

Article

Effects of an *Ascophyllum nodosum*-Based Biofertilizer Applied Through Different Application Methods on Growth, Yield and Nutritional Quality of *Phaseolus vulgaris* L. var. *Opus* Under a Controlled Aeroponic System

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Abstract

The study evaluated the effect of an *Ascophyllum nodosum*-based biofertilizer applied under three treatments: foliar (FA, 0.5 g/L every 15 days), root (RA, 0.5 g/L every 30 days), and combined (F&RA, 0.5 g/L, Foliar every 15 days and Root every 30 days) compared to a control (C) in aeroponic cultivation of *Phaseolus vulgaris* L. var. *Opus*. Environmental conditions were continuously monitored using an IoE-based system, ensuring consistent microclimatic characterization throughout the experimental period. A non-parametric statistical approach was applied due to non-normal data distribution. Most growth and yield variables did not show statistically significant differences; however, significant treatment effects were observed for leaf temperature, root temperature, calcium, iron, and manganese. Significant differences among treatments ($\alpha = 0.05$) were identified using Kruskal–Wallis test for calcium ($p = 0.0002$), iron ($p = 0.0067$), and manganese ($p = 0.0439$), while other micronutrients showed no statistical differences. Descriptive statistics indicated moderate shifts in central tendency, particularly for calcium and iron, with higher values observed in the combined treatment (F&RA). Effect size analysis using Cliff's delta (δ) revealed moderate to large differences for calcium, iron, and manganese, although these estimates were interpreted cautiously given the unequal replication among treatments. Spearman correlation analysis showed moderate to strong associations, although none were statistically significant ($p > 0.05$). Overall, results indicate limited effects of the biofertilizer on growth and yield variables but treatment-associated changes in selected nutritional components, suggesting element-dependent responses to biofertilizer application.

Keywords: agricultural practices; aeroponic cultivation systems; *Ascophyllum nodosum* biofertilizer; internet of everything (IoE) monitoring



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1. Introduction

The continuous expansion of land use for residential and commercial purposes represents one of the main threats to the availability of agricultural land and the sustainability of food production systems [1]. This tendency, combined with soil degradation, deforestation, and overgrazing, has intensified pressure on natural resources and contributed to biodiversity loss. Currently, approximately 38% of the Earth's land surface is dedicated to agriculture, and nearly 95% of global food production depends directly on land use [2]. At the same time, the global population is projected to reach 8.5 billion by 2030 and 9.7 billion by 2050 [3], significantly increasing the demand for food, water, and energy resources.

In addition to these pressures, climate change introduces further challenges to agricultural productivity. Environmental stressors such as water scarcity, soil salinity, high temperatures, and the presence of heavy metals negatively affect plant physiological processes, microbial soil balance, and crop resilience [4]. These conditions reduce plant tolerance to biotic and abiotic stress, ultimately compromising yield and food quality. Therefore, improving plant resilience under adverse environmental conditions has become a critical factor in achieving sustainable agriculture and ensuring food security [5].

Conventional agricultural practices have relied heavily on synthetic fertilizers and pesticides to enhance productivity and protect crops. While effective in the short term, the excessive or improper use of these inputs may lead to soil degradation, environmental contamination, and potential risks to human health [4]. In this context, there is increasing interest in sustainable alternatives such as biofertilizers, biostimulants, and regenerative agricultural practices that promote soil health, reduce environmental impact, and maintain crop productivity.

Over recent decades, concerns have also emerged regarding the decline in the nutritional quality of food, attributed to intensive agricultural practices, the use of high-yield crop varieties, and reduced soil fertility. This decline has contributed to global nutritional imbalances, where malnutrition and micronutrient deficiencies coexist with obesity and overnutrition [6,7]. Thus, ensuring food security requires not only increasing production but also improving the nutritional quality of crops.

Leguminous plants are essential in this context due to their high nutritional value and functional properties. They are rich in proteins, dietary fiber, essential minerals, vitamins, and bioactive compounds such as polyphenols, saponins, and resistant starch, which contribute to antioxidant, metabolic and health-promoting effects [8,9]. Among them, *Phaseolus vulgaris* L. is widely cultivated and consumed globally, making it a key crop for improving dietary quality and food security.

In recent years, biofertilizers derived from algae have gained attention as sustainable agricultural inputs. These products have been reported to improve soil quality by enhancing microbial activity, contributing to nutrient availability, and promoting processes such as nitrogen fixation and phosphate solubilization, as well as improving soil structure and pH balance [10,11]. Furthermore, they contain bioactive compounds, including carbohydrates, proteins, lipids, micronutrients, and phytohormones such as auxins, cytokinins, and gibberellins, which can enhance plant growth and stress tolerance [12]. In particular, the brown macroalga *Ascophyllum nodosum* has been extensively studied for its biostimulant properties. Extracts from this species have been reported to improve photosynthesis, nutrient uptake, stress tolerance, and crop yield across various agricultural systems [13–20]. Additionally, these extracts can enhance antioxidant activity and regulate physiological and molecular responses under stress conditions [21–23].

Despite the growing body of evidence supporting the benefits of *A. nodosum*-based biofertilizers, most studies have been conducted under conventional soil-based cultivation systems. Limited research has evaluated their effects under controlled environments such

as aeroponic systems, where nutrient delivery, water use efficiency and root exposure differ significantly from traditional methods. Aeroponics has emerged as an innovative and sustainable cultivation technique that allows plant growth without soil, using nutrient-rich solutions applied directly to the roots. This system can reduce water use by up to 95%, minimize agrochemical inputs, and improve crop quality and productivity [24,25]. Furthermore, aeroponics offers significant potential for urban agriculture and controlled-environment food production [26,27].

Although both biofertilizers and aeroponic systems have demonstrated individual benefits, there is still a lack of studies evaluating their combined effect on crop performance, particularly in leguminous species. Moreover, limited information is available regarding the impact of algal-based biofertilizers on both agronomic and nutritional parameters under these conditions.

Furthermore, previous studies have predominantly focused on growth promotion or yield responses under conventional cultivation systems, whereas the combined evaluation of growth, physiological responses, yield dynamics, and nutritional composition under aeroponic conditions remains limited. The novelty of the present study lies in the simultaneous assessment of different application methods (foliar, root, and combined foliar and root application) of an *Ascophyllum nodosum*-based biofertilizer in *Phaseolus vulgaris* L. var *Opus* cultivated in a controlled aeroponic system. This approach allows the comparison of application routes while integrating agronomic, physiological, and nutritional indicators within a single experimental framework.

Therefore, the aim of this study was to evaluate the effect of a biofertilizer based on *Ascophyllum nodosum* extract on the vegetative development, yield and nutritional quality of *Phaseolus vulgaris* L. var. *Opus* (green bean) cultivated under controlled conditions, selected due to its nutritional relevance and widespread consumption [28]. This research seeks to contribute to the development of sustainable agricultural strategies that enhance crop productivity and nutritional value while reducing environmental impact.

2. Materials and Methods

The crops were grown in a controlled environment located at coordinates 20°32'16.0" N 100°48'54.4" W in the city of Celaya, Guanajuato, inside a greenhouse with an aeroponic system. The experimental crop was *Phaseolus vulgaris* L. var. *Opus* (green bean), acquired from the Seminis company (Seminis México Vegetables by Bayer®, Mexico City, Mexico). The objective of the experiment was to evaluate the effect of applying a biofertilizer based on *Ascophyllum nodosum* (macroalgae extract) on three aeroponic treatment groups, including one untreated control treatment. The biofertilizer was applied to one treatment through root application, another through foliar application, and the third in both root and foliar applications, in order to evaluate the phenological and productive response of the treatments.

The factors determined for the experiments were three experimental groups, two with 30 replicates each and one with 5 replicates, along with a control group of 5 replicates for experimental validation. The study was conducted using a completely randomized design with four treatments: foliar application (FA), root application (RA), combined foliar and root application (F&RA), and control treatment (C). Plants were randomly assigned to treatment positions within the aeroponic chambers prior to the first biofertilizer application. Because all plants received identical germination and fertigation management before treatment initiation, no pre-existing treatment effects were present at the time of allocation. After treatment allocation, all plants were maintained under identical greenhouse and fertigation conditions throughout the cultivation cycle. The experimental unit was an individual plant grown in the aeroponic system. A total of 70 plants were evaluated: 30 plants for FA, 30 plants for F&RA, 5 plants for RA, and 5 plants for the control treatment.

The unequal number of replicates resulted from physical limitations associated with the aeroponic infrastructure and the availability of cultivation space during the experimental period. Treatments FA and F&RA were prioritized because they represented the main application strategies under evaluation, whereas RA and the control were included as reference treatments.

The treatments were grown on horizontal aeroponic structures with an automatic fertigation system, set to a timer with cycles of 24 min of rest followed by 30 s of irrigation. This frequency was maintained in all three experimental groups and the control group. The crops were grown in a greenhouse where variable environmental conditions were recorded. The average temperature reached 19.16 °C during the day and rose to 41.23 °C at sunset. Regarding relative humidity, average values of 37.82% were observed during the day and minimum values approached 5.2% at sunset. Likewise, the recorded light radiation was 5878.42 lux during the day and 25,760 lux at sunset. These environmental parameters were key factors in the phenological development of the plants, as well as in the interpretation of the results obtained from the treatments applied.

During the first 15 days after transplanting from the germination stage to the aeroponic chamber, the plants were fertigated with tap water at a pH of 6.5, supplemented with a diluted Steiner universal nutrient solution at a concentration of 2.5 mL/L. Subsequently, for the remainder of the phenological cycle, the concentration of the nutrient solution was increased to 5 mL/L of tap water, ensuring an adequate supply of nutrients throughout the rest of the cycle, maintaining 1200 ppm.

2.1. Description of the Phenological Cycle

Phaseolus vulgaris L., commonly known as green bean, is a shrubby leguminous plant with a phenological cycle of 90 to 100 days from planting to harvest. Factors such as temperature, relative humidity, and radiation significantly influence the crop's phenological development. It is a source of protein that meets the caloric needs of the world's population [29]. Its cultivation is divided into two main phases: vegetative and reproductive. The vegetative phase extends from seed sowing to the beginning of flowering. During this period, the plant experiences a rapid increase in dry matter, focusing on the synthesis of new tissues. This growth is influenced by environmental factors such as temperature and the availability of nutrients. The developing pods extract essential nutrients from the plant for their growth and subsequent development. The reproductive phase begins with the appearance of the first flowers and culminates when the fruits reach the necessary maturity for harvesting.

