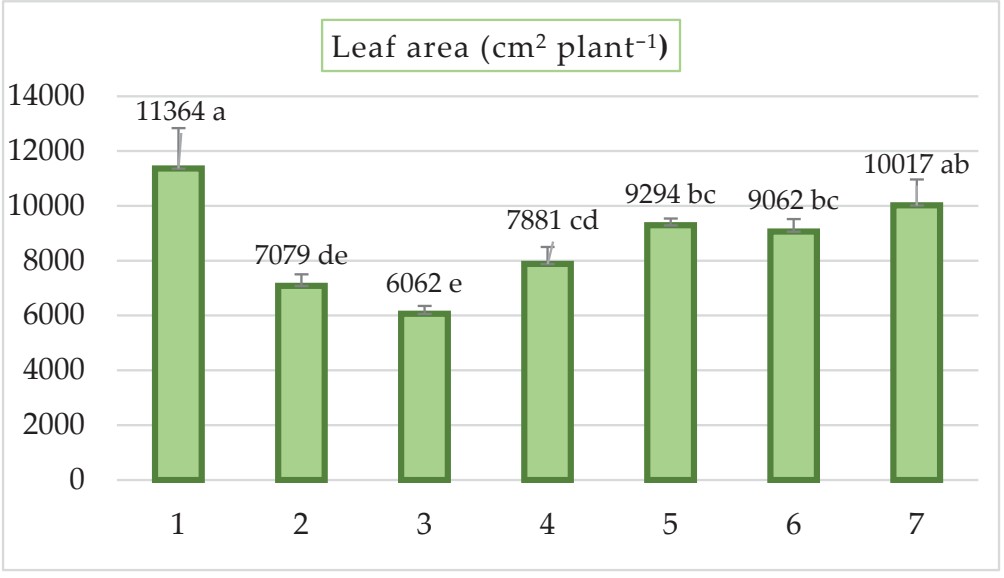

Figure 2. Effects of biofertilizers on pepper plant leaf area 164 DAT at the end of cultivation. DAT: day after transplanting. 1:100% MF, 2:60% MF + PGPR, 3:60% MF + AMF, 4:60% MF + PGPR + AMF, 5: 80% MF + PGPR, 6:80% MF + AMF, 7:80% MF + PGPR + AMF. MF: mineral fertilizer; PGPR: plant-growth-promoting rhizobacteria; AMF: arbuscular mycorrhizal fungi. Different letters within a column indicate significant differences, $LSD_{0.05}$:1413, $p < 0.0001$.

3.2. Effects of Bio-Fertilizers on Total Fruit Yield and Fruit Number

Total pepper fruit yield ranged from 3.30 to 5.80 kg m^{-2}. The application of bacteria and mycorrhiza to 80% mineral fertilizer induced the highest yield, 32.4% higher than the control (4.38 kg m^{-2}). The second- and third-highest yields were 24.2% and 11.2% for the 80% MF + PGPR and 80% MF + AMF treatments, respectively (Figure 3). In bio-fertilized plants with the consortium of PGPR + AMF, better plant growth and biomass production could promote photosynthesis more effectively. Therefore, accumulated supply facilitates fruit development and contributes to a higher total yield. According to Dere et al. [2], bacteria and mycorrhiza can enhance plant nutrient uptake and, in turn, photosynthesis. The lowest yield was obtained from 60% MF + AMF with 3.30 kg m^{-2} with a 16.4% yield decrease compared to the control. Maboko et al. [28] grew soilless tomato plants at 25% and 50% low nutrient levels with mycorrhiza in heated and unheated tunnels. Mycorrhiza worked more effectively in heated tunnels and increased tomato yields at the reduced nutritional treatments. Perhaps the temperatures in heated tunnels contributed to better-establishing mycorrhiza fungus. Baum et al. [29] reported that mycorrhizal inoculation increased pepper plant growth, fresh biomass, and total yield. Aini et al. [5] found that soilless-grown lettuce associated with PGPR + AMF increased the synthesis of growth-promoting plant hormones, primarily cytokines, which enhances leaf growth. While leaf growth contributes to canopy development, a greater photosynthetically active surface area becomes available, improving plant growth and yield. El-Tohamy et al. [9] reported similar results. Bio-fertilization resulted in higher N, P, and K contents of tomato leaves and higher indole acetic acid, gibberellins, and cytokines. Backer et al. [16] reported that mixing bacteria with mycorrhizal fungi improved corn, tomato, and soybean yields.

