

Article

The Effect of Biochar on the Biological Properties of Soil, Growth Vigour, and Quality of Strawberries After Replantation

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Abstract

Apple replant disease (ARD) is a significant problem in regions with intensive fruit production. ARD deteriorates the production properties of soil, reduces yield, and results in lower fruit quality. The effects of ARD can be mitigated with organic amendments applied to soil to improve its productivity. Our experiment was conducted on a farm in western Poland. Biochar was added to replanted soil at 5%, 10%, and 20% by volume and its effect on the physicochemical and biological properties of the soil, the growth vigour, and the quality of strawberries was analysed. Strawberry plants were planted in soil which either had been used for nursery production (replanted soil) or had never been used for this purpose (agricultural soil). The results of our study confirmed earlier reports on the negative consequences of ARD. The replanted soil was more saline and contained less organic matter than the agricultural soil. The plants growing on the replanted soil developed worse. When biochar was added to the replanted soil, depending on its percentage content, the organic matter content and soil respiration rate almost doubled, whereas the count of bacterial taxonomic units increased. As a result of these changes, the root mass of the plants growing in the replanted soil increased by nearly 50%, whereas the root collar mass of shrubs and their leaf area increased by 25 to 30%. The average fruit weight also increased by about 40%. The optimal biochar content in the replanted soil was 10%. The biochar content of 20% did not cause significant changes in most of the parameters under analysis.

Keywords: apple replant disease; strawberry; biochar; soil enzyme and respiration activity; fruit quality



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1. Introduction

The strawberry (*Fragaria × ananassa* Duch) is one of the most popular horticultural crops worldwide. In the last decade, global strawberry production has increased by about 20% [1]. Poland is one of the largest European strawberry producers, as according to FAOSTAT, in 2024, the annual production volume of this fruit amounted to 173,400 tonnes. Consumers around the world value strawberries due to their distinct flavour, attractive appearance, and health benefits. These benefits result from the content of bioactive substances such as phenolic compounds (phenolic acids, especially ellagic acid) and anthocyanins [2], which have anticarcinogenic [3], anti-inflammatory [4], and cardiovascular disease-preventing [5] properties.

The production cycle of strawberries is short. The market demand for new cultivars and fruit quality changes frequently. Due to the shortage of land for new plantations, strawberries are often grown in old plantations. This increases the risk of apple replant disease (ARD), also known as replant disease [6] or soil fatigue. Scientists continue to debate how we might identify the major causes of ARD. Researchers tend to agree that the causes of ARD are complex and multifaceted, with both biotic and abiotic factors. The former include nematodes [7], bacteria (*Bacillus*, *Pseudomonas*), and fungi [8]. The most common pathogenic fungi causing ARD are *Fusarium* [9], especially *F. proliferatum*. This is a soil-borne fungal pathogen that also occurs in strawberry plantations.

ARD has been studied for several decades. Therefore, its effects have been thoroughly investigated. In replanted soil, acidity increases, whereas the content of available macro- and micronutrients decreases [10]. The microbial diversity of soil (measured by soil enzyme and respiration activities) also decreases [11]. As a consequence, the production properties of the soil deteriorate. Plant growth is weaker, yield is lower, and fruit quality is worse [6]. ARD causes changes in the morphology of plant roots, which become discoloured and necrotic [12].

Thus far, researchers have developed several methods of mitigating the effects of replant disease. The population of biofactors causing ARD can be reduced through chemical or biological fumigation. Chemical fumigation causes significant changes to the structure of the soil microbiota [13], whereas biofumigation, though highly effective, is relatively resource-intensive and time-consuming. The abiotic factors of replant disease can be counteracted by enriching the soil with nutrients. Both mineral and organic fertilisers can be used for this purpose. Mineral fertilisers are widely used due to their high effectiveness, rapid action, and relatively low production cost. However, excessive use of mineral fertilisers may cause numerous environmental and agronomic problems, e.g., increased soil acidity, which may reduce soil microbial diversity [14] and lower the availability of nutrients [15], which may weaken the vegetative growth of plants.

Organic fertilisation can be used as an alternative to mineral fertilisation. Organic fertilisers not only provide organic matter to the soil but also improve its physicochemical properties [16] and increase the activity of soil enzymes [17]. In order to replace mineral fertilisers with organic ones, it is necessary to use large amounts of the latter. Soil productivity can also be improved with organic soil amendments such as biochar. This product is made from organic matter by exposing it to high temperatures with no access to oxygen [18,19]. As shown by results from earlier research, biochar can improve the physicochemical properties of soil [20], increase its water retention capacity [21,22], and increase the content of soil nutrients [23].

The aim of our study was to assess the effect of biochar applied to replanted soil on its biological and physicochemical properties as well as the growth vigour and quality of strawberries.

2. Materials and Methods

2.1. Experiment Location and Design

The experiment was conducted in 2022 on a nursery farm in Puszczykowo-Zaborze in western Poland. Strawberry tray plants of the Malling Centenary cultivar were grown in five-litre plastic containers. The plants were planted into soil from two different locations: a field in which agricultural crops had been grown for ten years (agricultural soil) and a nursery in which apple trees had been produced for two cycles (replanted soil). Before the experiment, the soil had the following physicochemical properties: replanted soil—pH(H₂O) 5.7; bulk density—1761 g dm⁻³; salinity—0.64 g NaCl dm⁻³; humus content—1.67%; mineral content (mg dm⁻³ of soil): N-NO₃—13; P—67; K—127; Ca—549; Mg—167;

agricultural soil: pH (H₂O)—5.9; bulk density—1714 g dm⁻³; salinity—0.96 g NaCl dm⁻³; humus content—2.12%; mineral content (mg dm⁻³ of soil): N-NO₃—25; P—52; K—176; Ca—623; Mg—174. At the beginning of the experiment, the biological properties of the soil were analysed by measuring its enzyme and respiration activities. The following values were measured: dehydrogenase activity (cm³ H₂ 24 h⁻¹ kg⁻¹ dry matter of soil): agricultural soil—0.29, replanted soil—0.23; protease activity (mg of tyrosine h⁻¹ kg⁻¹ dry matter of soil): agricultural soil—3.19, replanted soil—1.97; respiration activity (CO₂ in mg kg⁻¹ 24 h⁻¹): agricultural soil—32.17, replanted soil—26.07.

There were five variants in the experiment: agricultural soil (CRS—crop rotation soil); replanted soil (RS—replanted soil); replanted soil with biochar content of 5% (RS + 5% biochar), 10% (RS + 10% biochar), and 20% (RS + 20% biochar) by volume. The selection of biochar doses in the soil was based mainly on its manufacturer's recommendations and the organic matter content in soil in Poland. There were 10 replicates (pots) in each variant. Biochar was obtained through the anaerobic decomposition of wood pellets at a temperature of 400–500 °C. It contained humic acids, macronutrients (% dry matter): N—0.17; K—0.11; Ca—0.23; Mg—0.06 and micronutrients (mg kg⁻¹ dry matter): Zn—30.9; Cu—2.57; Mn—103; Fe—194; B—8.42. The salinity level was below 0.24 g NaCl dm⁻³, whereas pH was 7.68.