2.2. Description of the Biofertilizer

A commercial biofertilizer based on *Ascophyllum nodosum*, called Algae Plus® acquired from the M&T Organics® company (Valenzuela, Philippines), in powder form, was used. The manufacturer's technical data sheet indicates a guaranteed composition per kilogram of: total nitrogen (N) 1.0%; potassium (K₂O) 16%; total alginic acid 16%; organic matter 50%; total amino acids 2.0%; mannitol 3.0%; gibberellic acid 300 ppm; and cytokinin 300 ppm. The biofertilizer was stored in a cool, dry environment, protected from direct light, according to the manufacturer's recommendations. Each application consisted of 0.5 g L⁻¹, avoiding prolonged exposure to environmental conditions that could modify its physicochemical stability. Regarding product characterization, although the manufacturer provides general information indicating that the product is obtained from the water-soluble extract of *Ascophyllum nodosum* rich in alginates, mannitol, and phytohormone-like compounds, the specific extraction methodology used for biomass processing is not disclosed. Since the extraction methodology used for biomass processing is not disclosed by the

manufacturer, potential differences in extraction procedures may influence the preservation and bioavailability of bioactive compounds; this limitation should be considered when comparing the present results with those of other studies and when interpreting potential mechanistic effects.

2.3. Application of Biofertilizer

The first application of *A. nodosum* was carried out in three treatment groups at the beginning of flowering (at the beginning of the appearance of flower buds), which occurred 30 days after transplanting in all cases (including the control treatment). The second application was carried out 15 days after the first application in all three treatment groups. Experiment 1, designated F&RA, consisted of supplying *A. nodosum* extract to the foliar and root areas of the crop in 30 replicates. The foliar application in this experiment was carried out by aerial spraying, with a single application followed by a second application 15 days after the first. The root application was performed by diluting the *A. nodosum* extract directly in the main container holding tap water and the nutrient solution. The extract was completely diluted within 5 days of application, and this process was repeated 30 days after the second application for the root area in the experiment F&RA. For Experiment 2, designated FA, also with 30 replicates, the biofertilizer was applied by aerial spraying at the beginning of flowering, with a second application 15 days later. For Experiment 3, designated RA, with 5 replicates, the biofertilizer was applied by diluting *A. nodosum* extract in the container with tap water and the nutrient solution. The extract was completely dissolved 5 days after application, and the process was repeated 30 days after the first application. The experiments involved frequent fertigation of nutrients, meaning application was continuous. For the control treatment (C), also with 5 replicates, only constant fertigation of Steiner nutrients was carried out throughout the entire phenological cycle.

2.4. Data Collection

2.4.1. Environmental Variables and IoE-Based Monitoring System

Environmental variables were monitored from 5 February to 10 May 2025, using an Internet of Everything (IoE)-based sensing platform designed for continuous environmental characterization of the aeroponic cultivation system. Air temperature and relative humidity were measured using DHT22 digital sensors (-40 to 80 °C, ± 0.5 °C; 0–100% RH, $\pm 2\%$ accuracy), selected due to their reliability, accessibility, and suitability for distributed monitoring applications.

A total of eight monitoring units were deployed throughout the experimental system. Sensors were positioned at canopy level and within the plant growth. Additional sensors were installed inside the greenhouse to characterize ambient environmental conditions. Relative humidity within the aeroponic chambers remained above 80% during most of the experimental period.

Incident light intensity was measured using BHT1750 digital luminosity sensors (1–65,535 lx, 16-bit resolution). Sensors were distributed at different positions within each aeroponic chamber to characterize spatial variability in light availability. Measurements were validated using a calibrated UT-382 lux meter (UNIT-T[®], GreenTech Instruments, New Delhi, India).

Sensor data were acquired automatically at one-hour intervals using ESP32-based monitoring nodes connected to a cloud platform for storage and visualization. This IoE architecture enabled continuous monitoring, remote access to environmental information, and long-term data management throughout the cultivation cycle. Environmental alerts

were configured to notify users when the temperature exceeded 30 °C or the relative humidity dropped below 50%.

Sensor performance was verified through periodic calibration and comparison with reference instruments. Temperature and relative humidity measurements were validated against an HTC-2 digital thermohygrometer, while luminosity measurements were verified using the UT-382 lux meter. Calibration procedures were performed every 15 days using software-based correction factors when necessary. Observed deviations remained within the operational ranges reported by the manufacturers.

Additionally, fertigation water quality parameters, including temperature, pH, total dissolved solids (TDS), and electrical conductivity (EC), were monitored using a calibrated multiparameter meter according to the manufacturer's recommendations.

2.4.2. Morphological Variables

Morphological variables associated with product quality (weight, length, width, perimeter, and number of grains) were determined through direct measurement. Weight was recorded using a precision digital scale (range of 1 g to 10 kg), while longitudinal and transverse dimensions were measured using a stainless-steel digital caliper (Truper® brand, Mexico City, Mexico) with a range of 0–150 mm and millimeter resolution.

The vegetative stage was assessed by quantifying the number of flowers per plant, number of pods per plant (considering only pods larger than 1 cm after harvesting mature pods), and plant height through direct measurement and manual counting.

Leaf and root temperatures were determined using RGB and infrared imaging with a FLIR ONE Pro LT thermal camera (± 3 °C accuracy). Thermal images were processed using MATLAB R2019a software to calculate the average temperature per unit area of plant tissue, applying digital segmentation techniques to separate plant tissue from the background.

As a future improvement, the integration of MLX90640 thermal arrays (32×24 pixels) is proposed to enable continuous in situ thermal monitoring within the IoE framework.

2.4.3. Chemical and Biochemical Parameters

Experimental Design and Sample Preparation

Plant material samples were harvested at physiological maturity and transported to the laboratory under controlled conditions. Samples were washed with deionized water, dried at 60 °C to a constant weight in a forced-air oven (Mettler UF110, Mettler GmbH®, Schwabach, Germany), and ground to pass through a 0.5 mm stainless steel sieve using a laboratory mill (Retsch ZM200, Retsch GmbH®, Haan, Germany). Ground samples were stored in airtight polyethylene containers at room temperature under desiccated conditions until analysis. All chemical and biochemical analyses were performed in analytical triplicate for each sample. These analytical replicates were used exclusively for quality-control purposes and were averaged prior to statistical analysis. Therefore, analytical replicates were not considered independent biological replicates.

Proximate Composition Analysis

Moisture content was determined using the gravimetric method according to AOAC 925.10. Approximately 5 g of fresh sample was dried at 105 °C to a constant weight in a convection oven (Binder FD 115, Binder GmbH®, Tuttlingen, Germany). Moisture percentage was calculated based on weight loss before and after drying. Moisture content was analyzed on a fresh weight (FW) basis; all other analyses were performed on a dry weight (DW) basis. Therefore, variations in moisture content did not affect the results of these analyses. Total ash was quantified following AOAC 923.03. Approximately 2 g of dried sample was incinerated in a muffle furnace (Thermo Scientific® Thermolyne F6000, Waltham, MA, USA) at 550 °C for 6 h. Ash content was expressed as g 100 g⁻¹ DW. Crude

protein was determined by the Kjeldahl method (AOAC 2001.11). Approximately 0.5 g of dried sample was digested with concentrated H_2SO_4 in the presence of a catalyst mixture ($\text{K}_2\text{SO}_4\text{--CuSO}_4$). Digestion was carried out in a digestion block system (VELP® Scientifica DK 20, Usmate, Italy). Distillation and titration were performed using an automatic Kjeldahl analyzer (VELP® UDK 149, Usmate, Italy). Total nitrogen content was multiplied by a conversion factor of 6.25 to estimate crude protein. Crude fat content was determined using Soxhlet extraction (AOAC 920.39). Approximately 3 g of dried sample was extracted with petroleum ether (boiling point 40–60 °C) for 6 h using a Soxhlet apparatus (Büchi® Extraction Unit E-816, Flawil, Switzerland). The solvent was evaporated, and lipid content was determined gravimetrically.

Total carbohydrates were calculated by difference on a dry weight basis according to the following Equation (1):

$$\text{Carbohydrates (\% DW)} = 100 - (\% \text{ crude protein} + \% \text{ crude fat} + \% \text{ ash}), \quad (1)$$

(Note: Crude fiber was determined separately and is not subtracted here, as it is included within the carbohydrate fraction by this conventional method).

Total soluble sugars were quantified using the phenol-sulfuric acid method. Briefly, 100 mg of dried sample was extracted with 80% ethanol at 80 °C for 30 min. After centrifugation (Eppendorf® 5810R, Hamburg, Germany), aliquots of the supernatant were reacted with 5% phenol and concentrated sulfuric acid. Absorbance was measured at 490 nm using a UV-Vis spectrophotometer (Shimadzu® UV-1800, Kyoto, Japan). Glucose was used as a standard for calibration, and results were expressed as mg glucose equivalents g^{-1} DW. Crude fiber was determined following AOAC 978.10 using sequential acid and alkaline digestion. Approximately 2 g of defatted sample was subjected to digestion with 1.25% H_2SO_4 , followed by 1.25% NaOH in a fiber analyzer system (ANKOM 2000 Fiber Analyzer, ANKOM Technology®, Macedon, NY, USA). The residue was dried, incinerated at 550 °C, and the fiber content was calculated gravimetrically.

Mineral Element Determination

For mineral analysis, approximately 0.5 g of dried sample was subjected to wet acid digestion using a microwave digestion system (CEM® Mars 6, Matthews, MO, USA). Samples were digested with 10 mL of concentrated HNO_3 (trace metal grade) and 2 mL of H_2O_2 under controlled temperature and pressure conditions (ramp to 180 °C for 20 min, hold for 15 min). Digested solutions were diluted to 50 mL with ultrapure water.

Calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), potassium (K), and zinc (Zn) concentrations were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES) with an Agilent 5110 ICP-OES system (Agilent Technologies®, Santa Clara, CA, USA). Calibration curves were prepared using certified multi-element standard solutions (Merck, Darmstadt, Germany). Instrument operating conditions were optimized according to the manufacturer's specifications. The results were expressed as $\text{mg } 100 \text{ g}^{-1}$ DW (dry weight).

For phosphorus (P) determination, an aliquot of the digested solution was analyzed colorimetrically using the molybdenum blue method. Briefly, the aliquot was reacted with ammonium molybdate and ascorbic acid (reducing agent), and the absorbance of the blue complex was measured at 880 nm using a UV-Vis spectrophotometer (Shimadzu® UV-1800, Kyoto, Japan). Quantification was performed using a calibration curve prepared with KH_2PO_4 standard solutions. The results were expressed as $\text{mg } 100 \text{ g}^{-1}$ DW.

Quality Control

All reagents were of analytical grade or higher. Glassware was soaked in 10% HNO₃ for 24 h and rinsed with deionized water prior to use. Analytical accuracy was verified using certified reference materials (NIST SRM 1573A Tomato leaves, Sigma-Aldrich®, St. Louis, MO, USA). Method precision was evaluated through triplicate analyses and expressed as relative standard deviation (RSD). Calibration curves showed correlation coefficients ($R^2 \geq 0.995$).

2.4.4. Statistical Analysis

Statistical analyses were performed using a non-parametric approach due to the non-normal distribution of the data, previously evaluated using exploratory data analysis. Differences among treatments were assessed using the Kruskal–Wallis test ($\alpha = 0.05$), followed by post hoc pairwise comparisons based on median differences and effect size interpretation.