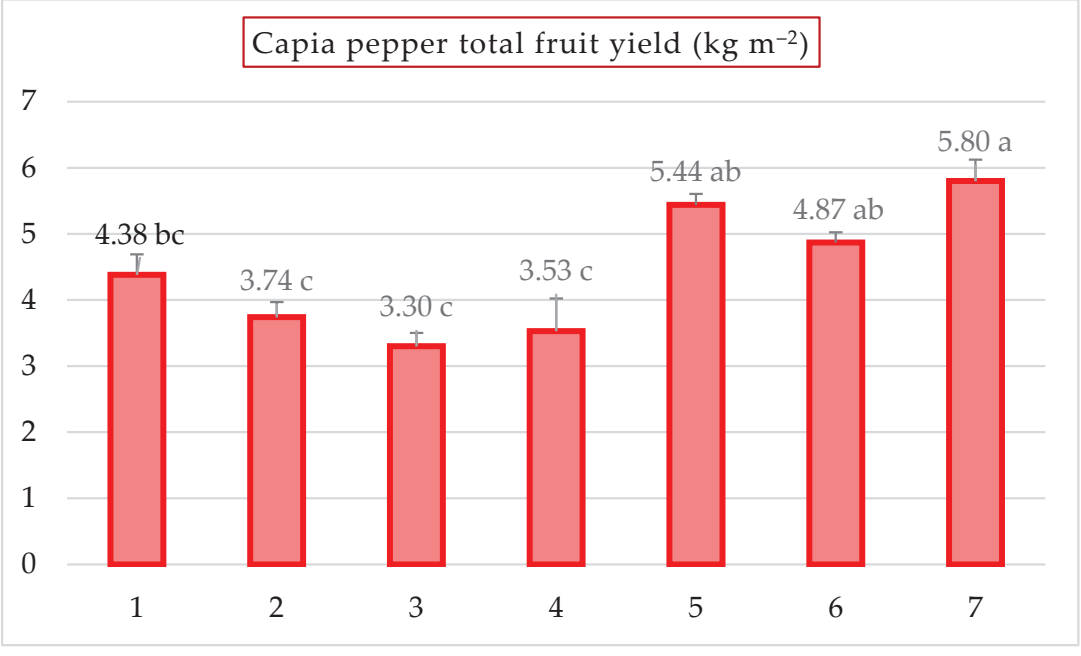

Figure 3. Effects of the bio-fertilizers on total fruit yield of soilless-grown capia pepper under reduced mineral fertilizers in the Mediterranean climate in spring greenhouse conditions. 1:100% MF, 2:60% MF + PGPR, 3:60% MF + AMF, 4:60% MF + PGPR + AMF, 5:80% MF + PGPR, 6:80% MF + AMF, 7:80% MF + PGPR + AMF. MF: mineral fertilizer; PGPR: plant-growth-promoting rhizobacteria; AMF: arbuscular mycorrhizal fungi. Different letters within a column indicate significant differences, $LSD_{0.05}$: 1.134, *p*: 0.0023.

The number of pepper fruit harvested per m^2 in our experiment varied from 35 to 48 fruit m^2. The highest number of fruits was obtained with the treatment using 80% mineral fertilizer combined with bacteria (Figure 4). The presence of 80% mineral fertilizer with bacteria provided better fruit set and development. The number of fruits per unit area of 80% MF + AMF is lower than 80% MF + PGPR and equal to 100% MF (Figure 4). Since the yields of the 80% MF + AMF and 80% MF + PGPR per unit area were in the same significance level and higher than the 100% MF (Figure 3), single-fruit weight of 80% MF + AMF was higher than that of the 80% MF + PGPR and 100% MF (Table 4). It seems that biofertilizer ingenuity induced better plant nutrition and promoted photosynthesis and fruiting.