During the growing season, the plants were treated according to recommendations for commercial strawberry plantations. The analysis of the climate conditions was based on data from a weather station (Pessl Instruments—iMETOS®, Weiz, Austria) located near the experiment site (Appendix A).

2.2. Measurements and Analyses

The experiment involved analyses of the physicochemical and biological properties of the soil, plant growth vigour, and fruit quality. The analyses of the physicochemical properties of the soil included measurements of the content of macro- and micronutrients, organic matter content, soil acidity, salinity, and bulk density. The soil samples collected from both sites were analysed twice: before (baseline analysis) and after the experiment (at the end of the growing season). A small amount of soil was collected from each container with plants. Next, the samples were mixed into a collective sample of about 0.5 kg representing each variant of the experiment. The following methods were used to measure the content of macro- and micronutrients: microdistillation (N-NO₃), atomic absorption spectrometry (Mg, Zn, Cu, Mn, Fe), colorimetric analysis (P), and flame photometry (K, Ca). Potentiometric titration was used to measure soil acidity.

The assessment of the biological properties of the soil was based on analyses of the dehydrogenase and protease activities, soil respiration activity, and the abundance of bacterial taxonomic units. The soil respiration and enzyme activities were analysed two times: in mid-June (17 June 2022) and in mid-July (19 July 2022). Colorimetric analysis developed by Thalmann [24] was used to determine the dehydrogenase activity. The spectrophotometric method developed by Ladd and Butler [25] was used to analyse the protease activity. The soil respiration activity was determined with the absorption method developed by Gołębiowska and Pędziwilk [26], which involved measurement of the amount of CO₂ released. The physicochemical and biological properties of the soil were analysed in four replicates for each variant of the experiment.

Analysis of the abundance of soil microorganisms was based on the serial dilution method [27]. A detailed description of the methodology was included in the authors' earlier publication [28]. Ready-made standard Merck agar was used to analyse the abundance of bacteria after three days of incubation at 25 °C. The results were calculated per 1 g of dry soil mass and expressed as colony forming units (CFUs) of 10⁵ g⁻¹ d.m.

The analysis of the plants' vegetative growth vigour was based on measurements of the increase in strawberry root collar diameter during the growing season, measurements of the average area (cm²) and mass (g) of strawberry leaves, and the mass of their root system. At the end of the experiment, 20 leaves were collected from each variant and weighed to the nearest 0.1 g. The assimilation surface of the collected leaves was determined with a scanner and DigiShape software (ver.1.9.19, Cortex Nowa, Bydgoszcz, Poland). At the end of the experiment, the strawberry root systems were rinsed under running water to remove soil particles and then dried. Then the roots were weighed to the nearest gram to calculate the average mass.

The following parameters were taken into consideration to assess the fruit quality: average weight, firmness, and soluble solid content. Fruits for these analyses were harvested in mid-June. Twenty fruits were collected from each variant and weighed to the nearest 0.1 g. Firmness (g mm⁻¹) was measured with a Fruit Pressure Tester, model FT 02 (Facchini Srl, Alfonsine, Italy). The fruits whose firmness was measured were then used for measurements of the total soluble solids (% Brix) with a PR 101 digital refractometer (ATAGO Co., Ltd., Fukaya-shi, Japan). The colour intensity was measured on the same fruits whose average weight was calculated. A Konika Minolta (Japan) digital colorimeter (Minolta Corp., Ramsey, NJ, USA) was used to determine three coordinates: L (where 0 = black and 100 = white), a (green and red), and b (blue and yellow). Due to unfavourable climatic conditions (frosts during the flowering period), no data on plant yields were presented.

2.3. Statistical Analysis

Data were analysed using one-way analysis of variance (ANOVA). Prior to the analysis, the assumptions of homogeneity of variances and normality of residuals were assessed using Levene's test and the Shapiro–Wilk test, respectively. When the ANOVA indicated significant differences, mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at a significance level of $\alpha = 0.05$. These statistical analyses were performed using STATISTICA version 12.1 (StatSoft Inc., Tulsa, OK, USA). Canonical variate analysis was used to assess the relationships between the experiment variants (treatments) and the fruit quality parameters, vegetative growth vigour, and soil biological properties. This method was based on both singular value decomposition (SVD) and linear transformation of the original variables. The extracted orthogonal canonical variates were used for comparison of the experiment variants with respect to the fruit quality parameters under study. Hierarchical cluster analysis based on the Euclidean distance and the single linkage method was used for this purpose. The structure of the resulting clusters is presented in a dendrogram.

3. Results and Discussion

3.1. Physicochemical Properties of Soil

The experiment showed differences in the physicochemical properties of the soil depending on its previous use. The replanted soil was more saline than the agricultural soil (0.66 and 0.40 g NaCl dm⁻³, respectively) and contained significantly less organic matter (1.69% and 2.12%, respectively) (Table 1).

High soil salinity negatively affects the availability of nutrients and water retention in the soil. In our experiment, biochar added to the replanted soil decreased its salinity significantly. In comparison with the variant without biochar (0.66 g NaCl dm⁻³), in the variants with 5% and 10% biochar content, salinity was almost three times lower (0.23 and 0.25 g NaCl dm⁻³) (Table 1). There was a similar result of the experiment by Yan et al. [29], who observed that biochar also reduced soil salinity by several dozen per cent. In our experiment, there were also differences in soil acidity. When biochar was added to

the replanted soil, regardless of its percentage content, its pH increased by about 20%, as compared with the soil without amendments. Abdelaal et al. [30] and Jia et al. [31] also observed that biochar reduced soil acidity. According to Hartemink and Barrow [32], low soil acidity increases the availability of nutrients, especially nitrogen and phosphorus. In comparison with the soil without amendments, 5% biochar added to the replanted soil significantly increased the organic matter content, which almost doubled (3.32% and 1.69%, respectively). It is noteworthy that as the amount of biochar in the soil increased, so did the organic matter content, from 3.32% (5% content) to 4.03% (20% content) (Table 1). In the latter case, the organic matter content in the replanted soil was almost twice as high as in the agricultural soil (4.03% and 2.12%, respectively). An increase in the organic matter content in the soil after the application of biochar was also observed by Abdelaal et al. [30] and Jia et al. [31] in their studies.

Table 1. Some physicochemical properties of the soil.

Treatments	Bulk Density (g dm ³)	pH (H ₂ O)	Salinity (g NaCl dm ³)	Organic Matter (%)
CRS	1750 a ± 84.17	6.4 a ± 0.20	0.40 c ± 0.03	2.12 b ± 0.24
RS	1770 a ± 69.54	5.8 a ± 0.19	0.66 d ± 0.11	1.69 a ± 0.41
RS + 5% biochar	1720 a ± 73.45	7.1 b ± 0.22	0.25 a ± 0.04	3.32 c ± 0.18
RS + 10% biochar	1730 a ± 69.34	7.2 b ± 0.24	0.23 a ± 0.06	3.80 c ± 0.16
RS + 20% biochar	1650 a ± 71.26	7.2 b ± 0.18	0.31 b ± 0.03	4.03 d ± 0.21

CRS = crop rotation soil; RS = replanted soil. Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

An adequate supply of macro- and micronutrients is crucial for both plants' resistance to stress factors and their productivity [33]. Our experiment showed that the content of minerals in the soil was influenced by its earlier use. In the replanted soil, there were lower levels of most macronutrients (N, K, Ca, Mg) and some micronutrients (Cu, Fe) than in the agricultural soil (Tables 2 and 3).