When significant treatment effects were detected by the Kruskal–Wallis test, post hoc pairwise comparisons were performed using Dunn’s multiple-comparisons procedure with Dunn–Šidák adjustment to identify specific treatment differences.

The experimental design included an unequal number of replicates among treatments (30 plants for FA, 30 for F&RA, and 5 each for RA and the control treatment). This unequal replication resulted from operational constraints associated with the aeroponic infrastructure and available cultivation space during the experimental period. To account for the resulting unbalanced design, non-parametric statistical methods were selected because they are less sensitive to unequal sample sizes and violations of normality assumptions. Nevertheless, unequal replication may reduce statistical power in some comparisons; therefore, all results were interpreted considering the differences in replication among treatments.

The experimental unit was an individual green bean plant grown in the aeroponic system. Growth, physiological, and yield variables were recorded at the plant level, and each plant was considered an independent replicate for statistical analyses. For nutritional composition analyses, laboratory measurements were performed on samples obtained from harvest pods associated with each treatment; however, statistical comparisons were conducted at the treatment-replicate level rather than using analytical measurements as independent biological replicates. This approach was adopted to avoid pseudoreplication and ensure consistency in the interpretation of treatment effects.

Because the number of harvest events differed among treatments (eight harvests for C and FA, and ten harvests for RA and F&RA), yield data were interpreted using two complementary perspectives: (i) cumulative yield measured over the entire production cycle and (ii) temporal production dynamics across successive harvest events. This distinction was adopted to avoid confounding treatment effects with differences in production duration and to facilitate the interpretation of productivity persistence over time.

Descriptive statistics included median and interquartile range (IQR) as primary measures of central tendency and dispersion, complemented by mean and standard deviation (SD) for reference.

Effect size estimation was conducted using Cliff’s delta (δ) as the primary metric to quantify the magnitude and direction of differences between treatments. This non-parametric measure was selected for its robustness to non-normal distributions and unequal sample sizes, providing a distribution-based interpretation of treatment effects.

Pairwise comparisons of effect sizes were calculated for all treatment combinations to support the interpretation of differences beyond statistical significance. The magnitude of δ was interpreted considering potential influences of sample size, within-group variability, and tied observations.

Particular caution was exercised when interpreting extreme δ values (close to ± 1), as these may be influenced by small sample sizes, low within-group variability, or a high proportion of tied observations. Therefore, Cliff's delta was used as a complementary measure of effect magnitude and not as definitive evidence of biological separation among treatments.

Additionally, Spearman's rank correlation coefficient (ρ) was used to evaluate associations between variables, given its suitability for non-parametric data. Correlation analyses were performed using individual observations within treatments; treatment-level summaries were used only for descriptive comparison. Given the unequal replication among treatments and the limited number of observations available for some comparisons, correlation analyses were considered exploratory and were used primarily to identify potential associations among variables rather than to establish causal relationships.

All statistical analyses and data processing were conducted using MATLAB R2019a, ensuring reproducibility of results.

3. Results

To evaluate the feasibility of using a macroalgae-based biofertilizer in aeroponic cultivation of *Phaseolus vulgaris* L. var. *Opus*, agronomic, physiological, and nutritional variables were analyzed across treatments. As most variables did not follow a normal distribution (Kolmogorov–Smirnov test), non-parametric methods were applied. Accordingly, results are primarily interpreted using rank-based approaches, with central tendency and dispersion described by the median and interquartile range (IQR).

Differences among treatments were assessed using the Kruskal–Wallis test ($\alpha = 0.05$). Effect sizes were estimated using Cliff's delta (δ), a non-parametric measure of distributional separation, allowing the evaluation of consistent differences between treatments beyond central tendency.

Overall, treatment responses were variable across traits. Most structural and yield-related variables did not show statistically significant differences ($p > 0.05$), whereas significant effects were detected in specific physiological parameters and nutritional composition ($p < 0.05$). These results suggest that treatment-associated responses were not uniformly expressed across plant traits and may be more readily detected in functional and compositional indicators than in conventional growth or yield metrics under the evaluated conditions.

3.1. Product Assessment

To evaluate product quality in each of the experimental treatments, five key variables were defined: weight, length, width, perimeter, and number of grains per 100 g per pod [30]. These morphological characteristics represent fundamental indicators of yield and commercial quality of the *Phaseolus vulgaris* crop [31].

Table 1 presents the results of a non-parametric Kruskal–Wallis test used to compare medians among treatments due to the non-normal distribution of the data. The analysis showed that no statistically significant differences were observed for most of the evaluated quality variables ($p > 0.05$), indicating that the null hypothesis could not be rejected for weight, length, width, and perimeter. In contrast, the number of grains showed a statistically significant difference ($p < 0.05$), leading to rejection of the null hypothesis for this variable and suggesting differences among treatments.

The boxplot of the distribution of pod weight across treatments is shown in Figure 1, while the distribution of the number of grains per 100 g of pods is presented in Figure 2. These boxplots illustrate the variability and median differences among treatments and visually complement the statistical results reported in Table 1.

Table 1. Kruskal–Wallis test results for product assessment variables. Significance codes: * $p < 0.05$.

Variable	p -Value	H_0
Weight	0.5651	Not rejected
Length	0.2907	Not rejected
Width	0.1672	Not rejected
Perimeter	0.5939	Not rejected
Number of grains	0.0036 *	Rejected

Statistical significance was evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). “Rejected” indicates rejection of the null hypothesis (H_0), whereas “Not rejected” indicates insufficient evidence to reject H_0 . Variables with $p < 0.05$ were considered to show statistically significant treatment effects.

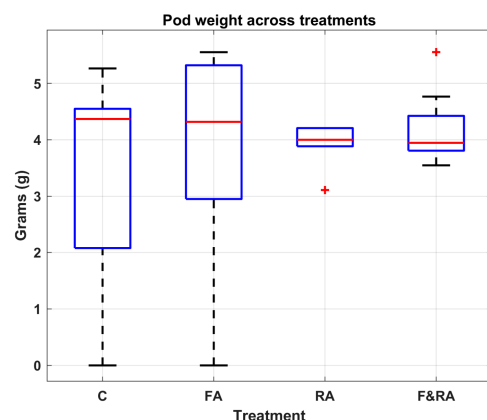


Figure 1. Distribution of pod weight across treatments represented as boxplots. The central red line indicates the median, the box represents the interquartile range (IQR), the whiskers (dashed lines) extend to the most extreme values within $1.5 \times \text{IQR}$, and the red ‘+’ symbols denote outliers. Differences among treatments were evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). Abbreviations: C = control treatment (Steiner nutrient solution only); FA = foliar application of *Ascophyllum nodosum* biofertilizer; RA = root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer.

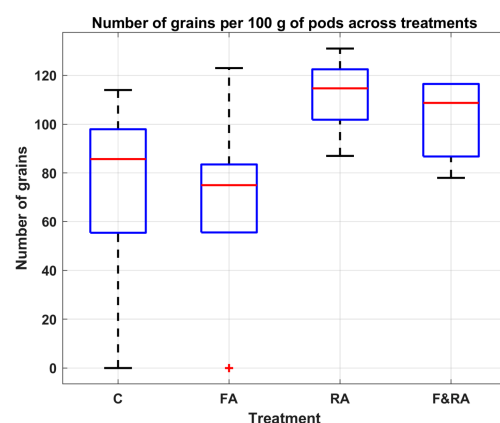


Figure 2. Distribution of number of grains per 100 g of pods across treatments represented as boxplots. The central red line indicates the median, the box represents the interquartile range (IQR), the whiskers (dashed lines) extend to the most extreme values within $1.5 \times \text{IQR}$, and the red ‘+’ symbols denote outliers. Differences among treatments were evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer.

In order to further explore the distribution of evaluated variables, Table 2 summarizes the descriptive statistics, including median and interquartile range (IQR) as primary measures of central tendency and dispersion, along with mean and standard deviation as

complementary metrics. Although no significant differences were found for most morphological characteristics, slight differences in median values were observed across treatments. The IQR values indicate relatively consistent dispersion across treatments for most variables, although the number of grains exhibited slightly higher variability, supporting the observed heterogeneity in this parameter.

Table 2. Descriptive statistics of product assessment variables (median, interquartile range, mean and standard deviation) for each treatment. Units: weight (g); length (mm); width (mm); perimeter (mm); number of grains (count).

Treatment	Variable	Median	IQR	Mean	SD
C	Weight	4.4545	0.7407	4.3563	0.7363
	Length	108.9583	12.3431	106.8043	8.2682
	Width	8.7692	0.3996	8.8709	0.3960
	Perimeter	27.5494	1.2917	27.8635	1.2482
	Number of grains	87.9310	32.1769	91.0873	14.1211
FA	Weight	4.9756	1.2404	4.8261	0.6051
	Length	116.4024	8.5580	116.3624	5.4499
	Width	8.9375	0.7415	8.8305	0.3274
	Perimeter	28.1	2.4	27.7428	1.0390
	Number of grains	75	23	85.2857	17.1773
RA	Weight	4	0.3223	3.9449	0.3227
	Length	110.7586	12.7250	110.9665	7.7599
	Width	8.9204	0.7826	8.9259	0.4450
	Perimeter	28	2.45	28.0222	1.3974
	Number of grains	114.7059	25.4971	112.1569	14.8069
F&RA	Weight	3.9459	0.9878	4.1605	0.4551
	Length	115.4902	16.3751	109.1680	8.2656
	Width	9.3311	0.8178	9.2146	0.4870
	Perimeter	29.3	2.55	28.9555	1.5217
	Number of grains	108.7379	31.7271	101.2861	14.7080

Abbreviations: C = control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer. Sample size (*n*): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants. The experimental unit was an individual plant. Descriptive statistics are presented for reference and do not imply statistical significance among treatments. Differences among treatments were evaluated separately using the Kruskal–Wallis test ($\alpha = 0.05$).

In some cases, discrepancies between median and mean values (e.g., FA treatment) suggest skewed distributions, where a high frequency of low values coexists with a few higher observations. This asymmetry should be considered when interpreting central tendency.

The FA treatment showed relatively higher median values for weight and length; however, these differences were not statistically significant and should therefore be considered descriptive tendencies rather than evidence of treatment effects. Similarly, the RA treatment presented higher median values for the number of grains, although without statistical significance. The F&RA exhibited intermediate values across most characteristics and relatively higher values for width and perimeter, although these differences were also not statistically significant. Overall, variability was consistent among treatments, with slightly greater dispersion observed for the number of grains. These results should be interpreted with caution, as most differences were not supported by statistical significance.

To complement the inferential and descriptive analyses, effect sizes were estimated using Cliff's delta (δ), allowing comparison of distribution-based measures of effect magnitude. As shown in Table 3, the effect sizes for pod weight varied across comparisons. According to Cliff's delta, moderate to large effects were observed in several comparisons, particularly between FA and RA ($\delta = 0.8412$), whereas smaller differences were found in

comparisons such as FA and F&RA ($\delta = -0.0864$). A similar pattern was observed with Cohen's d , where larger magnitudes were also associated with contrasts involving the FA treatment. Despite these effect size patterns, no statistically significant differences were detected by the Kruskal–Wallis test, suggesting that these differences may reflect variability within treatments or sample size limitations rather than consistent treatment effects.