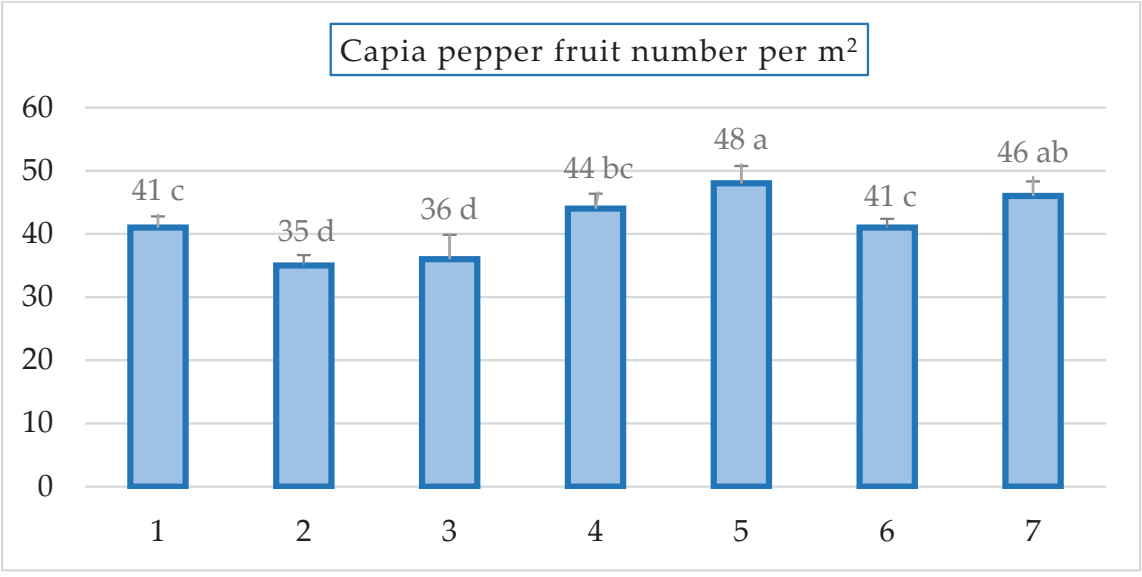

Figure 4. Effects of the bio-fertilizers on total fruit number of soilless-grown capia pepper under reduced mineral fertilizers in the Mediterranean climate in spring greenhouse conditions. 1:100% MF, 2:60% MF + PGPR, 3:60% MF + AMF, 4:60% MF + PGPR + AMF, 5:80% MF + PGPR, 6:80% MF + AMF, 7:80% MF + PGPR + AMF. MF: mineral fertilizer; PGPR: plant-growth-promoting rhizobacteria; AMF: arbuscular mycorrhizal fungi. Different letters within a column indicate significant differences, $LSD_{0.05}$: 4.119, *p*: <0.001.

3.3. Effects of Bio-Fertilizers on Capia Pepper Fruit Quality Properties

Using soilless culture systems to control nutrients and temperature in the rootzone and managing environmental and agronomic factors can improve product quality [28,30,31]. Dasgan et al. [13] showed that biofertilizers could affect the leaf yield, nitrate amount, and mineral and antioxidant content of basil (*Ocimum basilicum* L.) in a floating culture. In addition, Baum et al. [29] summarized that many research results prove the positive effects of AMF on plant growth, P crop physical and chemical characteristics, and produce quality. Our study's average pepper fruit weight ranged from 91.96 to 125.16 g. The treatment containing 80% mineral fertilizers with mycorrhiza and bacteria produced 19% heavier fruits than the control. As in the case of the total yield, the combined use of bacteria and mycorrhiza showed synergistic effects on fruit growth and physical properties, e.g., firmness and flesh thickness (Table 4). The second-heaviest fruit was from the 80% mineral fertilizers with mycorrhiza treatment (118.86 g), which was 13% heavier than the control. Effects of bio-fertilizers on pepper fruit height and diameter also increasingly affected the fruit weight. Dasgan et al. [15] reported that when the mineral fertilizers were reduced

by 20% and 40%, mycorrhiza, bacteria, and microalgae increased soilless-grown squash size. Although the effects of the treatments on pepper firmness and flesh thickness were insignificant, the effects on fruit volume were remarkable (Table 4). The maximum fruit volume was obtained from the 80% MF + PGPR + AMF treatment, with 246 cm^3 (Table 4).