Table 2. The content of macronutrients in the soil (mg dm⁻³).

Treatments	N-NO ₃	P	K	Ca	Mg
CRS	30 c ± 7.12	64 a ± 9.43	206 d ± 23.67	768 d ± 45.23	220 b ± 37.23
RS	17 b ± 3.56	79 a ± 10.28	148 b ± 17.29	540 b ± 32.16	160 a ± 28.56
RS + 5% biochar	15 b ± 2.89	76 a ± 9.37	187 c ± 21.52	597 b ± 41.32	201 b ± 34.87
RS + 10% biochar	15 b ± 2.74	84 b ± 8.41	144 b ± 17.34	620 c ± 39.82	186 b ± 31.84
RS + 20% biochar	10 a ± 2.13	74 a ± 10.11	99 a ± 14.28	485 a ± 37.48	133 a ± 28.51

CRS = crop rotation soil; RS = replanted soil. Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

Table 3. The content of micronutrients in the soil (mg dm⁻³).

Treatments	Zn	Cu	Mn	Fe
CRS	3.90 a ± 0.25	2.23 b ± 0.38	11.35 b ± 1.32	163.85 c ± 23.43
RS	4.14 a ± 0.68	1.80 a ± 0.42	11.45 b ± 1.05	111.45 b ± 17.45
RS + 5% biochar	3.94 a ± 0.63	1.68 a ± 0.61	7.65 a ± 0.96	76.64 a ± 21.68
RS + 10% biochar	4.07 a ± 0.74	1.68 a ± 0.53	6.85 a ± 1.45	80.41 a ± 19.34
RS + 20% biochar	4.64 a ± 0.62	1.66 a ± 0.49	8.02 a ± 1.03	100.60 b ± 20.29

CRS = crop rotation soil; RS = replanted soil. Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

The biggest differences, of about 75%, were observed in the content of nitrogen, an element essential for the synthesis of enzymes and amino acids responsible for cell structure [34].

The authors of many studies observed that biochar increased the content and availability of soil nutrients such as nitrogen [35], phosphorus [36], calcium, and magnesium [37]. The increase in the content of minerals in the soil with biochar may be caused by the fact that biochar itself is a source of these nutrients and its porous structure increases the ability of soil to retain macro- and microelements [29,37]. In our experiment, the variants with the 5% and 10% biochar content in the replanted soil did not differ significantly in their nitrogen content from the soil without biochar (Table 2). However, in the variant with biochar content of 20%, the nitrogen content decreased by about 40% (10 and 30 mg dm⁻³). In the same variant, the soil content of K, Ca, Mn (Table 2), and Fe (Table 3) also decreased by several dozen per cent. However, there was a significant increase in the content of K and Mg (about 5%), as well as P, Ca, and Mg (about 10%). An increase in the calcium content is particularly important in strawberry cultivation because this element plays a key role in maintaining the structure of cell membranes and walls. Singh et al. [38] observed that the foliar treatment of strawberry plants with calcium before harvest resulted in higher marketable yields and better firmness of the fruit [39]. Micronutrients, which are components of most enzymes, regulate biochemical processes in plants. In our experiment, there were no significant changes in the Zn or Cu content regardless of the percentage of biochar in the soil (Table 3).

3.2. Biological Properties of Soil

3.2.1. Enzyme and Respiration Activities

Microorganisms are important components of the soil microhabitat. They maintain soil fertility by regulating the nutrient cycle [40]. It is estimated that about 90% of soil microorganisms are bacteria and fungi. The soil microbial activity can be effectively assessed through the activity of soil enzymes, especially dehydrogenases [41]. In our experiment, the previous use of the soil significantly affected its biological properties, measured with the enzyme and respiratory activities. In the replanted soil, both of these parameters were significantly lower than in the agricultural soil. The difference in the dehydrogenase activity amounted to about 35% (0.33 and 0.9 cm⁻³ H₂ 24 h⁻¹ kg⁻¹ DM), whereas the difference in the protease was almost two times greater (1.94 and 4.87 mg tyrosine h⁻¹ kg⁻¹ DM) (Table 4).

Table 4. The effect of biochar on the enzyme and respiration activities of the soil (average for two terms).

Treatments	Dehydrogenase Activity (cm ⁻³ H ₂ 24 h ⁻¹ kg ⁻¹ d.m.)	Protease Activity (mg Tyrosine h ⁻¹ kg ⁻¹ d.m.)	Respiratory Activity (CO ₂ in mg kg ⁻¹ 48 h ⁻¹)
CRS	0.39 a ± 0.08	4.87 d ± 0.90	34.87 b ± 4.17
RS	0.33 a ± 0.05	1.94 a ± 0.81	24.01 a ± 3.62
RS + 5% biochar	0.37 a ± 0.02	2.51 ab ± 0.74	33.78 b ± 3.04
RS + 10% biochar	0.39 a ± 0.04	3.67 c ± 0.97	41.22 c ± 3.13
RS + 20% biochar	0.35 a ± 0.03	3.15 bc ± 0.45	40.73 c ± 2.85

CRS = crop rotation soil; RS = replanted soil. Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

The biochar used in our experiment had a beneficial effect on the biological properties of the replanted soil. The analysis of the soil respiration activity, which is considered a very effective method of assessing the overall activity of soil microorganisms, revealed the greatest differences. In comparison with the variant without biochar, even a 5% biochar content

resulted in differences of about 40% (33.78 and 24.01 mg kg⁻¹ 24 h⁻¹) (Table 4). When the biochar content was 10%, the respiration activity of the replanted soil also increased significantly (it almost doubled to 41.22 and 24.01 mg kg⁻¹ 24 h⁻¹, respectively). However, a further increase in the soil biochar content to 20% did not increase the respiration activity of the replanted soil significantly (Figure 1). Zaheer et al. [42] also observed higher soil respiration intensity in a variant with biochar.