Table 3. Pairwise effect size estimates (Cliff's delta, δ) of pod weight among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−0.2653
C	RA	0.5873
C	F&RA	0.3015
FA	RA	0.8412
FA	F&RA	0.6190
RA	F&RA	−0.0864

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer. Sample size (n): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants.

Likewise, Table 4 presents the effect size estimates for the number of grains per 100 g of pods. In agreement with the Kruskal–Wallis results, several comparisons showed moderate to large effect sizes. The largest contrast involved the RA treatment, particularly relative to C ($\delta = -0.6825$) and FA ($\delta = -0.6825$), while smaller effects were observed between C and FA. These patterns were consistent with those obtained using Cohen's d . Overall, these results suggest that grain number may be one of the variables most responsive to treatment differences under the evaluated conditions.

Table 4. Pairwise effect size estimates (Cliff's delta, δ) of number of grains per 100 gr of pods among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	0.1836
C	RA	−0.6825
C	F&RA	−0.4603
FA	RA	−0.6825
FA	F&RA	−0.5873
RA	F&RA	0.3827

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer. Sample size (n): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants.

To further understand the relationships between the morphological characteristics and grain production, a Spearman correlation analysis was conducted (Table 5). For the control treatment (C), significant negative correlations were found between length and number of grains ($\rho = -0.79$, $p < 0.05$), and between perimeter and number of grains ($\rho = -0.79$, $p < 0.05$), suggesting an inverse association between pod size and grain number under the evaluated conditions. No significant correlation was observed with weight.

Table 5. Spearman correlation coefficients between morphological variables and number of grains per treatment; * $p < 0.05$.

Treatment	Variable 1	Variable 2	ρ	p -Value
C	Weight	Number of grains	−0.6071	0.1667
	Length	Number of grains	−0.7857	0.0480 *
	Perimeter	Number of grains	−0.7857	0.0480 *
FA	Weight	Number of grains	−0.5092	0.2492
	Length	Number of grains	0.0182	0.9841
	Perimeter	Number of grains	0.2	0.6698
RA	Weight	Number of grains	0.5236	0.1489
	Length	Number of grains	0.0944	0.8073
	Perimeter	Number of grains	−0.6095	0.0882
F&RA	Weight	Number of grains	0.0513	0.8980
	Length	Number of grains	0.6696	0.0571
	Perimeter	Number of grains	0.6696	0.0571

Correlations were calculated using average values obtained from successive harvest events within each treatment. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascochyllum nodosum* biofertilizer; RA = Root application of *Ascochyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascochyllum nodosum* biofertilizer.

In contrast, no statistically significant correlations were observed for FA and RA treatments, ($p > 0.05$), indicating insufficient evidence to support consistent relationships between morphological characteristics and grain number under these conditions. These results should be interpreted cautiously, as the lack of significance may be influenced by sample size, particularly in RA treatment ($n = 5$), and within-treatment variability and therefore does not necessarily imply the absence of a biological relationship. For the F&RA treatment, moderate positive correlations were observed between length and number of grains ($\rho = 0.67$) and between perimeter and number of grains ($\rho = 0.67$); however, these associations were not statistically significant ($p > 0.05$) and should therefore be interpreted with caution. Although the observed correlation coefficients suggest a potential positive relationship, the available sample size limits statistical power and precludes definitive conclusions.

To obtain a more robust evaluation of relationships among morphological variables, a second Spearman correlation analysis was conducted using all individual observations (Table 6). Strong and statistically significant positive correlations were consistently observed between weight and length across all treatments, indicating a positive correlation pattern between pod length and weight. Moderate correlations were also observed between weight and both width and perimeter.

Moreover, length showed moderate positive correlations with width and perimeter, which is consistent with the geometric relationships among pod dimensions. The extremely high correlations observed between width and perimeter likely reflect geometric dependence rather than independent biological relationships. Perfect correlations ($\rho = \pm 1$) likely reflect limited sample size, tied ranks, or reduced within-group variability and should not be interpreted as exact deterministic relationships. These values should not be interpreted as evidence of exact biological dependence.

Overall, morphological variables exhibited a high degree of interrelation, particularly those associated with pod dimensions. However, the relationship between morphological variables and grain number was generally weak, suggesting that grain production may be associated with factors beyond the morphological variables evaluated in this study.

Table 6. Spearman correlation coefficients between morphological variables; ** $p < 0.01$.

Treatment	Variable 1	Variable 2	ρ	p -Value
C	Weight	Length	0.8514	4.06×10^{-37} **
	Weight	Width	0.5211	2.87×10^{-10} **
	Weight	Perimeter	0.5212	2.85×10^{-10} **
	Length	Width	0.3248	1.84×10^{-4} **
	Length	Perimeter	0.3171	2.66×10^{-4} **
	Width	Perimeter	0.9922	7.93×10^{-116} **
FA	Weight	Length	0.8039	9.20×10^{-126} **
	Weight	Width	0.6205	7.57×10^{-60} **
	Weight	Perimeter	0.6098	2.65×10^{-57} **
	Length	Width	0.4317	2.24×10^{-26} **
	Length	Perimeter	0.4225	3.23×10^{-25} **
	Width	Perimeter	0.9940	0
RA	Weight	Length	0.6575	5.14×10^{-26} **
	Weight	Width	0.3510	3.71×10^{-7} **
	Weight	Perimeter	0.3510	3.71×10^{-7} **
	Length	Width	0.2174	2.04×10^{-3} **
	Length	Perimeter	0.2174	2.04×10^{-3} **
	Width	Perimeter	1	0
F&RA	Weight	Length	0.7473	3.12×10^{-108} **
	Weight	Width	0.5049	3.98×10^{-40} **
	Weight	Perimeter	0.5049	3.98×10^{-40} **
	Length	Width	0.2840	1.35×10^{-12} **
	Length	Perimeter	0.2840	1.35×10^{-12} **
	Width	Perimeter	1	0

Correlations were calculated using individual observations within each treatment. The results should be interpreted as exploratory due to unequal replication among treatments and the limited sample sizes available for some treatment groups; therefore, correlations should not be interpreted as evidence of a causal relationship. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascochyllum nodosum* biofertilizer; RA = Root application of *Ascochyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascochyllum nodosum* biofertilizer. Sample size (n): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants.

The apparent discrepancy between Tables 5 and 6 may be related to differences in the scale of analysis. Table 5 is based on averaged values per treatment, resulting in a limited number of observations, whereas Table 6 was calculated using individual plant observations within each treatment. Consequently, Table 6 had greater statistical power to detect associations among variables. The stronger and more consistent correlations observed in Table 6 may therefore reflect both the larger sample size and the intrinsic relationships among morphological traits. Nevertheless, all correlation analyses should be interpreted as exploratory due to the unequal replication among treatments and the limited number of observations available for some groups.

3.2. Vegetative State

Following the morphological analysis, variables were defined to quantify aspects associated with vegetative development and physiological status throughout the phenological cycle. These variables were selected for their agronomic relevance and their ability to capture potential differences in plant responses among treatments.

The evaluated variables include the number of flowers per plant, number of pods per plant (considering only pods larger than 1 cm were counted after harvesting mature pods), plant height (cm), leaf temperature (°C), and root temperature (°C). Together, these

variables provide information on potential productivity, structural development, and physiological response to biofertilizer application.

The Kruskal–Wallis test is presented in Table 7. No statistically significant differences were observed for the number of flowers, number of pods, and plant height ($p > 0.05$), indicating that the null hypothesis could not be rejected for these variables. In contrast, leaf temperature and root temperature showed statistically significant differences among treatments ($p < 0.01$), indicating treatment-related differences in physiological responses.

Table 7. Kruskal–Wallis test results for vegetative variables. Significance codes: ** $p < 0.01$.

Vegetative	<i>p</i> -Value	H_0
Number of flowers on plant	0.9755	Not rejected
Number of pods on plant	0.9240	Not rejected
Plant height	0.1333	Not rejected
Leaf temperature	8.8277×10^{-5} **	Rejected
Root temperature	9.7836×10^{-4} **	Rejected

Statistical significance was evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). “Rejected” indicates rejection of the null hypothesis (H_0), whereas “Not rejected” indicates insufficient evidence to reject H_0 . Variables with $p < 0.05$ were considered to show statistically significant treatment effects.

These results indicate that while the treatments did not significantly influence structural or yield-related vegetative variables, they were associated with differences in physiological parameters.

The distribution of the number of flowers per plant across treatments is illustrated in Figure 3, which shows the average number of flowers per plant across multiple harvest events. A total of eight harvest events were carried out for the control (C) and FA treatments, while ten harvest events were conducted for the RA and F&RA treatments. Although no statistically significant differences were detected, the figure provides a visual representation of variability and tendencies among treatments.

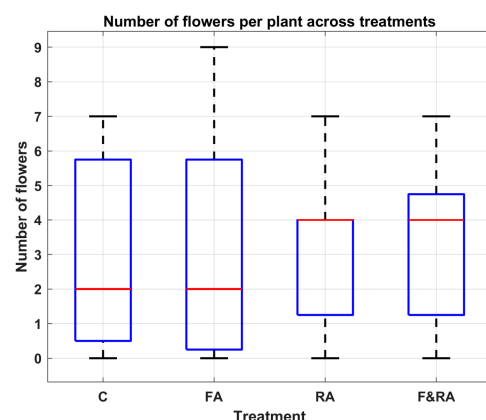


Figure 3. Distribution of number of flowers per plant across treatments represented as boxplots. The central red line indicates the median, the box represents the interquartile range (IQR), the whiskers (dashed lines) extend to the most extreme values within $1.5 \times \text{IQR}$, and the red ‘+’ symbols denote outliers. Differences among treatments were evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer.

The average number of pods produced per harvest for all the treatments is presented in Figure 4. Although no significant differences were observed in the total number of pods per plant (Table 7), the temporal pattern of yield showed distinct tendencies. For instance, RA and F&RA treatments remained productive harvest events. This pattern

became apparent from approximately the fourth harvest for RA and the fifth harvest for F&RA. Additionally, RA and F&RA included two additional harvest events compared to C and FA.

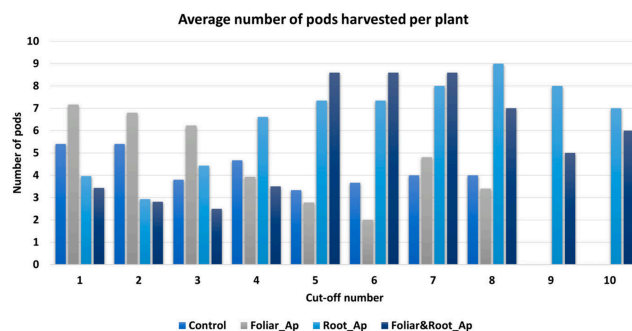


Figure 4. Values represent mean pod counts recorded during successive harvests throughout the production cycle. Harvest events correspond to sequential cutting dates. Treatments C and FA were evaluated during eight harvest events, whereas RA and F&RA were evaluated during ten harvest events. Abbreviations: Control = Control treatment (Steiner nutrient solution only); Foliar_Ap = Foliar application of *Ascophyllum nodosum* biofertilizer; Root_Ap = Root application of *Ascophyllum nodosum* biofertilizer; Foliar&Root_Ap = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer.