Table 4. Effects of the treatments on the physical properties of capia pepper fruit.

Treatments	Weight (g)	Height (cm)	Diameter (mm)	Volume (cm^3)	Firmness ($kg\ cm^{-3}$)	FLESH Thickness (mm)
100%MF	105.11 c	20.33 a	49.69 cd	201 bc	4.21	4.33
60%MF + PGPR	109.70 bc	19.95 a	51.59 bc	208 ab	4.00	4.23
60%MF + AMF	91.96 d	19.25 a	47.68 d	163 c	4.13	3.93
60%MF + PGPR + AMF	81.81 d	16.78 b	48.24 d	168 c	4.16	3.65
80%MF + PGPR	112.54 bc	19.70 a	55.40 a	231 ab	3.81	4.03
80%MF + AMF	118.86 ab	19.40 a	54.88 ab	230 ab	4.04	4.05
80%MF+ PGPR + AMF	125.16 a	20.73 a	54.94 a	246 a	3.98	4.12
$LSD_{0.05}$	11.382	1.845	3.321	38.531	NS	NS
P	<0.0001	0.0075	0.0001	0.0013	0.4228	0.3261

MF: mineral fertilizer; PGPR: plant-growth-promoting rhizobacteria; AMF: arbuscular mycorrhizal fungi; NS: not significant. Different letters within a column indicate significant difference.

Since mycorrhiza and bacteria increased the production of carbohydrates by enhanced photosynthesis and nutrient uptake, it may also have supported significantly increased electrical conductivity (EC) and total soluble solids of the pepper fruit (Table 5). The effects of the treatments on pH and titratable acidity were similar, although there were no statistically significant differences among the treatments (Table 5). Maboko et al. [28] found that inoculating mycorrhiza into soilless-grown tomatoes increased fruit quality, especially total soluble solids and total dry matter. Baum et al. [29] reported that mycorrhiza increased ascorbic acid in chili pepper, beta carotene in potatoes, and carotenoids, phenolics, anthocyanins, chlorophyll and some mineral nutrients in lettuce. In conclusion, bio-fertilizer affects the product quality of vegetables. Michałojć et al. [30] investigated the effect of mycorrhiza in two nutrient solutions with EC 2600 and 1900 $\mu S\ cm^{-1}$ on the quality of soilless-grown tomatoes. Tomato fruits produced with mycorrhiza contained significantly more total soluble solids than non-mycorrhizal ones.

Table 5. Effects of the treatments on chemical properties of capia pepper fruit.

Treatments	EC ($\mu S\ cm^{-1}$)	pH	TSS (%)	TA (%)
100%MF	2070 b	5.27	5.90 d	1.35
60%MF + PGPR	2642 a	5.01	7.00 a	1.79
60%MF + AMF	2671 a	5.04	7.10 a	1.67
60%MF + PGPR + AMF	2487 a	5.10	6.93 ab	1.78
80%MF + PGPR	2542 a	5.16	6.10 cd	1.61
80%MF + AMF	2577 a	5.00	6.23 bcd	1.71
80%MF+ PGPR + AMF	2445 a	5.11	6.85 ab	1.61
$LSD_{0.05}$	288.059	NS	0.326	NS
p	0.007	0.193	0.0222	0.242

MF: mineral fertilizer; PGPR: plant-growth-promoting rhizobacteria; AMF: arbuscular mycorrhizal fungi; NS: not significant; EC: electrical conductivity; TSS: total soluble solids; TA: titratable acidity. Different letters within a column indicate significant differences.

A sensory panel test was conducted by untrained amateur groups and reports showed that there was no significant difference among the treatments for the taste of the pepper such as spiciness, texture and flavor (unpublished data).