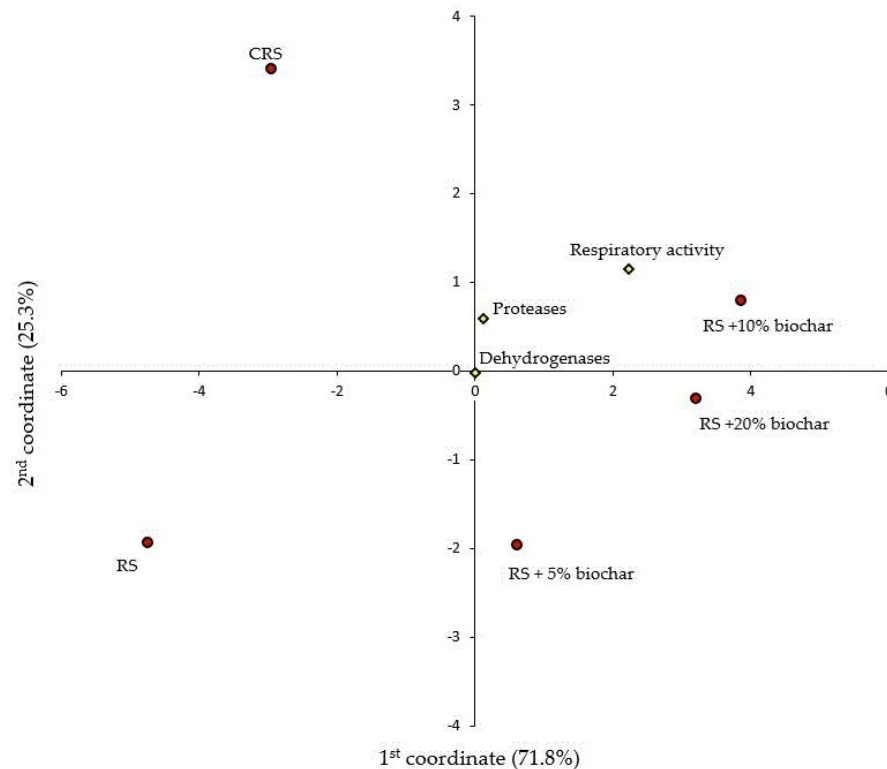


Figure 1. The results of canonical variate analysis, based on singular value decomposition, illustrating relationships between the variants of the experiment and biological properties of the soil (CRS = crop rotation soil; RS = replanted soil).

The biochar content of 5% added to the replanted soil did not significantly alter the activity of either dehydrogenases or proteases. Such changes in the activity of one of the enzymes were observed in the variant with a 10% biochar content in the soil. In comparison with the variant without biochar, the differences in the protease activity amounted to 58% (3.07 vs. 1.94 mg tyrosine h⁻¹ kg⁻¹ dry weight). These values were comparable to the activity of this enzyme in the agricultural soil. It is noteworthy that a further increase in the soil biochar content to 20% did not cause significant changes in the enzyme activity of the replanted soil (Figure 1). In accordance with results from a wide range of other studies, biochar added to the soil increased the activity of its enzymes [43,44], both dehydrogenases [45] and proteases [46]. The activation of enzymes in the variants with biochar may result from an increase in the organic matter content in the soil, which favours the growth of microorganisms.

Analysis of the data in Table 5 indicates that the date of analysis had a significant impact on the biological properties of the soil. The activity of both soil enzymes and soil respiratory activity in the middle of the vegetation period was significantly higher than at the beginning. The greatest differences (more than a twofold increase) were observed in the dehydrogenase activity (0.24 i 0.49 cm⁻³ H₂ 24 h⁻¹ kg⁻¹ DM).

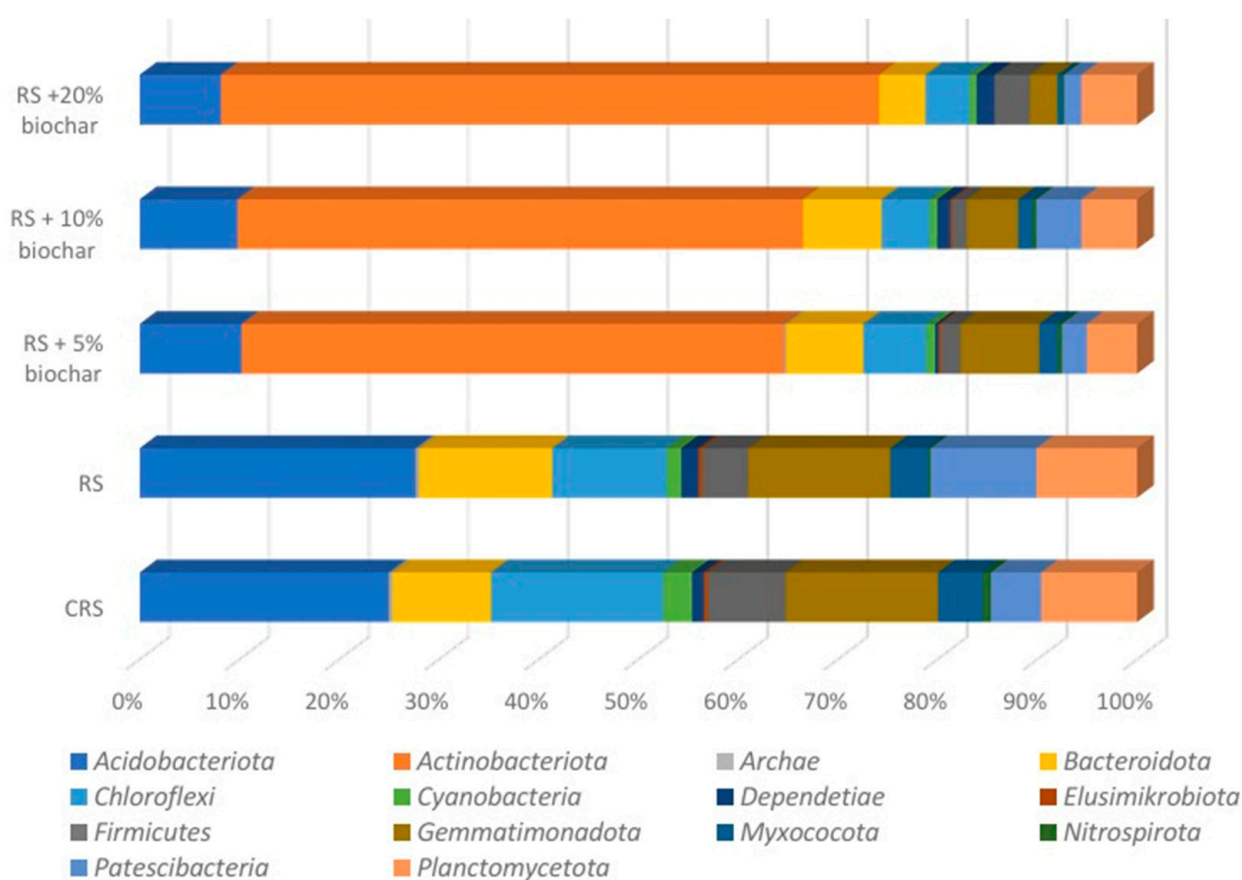
Table 5. The enzymatic and respiratory activity of the of soil during the vegetation period.

Tested Parameters	12 May 2022	19 July 2022
Dehydrogenase activity (in $\text{cm}^{-3} \text{H}_2 \text{ 24 h}^{-1} \text{ kg}^{-1} \text{ DM}$)	0.24 a	0.49 b
Protease activity (in $\text{mg tyrosine h}^{-1} \text{ kg}^{-1} \text{ DM}$)	2.28 a	4.18 b
Respiratory activity (CO_2 in $\text{mg kg}^{-1} \text{ 48 h}^{-1}$)	28.58 a	41.27 b

Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

3.2.2. Abundance of Soil Bacteria

The diversity of soil microorganisms is considered an effective indicator of soil quality and changes occurring in it. Increased activity and abundance of soil microorganisms increase strawberry production potential [47]. The metapopulation analysis based on the 16S rRNA sequence analysis revealed the presence of seventeen taxonomic units of soil bacteria (Figure 2).