Differences in harvest frequency among treatments represent a structural aspect of the experimental design and may influence cumulative comparisons of pod production.

To better understand the physiological basis of these patterns, leaf and root temperatures were analyzed (Figures 5 and 6). While structural variables did not show statistically significant differences among treatments, both temperature-related variables were able to show such differences (Table 7).

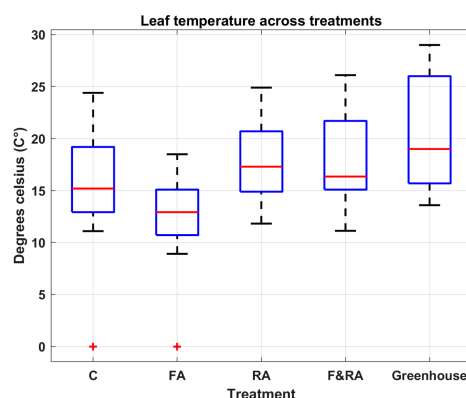


Figure 5. Distribution of leaf temperature across treatments represented as boxplots. Values are expressed in °C. The central red line indicates the median, the box represents the interquartile range (IQR), the whiskers (dashed lines) extend to the most extreme values within $1.5 \times \text{IQR}$, and the red '+' symbols denote outliers. Differences among treatments were evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer.

Figure 5 presents leaf temperature, and Figure 6 presents root temperature. These variables are associated with plant responses to environmental conditions. The observed differences suggest potential associations between treatments and physiological response; however, these results do not demonstrate causal mechanisms and should be interpreted within the limitations of the experimental design.

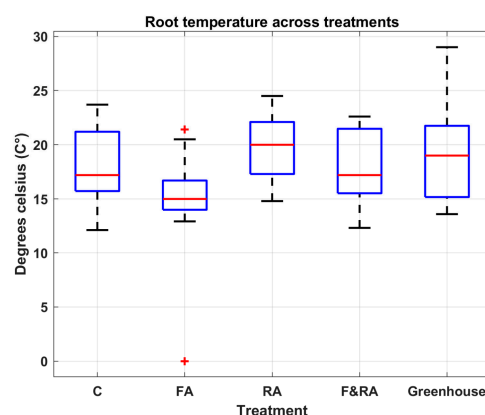


Figure 6. Distribution of root temperature across treatments represented as boxplots. Values are expressed in °C. The central red line indicates the median, the box represents the interquartile range (IQR), the whiskers (dashed lines) extend to the most extreme values within $1.5 \times \text{IQR}$, and the red '+' symbols denote outliers. Differences among treatments were evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer.

The combined results for yield dynamics (Figure 4) and physiological variables (Figures 5 and 6) suggest that differences in plant performance may be reflected more clearly in temporal production patterns and physiological responses than in cumulative structural variables.

The descriptive statistics of the vegetative variables are presented in Table 8, where median and IQR are used as primary descriptors, complemented by mean and standard deviation. In general, no substantial differences in median values were observed across treatments for most structural variables, such as plant height and the number of pods per plant, supporting the results of the Kruskal–Wallis test (Table 7).

Table 8. Descriptive statistics of vegetative variables (median, interquartile range, mean and standard deviation) for each treatment. Units: plant height (cm); leaf temperature (°C); root temperature (°C); number of flowers and pods (count).

Treatment	Vegetative Variable	Median	IQR	Mean	SD
C	Number of flowers on plant	1.4	1.55	1.0667	0.8138
	Number of pods on plant	6	6.8	6.2727	3.8160
	Plant height	29	19.47	33.9750	6.3693
	Leaf temperature	16.35	5.4	17.45	3.6409
	Root temperature	16.725	4.65	17.2843	2.8979
FA	Number of flowers on plant	0	0	3.9389	2.7894
	Number of pods on plant	3.6804	5.6984	4.8954	3.4157
	Plant height	23.37	18.3562	34.2131	11.5238
	Leaf temperature	13.725	3.425	14.0468	2.1394
	Root temperature	15.3	2.775	16.0375	2.2794
RA	Number of flowers on plant	3.5	2.6	2.8300	1.8272
	Number of pods on plant	2.625	4.7	4.5964	4.4229
	Plant height	30.9333	15.50	38.6444	10.4541
	Leaf temperature	18.5	8.5	19.0736	3.9503
	Root temperature	20	4.9	19.7394	3.1573

Table 8. Cont.

Treatment	Vegetative Variable	Median	IQR	Mean	SD
F&RA	Number of flowers on plant	4.0867	3.2333	3.2527	2.1288
	Number of pods on plant	2.9704	5.7815	5.0093	4.6234
	Plant height	24.1083	21.8680	32.1995	9.9851
	Leaf temperature	18.3	7.4	18.2876	4.0208
	Root temperature	17.2	6.1	18.1947	3.2305

Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; IQR = Interquartile range; SD = Standard deviation. Sample size (n): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants. The experimental unit was an individual plant. Descriptive statistics are presented for reference and do not imply statistical significance among treatments. Differences among treatments were evaluated separately using the Kruskal–Wallis test ($\alpha = 0.05$).

The IQR values indicate moderate variability across treatments, particularly for temperature-related variables, which is consistent with their statistically significant differences. Some differences in central tendency were observed; for instance, the foliar application (FA) treatment showed higher values in the number of flowers per plant. However, this variable exhibited a marked asymmetry in its distribution (median = 0; mean = 3.93), indicating a skewed distribution characterized by a high frequency of low values and a few higher values rather than a consistent increase across samples.

Although no statistically significant differences were detected for these variables, effect size analysis using Cliff's delta (δ) revealed variation in magnitude among treatments. For the number of flowers (Table 9), moderate to large effects were observed when comparing the control with the treatments (δ ranging from -0.5777 to -0.6319), whereas comparisons among treatments showed smaller effects (δ ranging from 0.0944 to 0.1444).

Table 9. Pairwise effect size estimates (Cliff's delta, δ) of number of flowers per plant among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	-0.6319
C	RA	-0.5777
C	F&RA	-0.5944
FA	RA	0.1444
FA	F&RA	0.0944
RA	F&RA	-0.2355

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta. Sample size (n): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants.

For the number of pods per plant (Table 10), effect sizes were generally small to moderate (δ ranging from 0.0649 to 0.3246), indicating substantial overlap between distributions. Overall, these results indicated limited differentiation among treatments despite some variation in effect magnitude.

The correlation analysis (Table 11) provides further insight into the relationship between vegetative and physiological variables. In the control group, few significant correlations were observed, except for the strong positive correlation between leaf and root temperature ($\rho = 0.77$, $p < 0.01$).

Table 10. Pairwise effect size estimates (Cliff's delta, δ) of number of pods on plant among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	0.2561
C	RA	0.3246
C	F&RA	0.2142
FA	RA	0.1298
FA	F&RA	0.0649
RA	F&RA	0.0969

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta. Sample size (n): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants.

Table 11. Spearman correlation coefficients between morphological variables; ** $p < 0.01$; * $p < 0.05$.

Treatment	Variable 1	Variable 2	ρ	p -Value
C	Number of flowers on plant	Number of pods on plant	−0.0830	0.7780
	Number of flowers on plant	Leaf temperature	0.2382	0.4121
	Number of flowers on plant	Root temperature	0.1957	0.5024
	Number of flowers on plant	Plant height	−0.1345	0.6465
	Number of pods on plant	Plant height	0.2561	0.3769
	Number of pods on plant	Leaf temperature	0.2589	0.3715
	Number of pods on plant	Root temperature	−0.1218	0.6783
	Plant height	Leaf temperature	0.1615	0.5812
	Plant height	Root temperature	0.0631	0.8303
	Leaf temperature	Root temperature	0.7703	0.0013 **
FA	Number of flowers on plant	Number of pods on plant	0.6475	0.0123 *
	Number of flowers on plant	Leaf temperature	0.5017	0.0676
	Number of flowers on plant	Root temperature	0.2497	0.3892
	Number of flowers on plant	Plant height	0.4004	0.1559
	Number of pods on plant	Plant height	0.7680	0.0013 **
	Number of pods on plant	Leaf temperature	0.9069	7.68×10^{-6} **
	Number of pods on plant	Root temperature	0.6851	0.0068 **
	Plant height	Leaf temperature	0.9234	2.47×10^{-6} **
	Plant height	Root temperature	0.8013	0.0006 **
	Leaf temperature	Root temperature	0.8004	0.0006 **
RA	Number of flowers on plant	Number of pods on plant	0.2631	0.3635
	Number of flowers on plant	Leaf temperature	0.2098	0.4715
	Number of flowers on plant	Root temperature	0.0578	0.8445
	Number of flowers on plant	Plant height	0.1659	0.5708
	Number of pods on plant	Plant height	0.9253	2.13×10^{-6} **
	Number of pods on plant	Leaf temperature	0.8961	1.45×10^{-5} **
	Number of pods on plant	Root temperature	0.8498	0.0001 **
	Plant height	Leaf temperature	0.8683	0.0001 **
	Plant height	Root temperature	0.9133	5.06×10^{-6} **
	Leaf temperature	Root temperature	0.8174	0.0004 **

Table 11. Cont.

Treatment	Variable 1	Variable 2	ρ	p -Value
F&RA	Number of flowers on plant	Number of pods on plant	0.8022	0.0006 **
	Number of flowers on plant	Leaf temperature	0.7223	0.0035 **
	Number of flowers on plant	Root temperature	0.5635	0.0358 *
	Number of flowers on plant	Plant height	0.7212	0.0036 **
	Number of pods on plant	Plant height	0.9523	1.54×10^{-7} **
	Number of pods on plant	Leaf temperature	0.8272	0.000 **
	Number of pods on plant	Root temperature	0.8137	0.0004 **
	Plant height	Leaf temperature	0.8817	3.05×10^{-5} **
	Plant height	Root temperature	0.8679	0.0001 **
	Leaf temperature	Root temperature	0.7759	0.0011 **

Correlations were calculated using individual observations within each treatment. The results should be interpreted as exploratory due to unequal replication among treatments and the limited sample sizes available for some treatment groups; therefore, correlations should not be interpreted as evidence of a causal relationship. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; ρ = Spearman correlation coefficient. Sample size (n): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants.