3.4. Effects of Bio-Fertilizers on Mineral Nutrient Concentration

Several studies reported that bacteria and mycorrhizae contribute to plant growth by increasing mineral nutrient uptake [25,30,32,33]. The concentrations of macro elements N, P, K, Ca, Mg, and microelements Fe, Mn, Zn, and Cu in the leaves of pepper plants provided with bio-fertilizers were higher than that of the leaves of the control treatment supplied with 100% mineral fertilizers (Tables 6 and 7). The bacteria and mycorrhiza used in this experiment improved plant nutrition by supplying and facilitating nutrient uptake. According to the pepper plant tissue analysis and interpretation of Hochmuth et al. [34], the mineral nutrition status of the plants fed with the biofertilizers was determined to be sufficient.

Table 6. Effects of the bio-fertilizers on pepper leaf macronutrient concentrations (%).

Treatments	N	P	K	Ca	Mg
100%MF	3.46 c	0.37 c	4.94 d	1.28 d	1.10 c
60%MF + PGPR	3.73 c	0.40 c	5.04 cd	1.94 ab	1.16 abc
60%MF + AMF	3.98 bc	0.61 a	5.32 bc	1.88 abc	1.21 ab
60%MF + PGPR + AMF	4.34 b	0.52 b	5.51 b	1.64 bc	1.11 bc
80%MF + PGPR	5.65 a	0.49 b	5.61 ab	1.59 c	1.22 a
80%MF + AMF	5.62 a	0.48 b	5.88 a	2.06 a	1.26 a
80%MF+ PGPR + AMF	5.57 a	0.42 c	5.89 a	1.88 abc	1.23 a
$LSD_{0.05}$	0.536	0.0496	0.341	0.296	0.104
p	<0.0001	<0.0001	<0.0001	0.0005	0.0255

MF: mineral fertilizer; PGPR: plant-growth-promoting rhizobacteria; AMF: arbuscular mycorrhizal fungi. Different letters within a column indicate significant differences.

Table 7. Effects of the bio-fertilizers on leaf micronutrient concentrations (mg kg^{-1}).

Treatments	Fe	Mn	Zn	Cu
100%MF	71.48 c	23.30 e	41.41 cd	8.36 b
60%MF + PGPR	82.65 bc	43.27 de	46.88 c	10.75 a
60%MF + AMF	171.67 a	86.04 ab	39.69 d	10.97 a
60%MF + PGPR + AMF	174.81 a	75.40 abc	43.44 cd	10.72 a
80%MF + PGPR	154.25 a	92.04 a	55.31 b	11.94 a
80%MF + AMF	147.06 a	64.70 bcd	58.44 ab	12.25 a
80%MF+ PGPR + AMF	107.63 b	54.22 cd	61.56 a	11.25 a
$LSD_{0.05}$	31.121	25.501	5.598	1.768
p	<0.0001	0.0002	<0.0001	0.0060

MF: mineral fertilizer; PGPR: Plant-growth-promoting rhizobacteria; AMF: arbuscular mycorrhizal fungi. Different letters within a column indicate significant differences.

Baum et al. [29], reported that the arbuscular mycorrhizal fungi with plant-growth-promoting bacteria, with a 50% reduction of P fertilizer during seedling transplanting, increased the growth and yield of pepper plants. Thus, biofertilizers could substitute P fertilizer in pepper cultivation. Ortas [35] showed that mycorrhizal application enhanced pepper plants' P and Zn content. Bio-fertilizers enrich the root zone with plant nutrients through N fixation, P, and K mineralization and stimulate plant growth regulators [24]. In chili pepper cultivation, mycorrhiza and bacteria have reported excellent synergistic effects on pepper plant nutrition by providing significant advantages to the uptake of P, Zn, Cu, Mn, and Fe nutrients [36]. In addition, some studies [37–39] noted that co-inoculation of mycorrhiza and bacteria improves nutrient uptake. Although the mechanism is not well known, Bharadwaj et al. [40] stated that the AMF secrete carbohydrates, amino acids, and unidentified compounds that could make the environment favorable for the growth of AMF-associated bacterial growth.