**Figure 2.** The effect of biochar on the abundance of bacterial taxonomic units in the soil (in $\text{CFU } 10^5 \text{ g}^{-1} \text{ d.m.}$) (CRS = crop rotation soil; RS = replanted soil).

The following bacterial phyla were the most abundant: *Acidobacteriota*, *Proteobacteria*, and *Actinobacteriota*. These are some of the most common bacterial phyla, especially in soils, which are crucial for organic matter decomposition [48]. The least abundant phyla were *Nitrospirota*, *Archae*, and *Elusimikrobiota*. The last-named phylum can be found in sewage sludge, toxic waste, and contaminated soils.

Apple replant disease (ARD) limits the growth of microorganisms in the soil environment. In our experiment, the previous use of the soil had a noticeable effect on the count of bacteria in it. With one exception, the count of all bacterial phyla in the replanted soil was lower than in the agricultural soil (Figure 2).

Depending on the phylum, the differences in the abundance reached up to several times. The largest differences of more than three times were observed for bacteria of the *Cyanobacteria* (152 and 545 CFU 105 g⁻¹ d.m.) and *Firmicutes* phyla (461 and 1445 CFU 105 g⁻¹ d.m.). The abundance of the following phyla was more than two times smaller: *Sumertaeota*, *Verrucomicrobiota*, *Myxococota*, and *Chloroflexi*. The last-named phylum includes nitrifying anaerobic or microaerophilic bacteria which can survive in rapidly changing, extreme conditions. A high share of *Chloroflexi* bacteria in ARD soil was also observed by Zheng et al. [49] and Tang et al. [50].

According to many researchers, biochar increases the soil microbial activity [43,49,51,52]. The beneficial effect of biochar on the development of soil microflora results from its porous structure, which provides a favourable environment for the growth of bacteria, fungi, and actinobacteria. In our experiment, biochar added to the replanted soil increased the abundance of bacterial taxonomic units. In the 5% biochar variant, the abundance of fifteen of the seventeen bacterial taxonomic units increased. The abundance of the *Firmicutes* bacteria was almost two times greater than in the replanted soil without biochar, whereas the abundance of eight other phyla (*Archae*, *Bacteroidota*, *Chloroflexi*, *Cyanobacteria*, *Gemmatimonadota*, *Myxococota*, *Planctomycetota*, and *Verrumicrobiota*) was more than two times greater (Figure 2). *Cyanobacteria* are an important indicator of soil quality. This phylum is responsible for fixing atmospheric nitrogen and synthesising exopolysaccharides, which translates into increased soil fertility and water retention as well as better soil structure and stability.

In the replanted soil with biochar, the abundance of bacteria of the *Dependetiae* and *Sumertaeota* phyla changed slightly. It is noteworthy that the abundance of some groups in the replanted soil with the 5% biochar content was comparable (*Acidobacteriota*, *Myxococota*, *Proteobacteria*) or greater (*Bacteroidota*, *Elusimicrobiota*) than in the agricultural soil. When the biochar content in the replanted soil increased, the abundance of bacteria changed. In the soil with 10% biochar content, the population of nine of the seventeen bacterial taxonomic units was more abundant than in the soil with 5% biochar content. The greatest differences were observed in the following phyla: *Dependetiae* (more than a double increase), *Patescibacteria* and *Verrucomicrobiota* (an increase of about 40%). The population of the other seven phyla of soil bacteria decreased. The differences ranged from a dozen or so per cent (*Nitrospirota*, *Myxococota*, *Cyanobacteria*) to about 50% (*Gemmatimonadota*) (Figure 2). The largest decrease was observed in the count of *Archae* bacteria (from 101 to 6 CFU 105 g⁻¹ d.m.). According to Wang et al. [8], a high dose of biochar in soil may negatively affect the development of soil microflora. In our experiment, when the biochar content in the replanted soil was increased to 20%, the abundance of the *Archae* and *Elusimicrobiota* bacterial phyla dropped to zero. In this variant, the count of another eight bacterial phyla was lower than in the soil with 10% biochar content. On the other hand, the count of the following bacterial phyla increased significantly: *Verrucomicrobiota*, *Firmicutes*, *Dependetiae*, *Sumertaeota*, and *Actinobacteriota*. According to Niewiadomska et al. [53], *Actinobacteriota* are a saprophytic group of actinobacteria which quickly adapt to adverse environmental conditions, such as desiccation. Therefore, they actively decompose organic matter when the soil moisture is low.

3.3. Vegetative Growth Vigour of Strawberry Plants

As the roots take up water and nutrients from the soil, they are of key importance to the aerial biomass of plants. In our experiment, the previous use of the soil did not have significant influence on the mass of strawberry roots. There were no significant differences between the replanted soil (9.70 g) and agricultural soil (11.73 g). As shown by results from reference publications, biochar added to the soil is beneficial for the growth of plant roots [54] because it increases their length [55] and mass [56]. There were similar

observations in our experiment. The mass of the strawberry root system in the soil variant with 5% biochar content did not differ significantly from the replanted soil without biochar. However, when the amount of biochar in the soil was increased to 10%, the difference was about 50% (14.51 and 9.70 g, respectively) (Figure 3). The 20% biochar content in the replanted soil did not change the mass of the strawberry root system significantly.

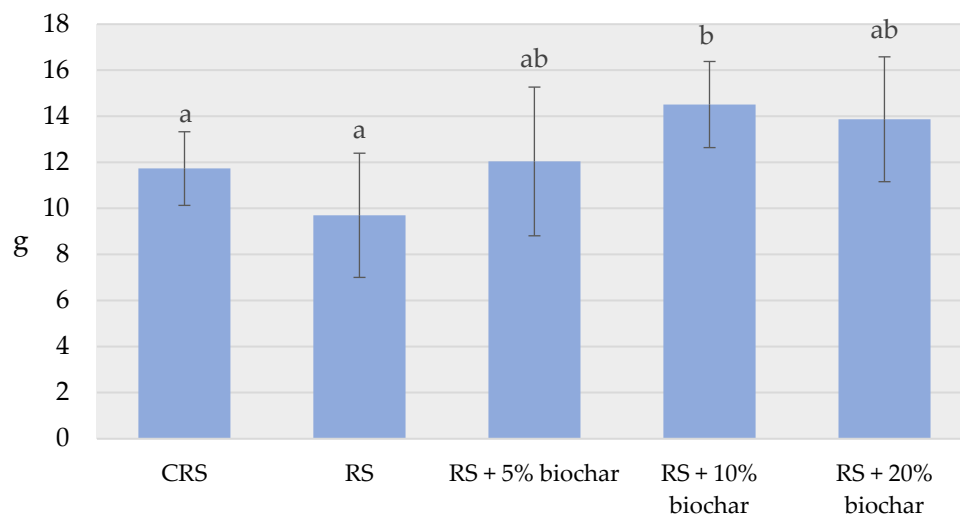


Figure 3. The effect of biochar on the mass of the strawberry root system (CRS = crop rotation soil; RS = replanted soil). Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

In the variants with organic carbon, some vegetative growth parameters improved due to the well-developed strawberry root system as well as higher levels of some macronutrients in the soil (Table 2). Regardless of the percentage content of biochar in the soil, it had a beneficial effect on the strawberry root collar mass. In comparison with the replanted soil without biochar, the differences ranged from 25% (20% biochar content) to 35% (5% biochar content) (Table 6). These values were greater than in the agricultural soil variant.