In contrast, a greater number of significant correlations were observed in the FA, RA, and F&RA treatments, particularly involving the number of pods, plant height, and temperature variables. Notably, in the F&RA treatment, the number of flowers and pods showed strong positive correlations with plant height and temperature variables. These relationships are consistent with the observed differences in temperature variables (Figures 5 and 6), which were the only vegetative parameters showing statistically significant differences among treatments (Table 7).

Overall, the strong associations among temperature, growth, and pod production suggest the involvement of physiological processes in the observed treatment responses. However, these relationships should be interpreted as associative rather than causal.

Temporal indicators such as production dynamics across harvest events, yield, accumulation patterns, and harvest duration, together with physiological variables such as leaf and root temperature, may provide a more integrative assessment of treatment effects than cumulative structural variables alone.

3.3. Agricultural Yield

Agricultural yield allows the estimation of the amount of product obtained in relation to the cultivated area. In this study, agricultural yield was determined using the average weight of pods harvested per plant per square meter (kg/m^2), providing an integrative indicator of treatment performance.

The Kruskal–Wallis test applied to agricultural yield did not detect statistically significant differences among treatments ($p > 0.05$), indicating that yield was statistically comparable under the evaluated conditions.

Despite the lack of statistical significance, differences in central tendency were observed (Table 12). The RA treatment showed higher mean yield values ($1.13 \text{ kg}/\text{m}^2$), followed by F&RA ($0.9492 \text{ kg}/\text{m}^2$), FA ($0.8325 \text{ kg}/\text{m}^2$), and the control ($0.6390 \text{ kg}/\text{m}^2$); however, these differences were not statistically significant and should therefore be interpreted cautiously. This pattern is consistent with the production dynamics illustrated in Figure 4.

Table 12. Average yield values per treatment, expressed as kg m^{−2}.

Treatment	Kg m ^{−2}
FA	0.8325
C	0.6390
RA	1.13
F&RA	0.9492

Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer. Sample size (n): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants. Yield values are expressed as Kg m^{−2}. Differences among treatments were evaluated using the Kruskal–Wallis test ($\alpha = 0.05$), and no statistically significant differences were detected ($p > 0.05$).

Figure 7 shows the distribution of yield across treatments. Although overlap among treatments is observed, the figure highlights the importance of considering both total yield and production patterns when evaluating treatment performance. Because treatments differed in the number of harvest events, yield performance was evaluated from two complementary perspectives: cumulative yield over the entire production cycle and production persistence across successive harvests. This distinction is important because treatment RA and F&RA remained productive for two additional harvest events compared with C and FA, which may influence cumulative yield comparisons.

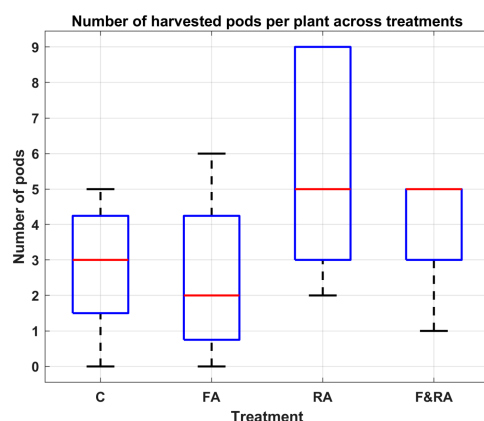


Figure 7. Distribution of the number of harvested pods per plant across treatments represented as boxplots. The central red line indicates the median, the box represents the interquartile range (IQR), the whiskers (dashed lines) extend to the most extreme values within $1.5 \times \text{IQR}$, and the red '+' symbols denote outliers. Differences among treatments were evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer.

Insights can be further supported by the descriptive statistics of total yield per plant (Table 13), which summarize cumulative pod weight over the phenology cycle. The RA treatment showed a higher mean yield per plant, followed by the control, FA, and F&RA treatments; however, differences were not statistically significant.

The differences should be interpreted considering the longer harvest period observed for RA and F&RA, which include two additional harvest events compared to C and FA. Differences in harvest frequency among treatments represent a structural aspect of the experimental design and may influence cumulative yield comparisons. Therefore, higher cumulative values, particularly RA, may partially reflect extended production duration rather than strictly increased productivity. Consequently, cumulative yield should not be interpreted as a direct indicator of treatment superiority. Instead, cumulative yield and production persistence were

considered complementary indicators of crop performance, allowing treatment responses to be evaluated while accounting for differences in harvest duration.

Table 13. Descriptive statistics of total yield per plant (median, interquartile range, mean and standard deviation) for each treatment. Units: pod weight (g plant^{−1}) over the phenological cycle.

Treatment	Median per Plant	IQR	Mean per Plant	SD
C	16.6	12.2	15.8857	6.0539
FA	10.7	14.9667	13.1429	8.6531
RA	20.2	16.2	17.7333	7.6023
F&RA	9.7333	2.8	9.1407	2.1287

Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; IQR = Interquartile range; SD = Standard deviation. Sample size (n): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants. The experimental unit was an individual plant. Descriptive statistics are presented for reference and do not imply statistical significance among treatments. Differences among treatments were evaluated separately using the Kruskal–Wallis test ($\alpha = 0.05$).

Overall, these results indicate that yield assessment may vary depending on the metric considered (per unit vs. per plant) and the temporal structure of production. Therefore, cumulative yield and production persistence should be interpreted as complementary indicators of crop performance, particularly when treatments differ in harvest duration.

The effect size analysis (Table 14) indicated small to moderate differences between treatments. According to Cliff’s delta (δ), most comparisons showed small effects (δ ranging from -0.2083 to 0.3448), with the largest contrast observed between FA and RA ($\delta = 0.3448$) and between FA and F&RA ($\delta = 0.2894$), while minimal differences were found between RA and F&RA ($\delta = -0.0351$). Overall, these results suggest limited separation between treatments in terms of cumulative yield and should be interpreted cautiously given the variability in sample sizes and differences in harvest duration among treatments.

Table 14. Pairwise effect size estimates (Cliff’s delta, δ) of comparing total yield (total pod weight accumulated over the phenological cycle) for each treatment.

Treatment	Treatment	Cliff’s δ
C	FA	-0.2083
C	RA	0.1129
C	F&RA	0.0726
FA	RA	0.3448
FA	F&RA	0.2894
RA	F&RA	-0.0351

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff’s δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer. Sample size (n): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants.

In this context, Cliff’s delta (δ) provides a distribution-based interpretation of affect magnitude, suggesting limited separation between treatments despite some differences in mean-based estimates.

Overall, no statistically significant differences in agricultural productivity were detected among treatments. Although the RA treatment showed a tendency toward higher values in both productivity per unit area and per plant, this pattern was not statistically significant. In contrast, the F&RA treatment, despite exhibiting a longer production period, showed the lowest mean productivity per plant, suggesting lower production efficiency per plant.

These results are consistent with previous observations indicating that treatment effects may be better captured through production dynamics and the physiological responses rather than cumulative yield alone. In particular, the distinction between production persistence and per-plant efficiency appears to be relevant for understanding yield behavior. The analysis of quality-related properties is addressed in the following section on nutritional quality.

Overall, these findings highlight the importance of considering both temporal production patterns and experimental structure when interpreting yield responses in aeroponic systems.

3.4. Nutritional Quality Analysis of Green Beans

The macro- and micronutrient composition of the samples was statistically analyzed to evaluate differences among treatments. This included significance testing ($\alpha = 0.05$), effect size estimation using Cliff's delta (δ), and Spearman correlation analysis. Additionally, comparisons of medians were performed to assess whether treatments influenced the nutritional composition of aeroponically grown green beans.

These approaches allowed the identification of treatment-related differences in nutritional composition.

3.4.1. Macronutrients

Macronutrient analysis was conducted to compare the effects of treatments using significance testing ($\alpha = 0.05$), effect size (Cliff's delta, δ), and correlation analysis. The null hypothesis (H_0) states that there are no significant differences in macronutrient content among treatments.

As shown in Table 15, statistically significant differences were detected among treatments for all macronutrients ($p < 0.01$), indicating that treatment effects were present, although the magnitude of the response varied across variables.

Table 15. Kruskal–Wallis test results for macronutrient variables. Significance codes: ** $p < 0.01$.

Macronutrients	<i>p</i> -Value	H_0
Total Carbohydrate	0.0013 **	Rejected
Sugar	0.0011 **	Rejected
Fiber	6.7683×10^{-4} **	Rejected
Fat	3.4095×10^{-5} **	Rejected
Protein	4.2469×10^{-5} **	Rejected
Water	2.7817×10^{-5} **	Rejected

Statistical significance was evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). “Rejected” indicates rejection of the null hypothesis (H_0), whereas “Not rejected” indicates insufficient evidence to reject H_0 . Variables with $p < 0.05$ were considered to show statistically significant treatment effects.

The descriptive statistics (Table 16) show a consistent pattern in which all treatments (FA, RA, and F&RA) exhibited higher median values than the control for total carbohydrates, sugars, fiber, fat, and protein, while lower values were observed for water content. IQR values indicated relatively low variability within treatments.

Among treatments, F&RA and RA generally exhibited higher values for most macronutrients; however, these observations are descriptive and should be interpreted in conjunction with the statistical results.

Effect size matrices (Tables 17–22) describe the magnitude and direction of differences among treatments using Cliff's delta (δ). Overall, a consistent pattern of strong separation between the control and all biofertilizer treatments was observed across most macronutrients. In particular, total carbohydrates, sugars, fiber, fat, and protein showed extreme effect sizes ($\delta \approx \pm 1$), indicating a clear shift in distribution relative to the control. However, these

values should be interpreted cautiously, as they may reflect low within-group variability, tied ranks, or limited sample size rather than absolute biological separation.

Table 16. Descriptive statistics of macronutrient composition (median, interquartile range, mean, and standard deviation) for each treatment. Units: total carbohydrate (g 100 g^{−1} DW); sugar (g 100 g^{−1} DW); fiber (g 100 g^{−1} DW); fat (g 100 g^{−1} DW); protein (g 100 g^{−1} DW); water (g 100 g^{−1} FW).

Treatment	Macronutrient	Median	IQR	Mean	SD
C	Total Carbohydrate	6.3243	0.2715	6.3894	0.1590
	Sugar	3.2824	0.2397	3.2577	0.1176
	Fiber	3.0697	0.1787	3.1317	0.0857
	Fat	0.1727	0.0289	0.1679	0.0144
	Protein	1.5889	0.1314	1.5558	0.0630
	Water	90.9968	0.2512	90.8853	0.1502
FA	Total Carbohydrate	7.4186	0.5137	7.4580	0.2330
	Sugar	3.9567	0.1695	3.8708	0.1273
	Fiber	3.5533	0.4314	3.5871	0.1717
	Fat	0.2023	0.022	0.2128	0.0106
	Protein	1.9942	0.1183	2.0192	0.0506
	Water	89.4527	0.7406	89.3909	0.3098
RA	Total Carbohydrate	7.5881	0.2881	7.4604	0.1779
	Sugar	3.9689	0.1126	3.9084	0.1001
	Fiber	3.6192	0.117	3.5631	0.0730
	Fat	0.2430	0.0042	0.2431	0.0042
	Protein	1.8944	0.0733	1.8669	0.0422
	Water	90.1438	0.0622	90.2091	0.1303
F&RA	Total Carbohydrate	7.4263	0.4663	7.4927	0.1720
	Sugar	3.8352	0.1325	3.8287	0.0662
	Fiber	3.6459	0.2812	3.6639	0.1198
	Fat	0.2376	0.0086	0.2381	0.0060
	Protein	2.0473	0.1036	2.0329	0.0496
	Water	89.8957	0.3483	89.8508	0.2982

Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; IQR = Interquartile range; SD = Standard deviation. Sample size (n): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants. The experimental unit was an individual plant. Descriptive statistics are presented for reference and do not imply statistical significance among treatments. Differences among treatments were evaluated separately using the Kruskal–Wallis test ($\alpha = 0.05$).