4. Conclusions

Bacteria and mycorrhiza reduced the need for mineral fertilizers used in soilless-grown capia pepper by 20%. Furthermore, combining mycorrhiza and bacteria was more effective than their individual use. Thus, we observed a synergistic effect between the two biofertilizers. The application of the 80% MF + PGPR + AMF induced the highest yield of 5.80 kg m^{-2}, which is 32.4% higher than the 100% MF control yield (4.38 kg m^{-2}). The second- and third-highest yields were 24.2% and 11.2% higher than that of the control for the 80% MF + PGPR and 80% MF + AMF treatments, respectively. The results obtained from the study showed that the use of biofertilizers increased the yield. Therefore, using bacteria and mycorrhiza in soilless-grown capia pepper is an eco-friendly approach to a sustainable environment that reduces synthetic mineral fertilizers and protects the environment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9020188/s1, Figure S1: Views of the capia red peppers on the plants in greenhouse (a,b) and harvested in the lab for fruit analysis (c).

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References

1. Chen, W.; Guo, X.; Guo, Q.; Tan, X.; Wang, Z. Long-term chili monoculture alters environmental variables affecting the dominant microbial community in rhizosphere Soil. *Front. Microbiol.* **2021**, *12*, 681953.
2. Dere, S.; Coban, G.A.; Akhoundnejad, Y.; Ozsoy, Y.S.; Dasgan, H.Y. Use of mycorrhiza to reduce mineral fertilizers in soilless melon (Cucumis melo L) Cultivation. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2019**, *47*, 1331–1336. [CrossRef]
3. Gruda, N.S. Increasing sustainability of growing media constituents and stand-alone substrates in soilless culture systems. *Agronomy* **2019**, *9*, 298. [CrossRef]
4. Gulia, U.; Shukla, J.; Nishanth, S.; Kokila, V.; Bharti, A.; Singh, A.K.; Shivay, Y.S.; Prasanna, R. Fortifying nursery soil-less media with cyanobacteria for enhancing the growth of tomato. *S. Afr. J. Bot.* **2022**, *146*, 564–572. [CrossRef]
5. Aini, N.; Yamika WS, D.; Ulum, B. Effect of nutrient concentration, PGPR and AMF on plant growth, yield and nutrient uptake of hydroponic lettuce. *Int. J. Agric. Biol.* **2019**, *21*, 175–183.
6. Gul, A.; Kidioglu, F.; Tuzel, Y. Effects of nutrition and Bacillus amyloliquefaciens on tomato (*Solanum lycopersicum* L.). *Span. J. Agric. Res.* **2008**, *6*, 422–429. [CrossRef]
7. Dasgan, H.Y.; Kusvuran, S.; Ortas, I. Responses of soilless grown tomato plants to arbuscular mycorrhizal fungal (Glomus fasciculatum) colonization in re-cycling and open systems. *Afr. J. Biotechnol.* **2008**, *7*, 3606–3613.
8. Ikiz, O.; Abak, K.; Dasgan, H.Y.; Ortas, I. Effects of mycorrhizal inoculation in soilless culture on pepper plant growth. *Acta Hortic.* **2009**, *807*, 533–540. [CrossRef]
9. El-Tohamy, W.A.; El-Abagy, H.M.; El-Greadly NH, M.; Gruda, N. Hormonal changes, growth and yield of tomato plants in response to chemical and bio-fertilization application in sandy soils. *J. Appl. Bot. Food Qual.* **2009**, *82*, 179–182.
10. Abak, K.; Dasgan, H.Y.; Rehber, Y.; Ortas, I. Effect of vesicular arbuscular mycorrhizas on plant growth of soilless grown muskmelon. *Acta Hortic.* **2010**, *871*, 301–306. [CrossRef]
11. Aini, N.; Yamika, W.S.D.; Pahlevi, R.W. The effect of nutrient concentration and inoculation of PGPR and AMF on the yield and fruit quality of hydroponic cherry tomatoes (*Lycopersicum esculentum* Mill. var. cerasiforme). *J. Appl. Hortic.* **2019**, *21*, 116–122. [CrossRef]
12. Nasiri, A.; Yarnia, M.; Hassanpanah, D.; Farahvash, F.; Khalivand, E. The response of different potato cultivars to plant growth-promoting rhizobacteria (PGPRs) and chemical fertilizers in aeroponic culture conditions. *J. Plant Nutr.* **2022**, *45*, 2975–2985. [CrossRef]