Table 6. The effect of biochar on the vegetative growth of strawberry plants.

Treatments	Root Collar Diameter (mm)	Strawberry Root Collar Mass (g)
CRS	2.14 b $\pm$ 0.66	16.52 a $\pm$ 1.83
RS	1.98 a $\pm$ 0.38	14.95 a $\pm$ 1.68
RS + 5% biochar	2.17 b $\pm$ 0.34	20.15 b $\pm$ 2.51
RS + 10% biochar	1.94 a $\pm$ 0.48	20.31 b $\pm$ 1.97
RS + 20% biochar	2.00 ab $\pm$ 0.61	18.67 b $\pm$ 1.69

CRS = crop rotation soil; RS = replanted soil. Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

When the biochar content in the replanted soil was increased several times (from 5% to 20%), it did not translate into a significant increase in the root collar mass. Table 6 shows the results of measurements of the strawberry root collar diameter at the beginning and end of the experiment. As can be seen, biochar had a relatively small effect on this parameter. Among the three variants with biochar, the root collar diameter increased by about 9% only in the one with 5% content, as compared with the replanted soil without biochar (2.17 vs. 1.98 mm).

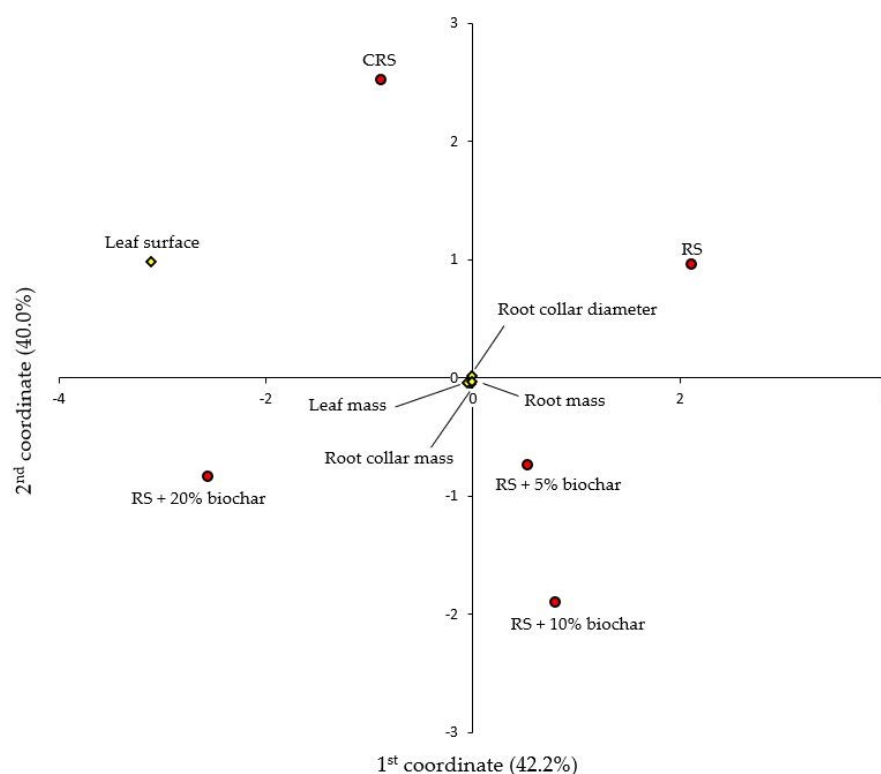
In our experiment, the strawberry leaf parameters varied significantly depending on the previous soil use. In the replanted soil variant, both the leaf surface area and average leaf mass were several dozen percent smaller than in the agricultural soil variant. The greatest differences, reaching 80%, were observed in the leaf area (278.43 vs. 499.13 cm², respectively) (Table 7).

Table 7. The effect of biochar on the strawberry leaf parameters.

Treatments	Leaf Surface Area (cm ²)	Average Leaf Mass (g)
CRS	499.13 c ± 62.59	17.12 b ± 2.34
RS	278.43 a ± 81.57	14.31 a ± 1.85
RS + 5% biochar	359.72 b ± 74.22	15.35 a ± 2.64
RS + 10% biochar	338.24 b ± 59.56	18.37 bc ± 2.53
RS + 20% biochar	405.66 c ± 71.45	19.66 c ± 3.58

CRS = crop rotation soil; RS = replanted soil. Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

The biochar used in our experiment had a beneficial effect on the parameters of strawberry leaves in the replanted soil variants. In the variant with 5% biochar content, the leaf area increased significantly (by about 30%), i.e., from 278.43 to 359.72 cm², as compared with the replanted soil without biochar (Table 7). This parameter did not change significantly in the variant with 10% biochar content in the soil. However, an increase in the leaf area was observed in the variant with 20% biochar content in the soil, and this value (405.66 cm²) was similar to the leaf area in the agricultural soil variant (499.13 cm²) (Table 7, Figure 4).

**Figure 4.** The results of canonical variate analysis, based on singular value decomposition, illustrating relationships between the variants of the experiment and the vegetative growth of strawberry plants (CRS = crop rotation soil; RS = replanted soil).

The analysis of the results of other studies led to similar conclusions. The studies conducted by Simiele et al. [57] and Wu et al. [58] showed that biochar added to the soil increased the leaf area of vegetables and strawberries by several dozen per cent [59]. Biochar also had a positive effect on the average mass of strawberry leaves. Measurements showed significant differences in the variant with 10% biochar content in the soil (Figure 4), where the average leaf mass (18.73 g) was about 28% greater than in the variant with replanted soil without biochar (14.31 g) (Table 7). Increasing the biochar content to 20% did not translate into a statistically proven increase in the strawberry leaf mass. However,

it is noteworthy that the leaf mass of plants in this variant (19.66 g) was significantly greater than in the variant with agricultural soil (17.12 g). The lack of significant growth of strawberry leaves, as well as the parameters of its root system, with an increase in the amount of biochar in the soil from 10 to 20% is explained by the results of previous analyses. In the experiment, when the amount of biochar in the replanted soil was doubled, no significant increase in its enzymatic and respiratory activity was observed (Table 4). This indicates that the activity of soil microorganisms, which determine soil productivity, also remained unchanged. This is confirmed by the results of previous studies, according to which a high dose of biochar inhibits the development of soil microflora [8]. The analysis of the abundance of soil bacteria performed in the experiment also showed that with an increase in the biochar content in the soil from 10 to 20%, the number of most taxonomic units of bacteria decreased (Figure 2).

3.4. Fruit Quality

Fruit quality is determined by a wide range of characteristics: aroma, shape, colour, solid content, and firmness. Experiments have shown that biochar positively affects not only vegetative growth but also fruit quality. It reduces fruit acidity and increases the content of ascorbic acid [60], glucose, and fructose [61]. In our experiment, previous soil use significantly influenced some strawberry quality parameters. The average weight of fruit harvested from the plants growing in the replanted soil was over 30% lower than that of the plants growing in the agricultural soil (10.51 vs. 14.31 g) (Table 8).