Table 17. Pairwise effect size estimates (Cliff's delta, δ) of total carbohydrate among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−1
C	RA	−1
C	F&RA	−1
FA	RA	−0.0793
FA	F&RA	−0.3333
RA	F&RA	0.0617

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

Table 18. Pairwise effect size estimates (Cliff's delta, δ) of sugar among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−1
C	RA	−1
C	F&RA	−1
FA	RA	−0.1428
FA	F&RA	0.1428
RA	F&RA	0.6172

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

Table 19. Pairwise effect size estimates (Cliff's delta, δ) of fiber among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−1
C	RA	−1
C	F&RA	−1
FA	RA	−0.1111
FA	F&RA	−0.4285
RA	F&RA	−0.5802

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

Table 20. Pairwise effect size estimates (Cliff's delta, δ) of fat among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−1
C	RA	−1
C	F&RA	−1
FA	RA	−1
FA	F&RA	−1
RA	F&RA	0.4567

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

Table 21. Pairwise effect size estimates (Cliff's delta, δ) of protein among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−1
C	RA	−1
C	F&RA	−1
FA	RA	1
FA	F&RA	−0.1428
RA	F&RA	−1

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

Table 22. Pairwise effect size estimates (Cliff's delta, δ) of water among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	1
C	RA	1
C	F&RA	1
FA	RA	−1
FA	F&RA	−0.6824
RA	F&RA	1

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

In contrast, comparisons among treatments generally showed smaller and less consistent differences, suggesting partial overlap in their distributions. Notable exceptions were observed for fat and protein, where some treatment contrasts exhibited moderate to large effect sizes, indicating a greater degree of differentiation under specific application modes.

Water content displayed an inverse pattern, with all treatments consistently showing lower values than the control ($\delta \approx 1$), reinforcing the observed compositional shift.

Overall, these results indicate that biofertilizer treatments are associated with marked changes in macronutrient composition relative to the control, while differences among treatments remain more variable and dependent on the specific nutrient considered.

These patterns are consistent with the descriptive statistics (Table 16), supporting the observed shifts in central tendency.

Taken together, these patterns indicate a consistent compositional shift between control and treated samples rather than isolated nutrient-specific effects.

Further insight is provided by the correlation analysis (Table 23), which indicates treatment-dependent patterns in the relationships among macronutrients. Positive correlations between total carbohydrates, sugar, and fiber in RA and F&RA suggest coordinated variation of carbon-related compounds, potentially reflecting shared metabolic pathways or allocation processes.

Table 23. Spearman correlation coefficients between macronutrients; * $p < 0.05$.

Treatment	Variable 1	Variable 2	ρ	p -Value
C	Total Carbohydrate	Sugar	0.8	0.1333
	Total Carbohydrate	Fiber	0.7	0.2333
	Total Carbohydrate	Fat	−0.1	0.95
	Total Carbohydrate	Protein	−0.8	0.1333
	Total Carbohydrate	Water	0	1
	Sugar	Fiber	0.3	0.6833
	Sugar	Fat	−0.1	0.95
	Sugar	Protein	−1	0.0167 *
	Sugar	Water	−0.2	0.7833
	Fiber	Fat	−0.4	0.5167
	Fiber	Protein	−0.3	0.6833
	Fiber	Water	−0.3	0.6833
	Fat	Protein	0.1	0.95
	Fat	Water	0.9	0.0833
	Protein	Water	0.2	0.7833

Table 23. Cont.

Treatment	Variable 1	Variable 2	ρ	p -Value
FA	Total Carbohydrate	Sugar	0.7	0.2333
	Total Carbohydrate	Fiber	0.7	0.2333
	Total Carbohydrate	Fat	−0.4	0.5167
	Total Carbohydrate	Protein	0.1	0.95
	Total Carbohydrate	Water	−0.4	0.5167
	Sugar	Fiber	0.3	0.6833
	Sugar	Fat	−0.9	0.0833
	Sugar	Protein	0.1	0.95
	Sugar	Water	−0.1	0.95
	Fiber	Fat	0.1	0.95
	Fiber	Protein	0.4	0.5167
	Fiber	Water	−0.9	0.0833
	Fat	Protein	0.2	0.7833
	Fat	Water	−0.3	0.6833
	Protein	Water	−0.7	0.2333
RA	Total Carbohydrate	Sugar	1	0.0167
	Total Carbohydrate	Fiber	0.7	0.2333
	Total Carbohydrate	Fat	−0.3	0.6833
	Total Carbohydrate	Protein	0.6	0.35
	Total Carbohydrate	Water	−1	0.0167
	Sugar	Fiber	0.7	0.2333
	Sugar	Fat	−0.3	0.6833
	Sugar	Protein	0.6	0.35
	Sugar	Water	−1	0.0167
	Fiber	Fat	−0.2	0.7833
	Fiber	Protein	0.4	0.5167
	Fiber	Water	−0.7	0.2333
	Fat	Protein	0.5	0.45
	Fat	Water	0.3	0.6833
	Protein	Water	−0.6	0.35
F&RA	Total Carbohydrate	Sugar	0.8571	0.0238
	Total Carbohydrate	Fiber	0.8929	0.0123
	Total Carbohydrate	Fat	0.75	0.0663
	Total Carbohydrate	Protein	−0.3214	0.4976
	Total Carbohydrate	Water	−0.2857	0.5560
	Sugar	Fiber	0.6071	0.1667
	Sugar	Fat	0.6786	0.1095
	Sugar	Protein	−0.1071	0.8397
	Sugar	Water	−0.6429	0.1389
	Fiber	Fat	0.7143	0.0881
	Fiber	Protein	−0.2143	0.6615
	Fiber	Water	−0.0714	0.9063
	Fat	Protein	0	1
	Fat	Water	−0.4286	0.3536
	Protein	Water	−0.3214	0.4976

Correlations were calculated using individual observations within each treatment. The results should be interpreted as exploratory due to unequal replication among treatments and the limited sample sizes available for some treatment groups; therefore, correlations should not be interpreted as evidence of a causal relationship. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascochyllum nodosum* biofertilizer; RA = Root application of *Ascochyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascochyllum nodosum* biofertilizer; ρ = Spearman correlation coefficient.

In the control group, a significant negative correlation was observed between sugar and protein ($\rho = -1$, $p < 0.05$). Perfect correlations ($\rho = \pm 1$) likely reflect limited sample size or tied ranks and should not be interpreted as exact deterministic relationships.

The similarity in central tendency and dispersion (Table 16) is illustrated in Figures 8 and 9, which show median values across treatments. Figure 8a,b highlights higher median values for fat and total carbohydrates, particularly in RA and F&RA, while Figure 9a,b shows a similar pattern for protein and fiber. These graphical representations support the observed differences in central tendency.

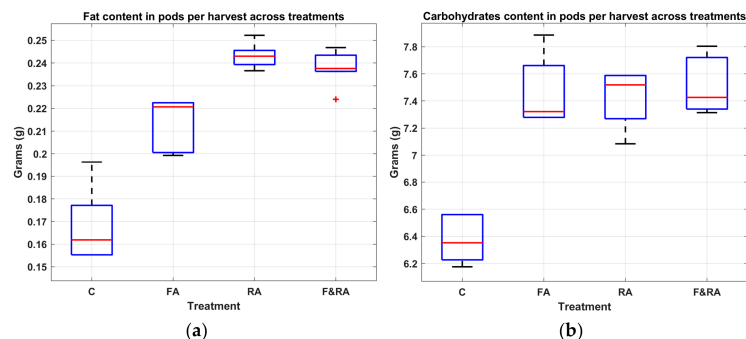


Figure 8. Distribution of fat content (a) and total carbohydrate content (b) in pods across treatments represented as boxplots. Values are expressed as $\text{g } 100 \text{ g}^{-1} \text{ DW}$. The central red line indicates the median, the box represents the interquartile range (IQR), the whiskers (dashed lines) extend to the most extreme values within $1.5 \times \text{IQR}$, and the red '+' symbols denote outliers. Differences among treatments were evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer.

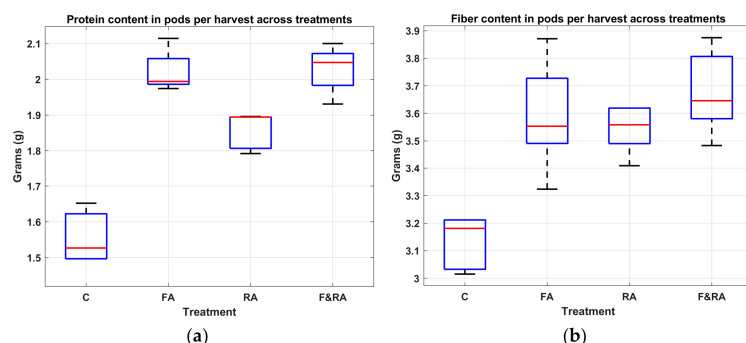


Figure 9. Distribution of protein content (a) and fiber content (b) in pods across treatments represented as boxplots. Values are expressed as $\text{g } 100 \text{ g}^{-1} \text{ DW}$. The central red line indicates the median, the box represents the interquartile range (IQR), the whiskers (dashed lines) extend to the most extreme values within $1.5 \times \text{IQR}$, and the red '+' symbols denote outliers. Differences among treatments were evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer.

These results indicate statistically supported differences in several nutritional parameters; however, biological relevance should be interpreted considering the magnitude of the effect, sample size, and experimental conditions.

3.4.2. Micronutrients

The analysis of micronutrient composition was conducted to evaluate differences among treatments using a significance test ($\alpha = 0.05$), effect size estimation (Cliff's delta, δ) and descriptive statistics. These complementary approaches allow comparison from both statistical and practical perspectives.

Based on the significance test (Table 24), only calcium, iron, and manganese showed statistically significant differences among treatments ($p < 0.05$), whereas magnesium, phosphorus, potassium, and zinc did not. This indicates a selective response pattern among micronutrients, rather than a uniform treatment effect.

Table 24. Kruskal–Wallis test results for micronutrient variables. Significance codes: ** $p < 0.01$; * $p < 0.05$.