13. Dasgan, H.Y.; Aldiyab, A.; Elgudayem, F.; Ikiz, B.; Gruda, N.S. Effect of biofertilizers on leaf yield, nitrate amount, mineral content and antioxidants of basil (*Ocimum basilicum* L.) in a floating culture. *Sci. Rep.* **2022**, *12*, 20917. [CrossRef]
14. Gruda, N.; Savvas, D.; Colla, G.; Rouphael, Y. Impacts of genetic material and current technologies on product quality of selected greenhouse vegetables—A review. *Eur. J. Hortic. Sci.* **2018**, *83*, 319–328. [CrossRef]
15. Dasgan, H.Y.; Aydoner, G.; Akyol, M. Use of some microorganisms as bio-fertilizers in soilless grown squash for saving chemical nutrients. *Acta Hortic.* **2012**, *927*, 155–162. [CrossRef]
16. Backer, R.; Rokem, J.S.; Ilangumaran, G.; Lamont, J.; Praslickova, D.; Ricci, E.; Smith, D.L. Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Front. Plant Sci.* **2018**, *9*, 1473. [CrossRef]
17. Raffo, A.; Baiamonte, I.; Nardo, N.; Paoletti, F. Internal quality and antioxidants content of coldstored red sweet peppers as affected by polyethylene bag packaging and hot water treatment. *Eur. Food Res. Technol.* **2007**, *225*, 395–405. [CrossRef]
18. Karaagac, O.; Balkaya, A. Bafra red pepper populations (*Capsicum annuum* L. var. conoides (Mill.) Irish] identification and evaluation of variation available. *Anadolu J. Agric. Sci.* **2010**, *25*, 10–20.
19. Tüik. Turkish Statistical Institute. 2022. Available online: https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-Istatistikleri-2022-45504 (accessed on 21 January 2023).
20. Akyol, M.; Dasgan, H.Y. The Effects of partial root drying in greenhouse soilless bell pepper cultivation. *9th Natl. Veg. Symp. Proc.* **2012**, *1*, 305–309.
21. Schröder, F.G.; Lieth, J.H. Irrigation Control in Hydroponics. In *Hydroponic Production of Vegetables and Ornamentals*; Savvas, D., Passam, H., Eds.; Embryo Publishers: Athens, Greece, 2022; pp. 263–298.
22. Jones, J.B. *Laboratory Guid for Conducting Soil Tests Plant Analysis*, 1st ed.; CRC Press: Boca Raton, FL, USA, 2001.
23. Lucas García, J.A.; Probanza, A.; Ramos, B.; Palomino, M.; Mañero, F.J.G. Effect of inoculation of Bacillus licheniformis on tomato and pepper. *Agronomie* **2004**, *24*, 169–176. [CrossRef]
24. Sinha, R.K.; Valani, D.; Chauhan, K.; Agarwal, S. Embarking on a second green revolution for sustainable agriculture by vermiculture biotechnology using earthworms: Reviving the dreams of Sir Charles Darwin. *J. Agric. Biotechnol. Sustain. Dev.* **2010**, *2*, 113–128.
25. Zayed, M.S. Improvement of growth and nutritional quality of Moringa oleifera using different biofertilizers. *Ann. Agric. Sci.* **2012**, *57*, 53–62. [CrossRef]
26. Prasad, M.R.; Srinivasan, M.; Chaudhary, M.; Choudhary, M.; Jat, L.K. Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture: Perspectives and challenges. In *PGPR Amelioration in Sustainable Agriculture*; Singh, A.K., Kumar, A., Singh, P.K., Eds.; Woodhead Publishing (Elsevier): Sawston, UK, 2019; pp. 129–157.