Table 8. The effect of biochar on the strawberry fruit quality parameters.

Treatments	Fruit Weight (g)	Firmness (g mm ^{−1})	Soluble Solid Content (% Brix)
CRS	14.31 b ± 4.31	174.17 a ± 44.46	9.75 a ± 1.85
RS	10.51 a ± 3.46	179.58 ab ± 31.15	11.71 b ± 1.36
RS + 5% biochar	14.89 b ± 4.02	191.25 ab ± 30.24	11.87 b ± 1.69
RS + 10% biochar	14.21 b ± 3.31	206.25 b ± 25.51	11.80 b ± 1.97
RS + 20% biochar	14.07 b ± 2.67	204.16 ab ± 32.36	11.58 b ± 1.23

CRS = crop rotation soil; RS = replanted soil. Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

However, the solid content was significantly higher (11.7 vs. 9.75 Brix), which is likely to be related to the lower weight of fruit harvested from the plants growing in the replanted soil (Figure 5).

The biochar used in our experiment had a positive effect only on some quality parameters of strawberries. The average weight of the fruit in the variant with a 5% biochar content was over 40% greater than in the variant with replanted soil without biochar (14.89 vs. 10.51 g) (Table 8, Figure 5). Tang et al. [59] and Jia et al. [31] also observed an increase in the average weight of strawberries after the use of biochar. In our experiment, increasing the biochar content in the replanted soil from 5% to 10% and 20% did not change the fruit weight significantly. In all variants with biochar, the weight of strawberries was comparable to that in the agricultural soil variant. As can be seen in Figure 5, which shows the distribution of experimental objects in the space of fruit quality parameters, the 5% biochar content in the soil caused the greatest variability in the strawberry fruit quality parameters. The analysis of the other two quality parameters, i.e., firmness and total soluble solid content, did not reveal significant differences in these parameters between the variants. This may result from the fact that some quality traits, particularly the total soluble solid content, are more influenced by climatic conditions, mostly temperature and sunlight. The canonical variate analysis revealed a negative correlation between the fruit weight, firmness, and total soluble solid content (Figure 5).

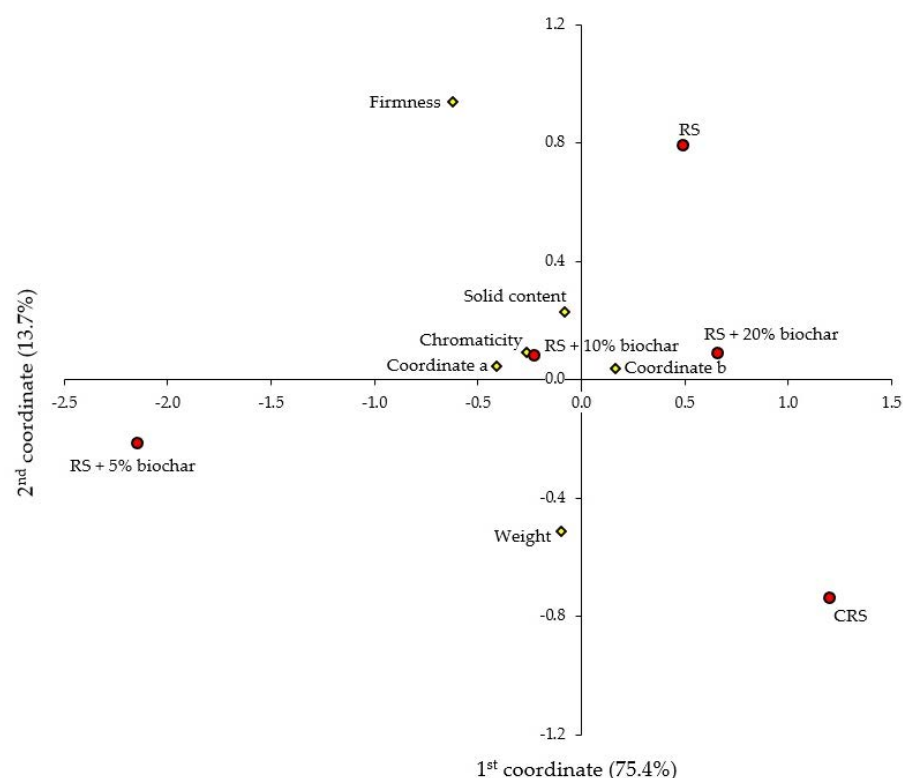


Figure 5. The results of canonical variate analysis based on singular value decomposition, illustrating relationships between the variants of the experiment and quality traits of strawberry fruit (CRS = crop rotation soil; RS = replanted soil).

The colour of strawberries was assessed with CIEL*a*b* and CIEL*C*h colorimetric analysis, which showed that the 5% biochar content in the replanted soil significantly increased the L coordinate, which determines colour brightness. The value of the a coordinate also increased, indicating greater intensity of the red colour of strawberries. The value of the b coordinate decreased significantly, indicating that the hue was more greenish and yellow (Table 9). The weaker colour intensity of strawberries may be caused by disrupted transport of phosphorus and potassium or deficiency of these nutrients in the soil.

Table 9. The effect of biochar on the parameters of strawberry leaves and the fruit colour.

Treatments	L	a	b	Chromaticity
CRS	37.31 a $\pm$ 5.51	31.24 a $\pm$ 4.17	17.65 b $\pm$ 2.57	35.42 a $\pm$ 3.70
RS	38.76 ab $\pm$ 2.09	33.23 a $\pm$ 2.38	17.19 b $\pm$ 2.29	37.02 ab $\pm$ 1.63
RS + 5% biochar	42.29 b $\pm$ 6.37	37.84 b $\pm$ 2.51	15.02 a $\pm$ 2.56	39.87 b $\pm$ 5.02
RS + 10% biochar	37.51 a $\pm$ 2.43	33.35 a $\pm$ 2.29	17.66 b $\pm$ 1.99	37.80 ab $\pm$ 3.06
RS + 20% biochar	37.09 a $\pm$ 3.61	30.96 a $\pm$ 3.07	17.95 b $\pm$ 3.34	35.82 a $\pm$ 3.12

CRS = crop rotation soil; RS = replanted soil. Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

The analysis of the positions of the experimental variants in relation to the fruit quality parameters revealed significant variation mostly due to relatively high values of colour chromaticity and the a coordinate and a low value of the b coordinate. The greatest similarity in the values of the fruit quality parameters was observed in the fruit harvested from plants grown in replanted soil with 10% and 20% biochar content (Figure 6).

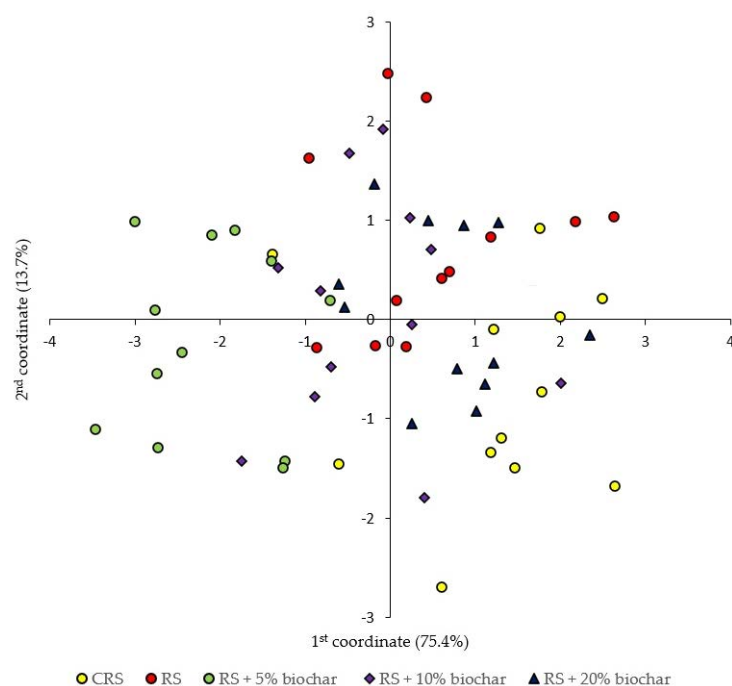


Figure 6. A graphic presentation of the results of canonical variate analysis (CVA), based on linear transformation, showing the diversification of experimental units within individual experimental variants in relation to the strawberry fruit quality parameters (CRS = crop rotation soil; RS = replanted soil).

As shown by results from the analysis of the dendrogram in Figure 7, the variants with 10% and 20% biochar content were the most similar to each other. They also differed significantly from the variants where strawberries were grown in replanted and agricultural soils.

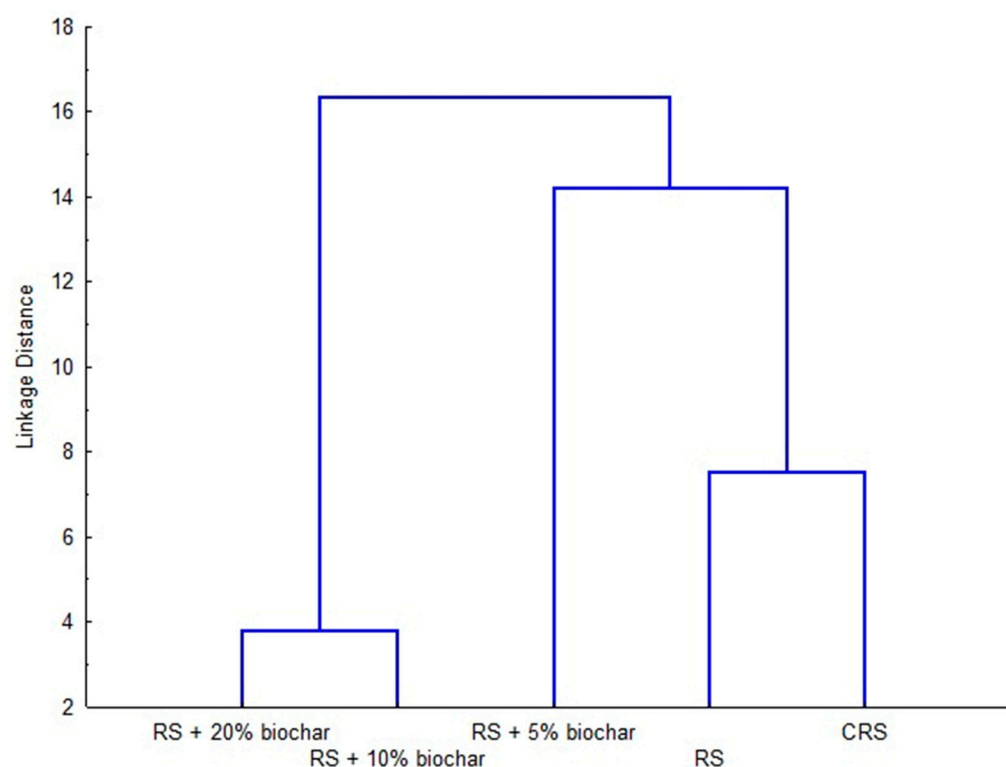


Figure 7. Dendrogram showing the structure of similarity between the experimental variants based on strawberry fruit quality parameters (CRS = crop rotation soil; RS = replanted soil).

4. Conclusions

The experiment showed that the previous use of soil had significant influence on both soil and plant parameters. In comparison with agricultural soil, the replanted soil was more saline and contained significantly less organic matter and some macronutrients. It was also characterised by poorer biological parameters such as enzyme activity and abundance of bacteria. This resulted in a smaller area and mass of strawberry leaves and a significantly lower average fruit weight.

Biochar added to the replanted soil improved some its physicochemical and biological properties, improved the vegetative growth of the plants, and improved some fruit quality parameters. In the variants with biochar, soil salinity decreased several times, and the organic matter content more than doubled, whereas the soil respiration activity nearly doubled. There were also changes in the vegetative growth of the plants. In the variants with biochar, the mass of the root collar and roots of the plants increased significantly. The 5% biochar content in the replanted soil caused the average fruit weight to increase by several dozen per cent. There were no significant differences in the fruit firmness, colour, or total content of soluble solids. The results varied depending on the percentage of biochar in the soil. The doubling of the biochar content in the replanted soil from 10% to 20% did not result in significant changes in the level of soil enzyme activity, root system mass, or average fruit weight. The 20% biochar content in the replanted soil caused a decrease in the abundance of nine of seventeen groups of bacteria. The analysis of the results showed that the 10% biochar content in the replanted soil is the optimal because it improved some of its properties. Such an amount of biochar may improve the parameters of strawberry leaves and increase the mass of fruit.

The threat of ARD in fruit plantations is increasing due to dynamic changes in the planting structure, also in berry plantations, and due to the shrinking area of land suitable for fruit growing. Owing to these circumstances, efficient and environmentally friendly methods of mitigating the consequences of ARD are increasingly important. The use of biochar may be an example of such solutions. And according to information from the manufacturer, when applying biochar to the soil, the estimated cost is from USD 260 to USD 470 per ha, depending on the dose.

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

Appendix A

Table A1. Temperature and precipitation trends in 2022.

Month	Total Rainfall (mm)		Average Temperature (°C)	
	Average for Years 1978–2020	In 2022	Average for Years 1978–2020	In 2022
January	30.9	29.7	−0.8	1.2
February	24.4	18.4	0.1	−0.1
March	34.3	34.8	3.5	8.4

Table A1. Cont.

Month	Total Rainfall (mm)		Average Temperature (°C)	
	Average for Years 1978–2020	In 2022	Average for Years 1978–2020	In 2022
April	29.8	45.6	9.3	10.6
May	49.5	35.8	14.5	15.1
June	62.5	37.3	17.2	17.9
July	78.2	49.7	19.5	22.5
August	60.0	94.3	18.9	18.5
September	40.9	8.4	14.1	15.8
October	34.2	4.9	9.0	9.2
November	37.5	12.8	3.9	5.8
December	37.0	22.8	0.6	2.6
Total/Average	516.3	377.5	9.1	10.7
Total/Average during the growing season	389.4	310.8	14.6	16.9

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