27. Kandiannan, K.; Sivaraman, K.; Anandaraj, M.; Krishnamurthy, K.S. Growth and nutrient content of black pepper (Piper nigrum L.) cuttings as influenced by inoculation with biofertilizers. *J. Spices Aromat. Crops* **2000**, *9*, 145–147.
28. Maboko, M.M.; Bertling, I.; Du Plooy, C.P. Arbuscular mycorrhiza has limited effects on yield and quality of tomatoes grown under soilless cultivation. *Acta Agric. Scand. Sect. B-Soil Plant Sci.* **2013**, *63*, 261–270. [CrossRef]
29. Baum, C.; El-Tohamy, W.; Gruda, N. Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: A review. *Sci. Hortic.* **2015**, *187*, 131–141. [CrossRef]
30. Michałojć, Z.; Jarosz, Z.; Pitura, K.; Dzida, K. Effect of mycorrhizal colonization and nutrient solutions concentration on the yielding and chemical composition of tomato grown in rockwool and straw medium. *Acta Sci. Pol. Hortorum Cultus* **2015**, *14*, 5–27.
31. Gruda, N.S. Advances in Soilless Culture and Growing Media in Today's Horticulture An Editorial. *Agronomy* **2022**, *12*, 2773. [CrossRef]
32. Chen, S.; Zhao, H.; Zou, C.; Li, Y.; Chen, Y.; Wang, Z.; Jiang, Y.; Liu, A.; Zhao, P.; Wang, M.; et al. Combined inoculation with multiple arbuscular mycorrhizal fungi improves growth, nutrient uptake and photosynthesis in cucumber seedlings. *Front. Microbiol.* **2017**, *8*, 2516. [CrossRef] [PubMed]
33. Priyanka, M.; Rajesh, K.; Khushboo, S.; Manoj, K. Evaluation of native PGPR isolates in bell pepper for enhanced growth, yield and fruit quality. *Int. J. Farm Sci.* **2018**, *2*, 28–35.
34. Hochmuth, G.; Maynard, D.; Vavrina, C.; Hanlon, E.; Simonne, E. *Plant Tissue Analysis and Interpretation for Vegetable Crops in Florida*; IFAS Extension; University of Florida: Gainesville, FL, USA, 2012.
35. Ortas, I. Do maize and pepper plants depend on mycorrhizae in terms of phosphorus and zinc uptake? *J. Plant Nutr.* **2012**, *35*, 1639–1656. [CrossRef]
36. Sreenivasa, M.N.; Krishnaraj, P.U. Synergistic interaction between VA mycorrhizal fungi and a phosphate solubilizing bacterium in chilli (*Capsicum annuum*). *Zent. Für Mikrobiol.* **1992**, *147*, 126–130. [CrossRef]
37. Kim, K.; Yim, W.; Trivedi, P.; Madhaiyan, M.; Deka Boruah, H.P.; Rashedul Islam, M.; Lee, G.; Sa, T.M. Synergistic effects of inoculating arbuscular mycorrhizal fungi and Methylobacterium oryzae strains on growth and nutrient uptake of red pepper (*Capsicum annuum* L.). *Plant Soil* **2010**, *327*, 429–440. [CrossRef]
38. Tajini, F.; Trabelsi, M.; Drevon, J.J. Co-inoculation with Glomus intraradices and Rhizobium tropici CIAT899 increases P use efficiency for N2 fixation in the common bean (*Phaseolus vulgaris* L.) under P deficiency in hydro-aeroponic culture. *Symbiosis* **2011**, *53*, 123–129. [CrossRef]

39. Nadeem, S.M.; Ahmad, M.; Zahir, Z.A.; Javaid, A.; Ashraf, M. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnol. Adv.* **2014**, *32*, 429–448. [CrossRef] [PubMed]
40. Bharadwaj, D.P.; Alström, S.; Lundquist, P.O. Interactions among Glomus irregulare, arbuscular mycorrhizal spore-associated bacteria, and plant pathogens under in vitro conditions. *Mycorrhiza* **2012**, *22*, 437–447. [CrossRef]

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