Micronutrients	<i>p</i> -Value	H ₀
Calcium	0.0002 **	Rejected
Iron	0.0067 **	Rejected
Magnesium	0.1286	Not rejected
Manganese	0.0439 *	Rejected
Phosphorus	0.2376	Not rejected
Potassium	0.1504	Not rejected
Zinc	0.3261	Not rejected

Statistical significance was evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). “Rejected” indicates rejection of the null hypothesis (H₀), whereas “Not rejected” indicates insufficient evidence to reject H₀. Variables with $p < 0.05$ were considered to show statistically significant treatment effects. For micronutrients, comparisons were subsequently performed using Dunn’s multiple-comparison procedure with Dunn–Šidák adjustment.

To further identify which treatment pairs contributed to the significant Kruskal–Wallis results, post hoc pairwise comparisons were performed using Dunn’s multiple-comparison procedure with Dunn–Šidák adjustment. For calcium, the combined foliar and root application treatment (F&RA) exhibited significantly higher concentrations than both the control treatment ($p = 0.0003$) and the foliar application treatment (FA) ($p = 0.0114$), whereas the remaining pairwise comparisons were not significant. For iron, a significant difference was detected only between F&RA and the control treatment ($p = 0.0079$). For manganese, a significant difference was observed between the control and foliar application treatment ($p = 0.0288$), while no significant differences were detected among the remaining treatment pairs.

The descriptive statistics (Table 25) show generally small differences in central tendency across treatments. Calcium exhibited a gradual increase from the control (C) to F&RA, while iron showed higher mean values in F&RA. In contrast, variables such as phosphorus and potassium displayed minimal variation, consistent with their lack of statistical significance. Median values suggest slight but consistent shifts, whereas IQR values indicate overlapping dispersion for most micronutrients, supporting the limited differentiation observed.

Table 25. Descriptive statistics of micronutrient composition (median, interquartile range, mean, and standard deviation) for each treatment. Units: calcium (mg 100 g^{−1} DW); iron (mg 100 g^{−1} DW); magnesium (mg 100 g^{−1} DW); manganese (mg 100 g^{−1} DW); phosphorus (mg 100 g^{−1} DW); potassium (mg 100 g^{−1} DW); zinc (mg 100 g^{−1} DW).

Treatment	Micronutrient	Median	IQR	Mean	SD
C	Calcium	35.0644	1.364	35.3153	0.6852
	Iron	1.0545	0.0506	1.0527	0.0243
	Magnesium	24.3735	2.1651	24.0662	0.8947
	Manganese	0.1896	0.0074	0.1891	0.0030
	Phosphorus	35.4741	3.0621	35.2301	1.3993
	Potassium	182.9513	17.8827	187.6101	7.9384
	Zinc	0.1970	0.0102	0.1930	0.0045

Table 25. Cont.

Treatment	Micronutrient	Median	IQR	Mean	SD
FA	Calcium	35.5735	1.3784	35.7760	0.7011
	Iron	1.0824	0.0398	1.0869	0.0168
	Magnesium	24.4516	2.1798	24.1107	0.9083
	Manganese	0.1942	0.0023	0.1946	0.0010
	Phosphorus	35.4744	3.0621	35.2304	1.3993
	Potassium	183.2511	18.0647	187.8945	8.0043
	Zinc	0.2	0.0116	0.1990	0.0053
RA	Calcium	36.0358	0.2402	36.1952	0.5683
	Iron	1.0724	0.0102	1.0669	0.0159
	Magnesium	25.2885	0.9187	25.0376	0.8549
	Manganese	0.1937	0.0023	0.1932	0.0027
	Phosphorus	35.8825	1.2928	36.3242	0.8715
	Potassium	199.8638	10.3592	196.5288	5.5340
	Zinc	0.1938	0.0083	0.1964	0.0043
F&RA	Calcium	36.5108	1.9743	37.3649	0.9666
	Iron	1.1129	0.0684	1.1084	0.0412
	Magnesium	24.4639	1.9521	24.9484	1.0166
	Manganese	0.1903	0.011	0.1918	0.0054
	Phosphorus	35.7235	2.5157	35.6400	1.9779
	Potassium	191.1648	27.0559	191.3496	11.1985
	Zinc	0.2016	0.0132	0.1984	0.0088

Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; IQR = Interquartile range; SD = Standard deviation. Sample size (*n*): C = 5 plants; FA = 30 plants; RA = 5 plants; F&RA = 30 plants. The experimental unit was an individual plant. Descriptive statistics are presented for reference and do not imply statistical significance among treatments. Differences among treatments were evaluated separately using the Kruskal–Wallis test ($\alpha = 0.05$).

Effect size analysis (Tables 26–32) revealed a differentiated response among micronutrients and was generally consistent with the significant pairwise differences identified through the post hoc comparisons for calcium, iron, and manganese. Calcium, iron, and manganese exhibited the most consistent and pronounced differences, with moderate to large effect sizes according to Cliff's delta (δ), particularly in comparisons involving F&RA, indicating a directional shift in their distributions. These findings are consistent with both the descriptive statistics (Table 25), the significant differences identified (Table 24), and the treatment-specific differences revealed by the post hoc analyses.

Table 26. Pairwise effect size estimates (Cliff's delta, δ) of total calcium among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−0.5918
C	RA	−0.7460
C	F&RA	−0.8730
FA	RA	−0.6825
FA	F&RA	−0.8412
RA	F&RA	−0.8518

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

Table 27. Pairwise effect size estimates (Cliff's delta, δ) of total iron among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−0.8367
C	RA	−0.5238
C	F&RA	−0.8412
FA	RA	0.5238
FA	F&RA	−0.2698
RA	F&RA	−0.4814

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

Table 28. Pairwise effect size estimates (Cliff's delta, δ) of total magnesium among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−0.2653
C	RA	−0.5238
C	F&RA	−0.5238
FA	RA	−0.5238
FA	F&RA	−0.3650
RA	F&RA	−0.0617

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

Table 29. Pairwise effect size estimates (Cliff's delta, δ) of total manganese among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−0.8775
C	RA	−0.4920
C	F&RA	−0.3333
FA	RA	0.5714
FA	F&RA	0.3650
RA	F&RA	0.2098

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

Table 30. Pairwise effect size estimates (Cliff's delta, δ) of total phosphorus among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−0.2653
C	RA	−0.4920
C	F&RA	−0.0793
FA	RA	−0.4920
FA	F&RA	−0.0158
RA	F&RA	0.4074

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

Table 31. Pairwise effect size estimates (Cliff's delta, δ) of total potassium among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−0.2653
C	RA	−0.6190
C	F&RA	−0.0158
FA	RA	−0.6190
FA	F&RA	0.0476
RA	F&RA	0.3580

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascochyllum nodosum* biofertilizer; RA = Root application of *Ascochyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascochyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

Table 32. Pairwise effect size estimates (Cliff's delta, δ) of total zinc among experimental treatments.

Treatment	Treatment	Cliff's δ
C	FA	−0.5102
C	RA	−0.4285
C	F&RA	−0.2381
FA	RA	0.5238
FA	F&RA	−0.0317
RA	F&RA	−0.1358

Positive and negative δ values indicate the direction of distributional differences between treatments. Effect sizes should be interpreted cautiously because Cliff's δ values may be influenced by unequal sample size, tied observations, and within-group variability. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascochyllum nodosum* biofertilizer; RA = Root application of *Ascochyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascochyllum nodosum* biofertilizer; Cliff's δ = Cliff's delta.

In contrast, magnesium, phosphorus, and potassium exhibited smaller and less consistent effect sizes (δ values closer to zero), indicating substantial overlap among treatments. Zinc presented small to moderate but inconsistent effects, suggesting a tendency toward variability that is not statistically supported. Overall, these results indicate that treatment effects on micronutrient composition are selective and element-dependent.

These patterns should be interpreted with caution, as relatively large effect sizes may arise from low within-group variability or limited sample size, rather than strong biological separation.

Further insight is provided by the correlation analysis (Table 33), which indicates treatment-dependent relationships among micronutrients. Some relatively strong correlation coefficients were observed, such as negative associations between calcium and manganese and between magnesium and phosphorus in FA and RA, suggesting potential interactions among these elements. Additionally, the RA treatment showed a strong negative correlation between iron and zinc, while the control group exhibited a positive association between phosphorus and zinc.

However, no statistically significant correlations were detected ($p > 0.05$). Therefore, these relationships should be interpreted cautiously and considered exploratory. Moreover, high or perfect correlations ($\rho = \pm 1$), when present, may reflect limited sample size or tied ranks rather than exact deterministic relationships.

The tendencies observed in Tables 24 and 25 are illustrated in Figures 10 and 11. Figure 10a,b. Greater separation of median values for calcium and iron, particularly in F&RA, consistent with the statistically significant differences observed. In contrast, in Figure 11a,b, greater overlap is observed for magnesium and manganese, despite the statistical significance detected for manganese, suggesting that its effect may be subtle and distribution-dependent.

Overall, micronutrient responses were more selective than those observed for macronutrients. Calcium, iron, and manganese were the most responsive elements, while the remaining micronutrients showed limited or inconsistent variation across treatments. These results suggest that biofertilizer effects on nutrient composition are element-specific and should be interpreted considering both statistical significance and distributional behavior.

Table 33. Spearman correlation coefficients between micronutrients.

Treatment	Variable 1	Variable 2	ρ	p -Value
C	Calcium	Iron	0.7	0.2333
	Calcium	Magnesium	−0.1	0.95
	Calcium	Manganese	−0.8	0.1333
	Calcium	Phosphorus	−0.1	0.95
	Calcium	Potassium	0.6	0.35
	Calcium	Zinc	−0.2	0.7833
	Iron	Magnesium	0.6	0.35
	Iron	Manganese	−0.7	0.2333
	Iron	Phosphorus	−0.5	0.45
	Iron	Potassium	0.1	0.95
	Iron	Zinc	−0.3	0.6833
	Magnesium	Manganese	0	1
	Magnesium	Phosphorus	−0.8	0.1333
	Magnesium	Potassium	−0.3	0.6833
	Magnesium	Zinc	−0.5	0.45
	Manganese	Phosphorus	0.1	0.95
	Manganese	Potassium	−0.4	0.5167
	Manganese	Zinc	0	1
	Phosphorus	Potassium	−0.3	0.6833
	Phosphorus	Zinc	0.9	0.833
	Potassium	Zinc	−0.6	0.35
FA	Calcium	Iron	0.3	0.6833
	Calcium	Magnesium	−0.1	0.95
	Calcium	Manganese	−0.9	0.0833
	Calcium	Phosphorus	−0.1	0.95
	Calcium	Potassium	0.6	0.35
	Calcium	Zinc	0.2	0.7833
	Iron	Magnesium	0.6	0.35
	Iron	Manganese	−0.1	0.95
	Iron	Phosphorus	−0.6	0.35
	Iron	Potassium	0.1	0.95
	Iron	Zinc	0	1
	Magnesium	Manganese	0	1
	Magnesium	Phosphorus	−0.8	0.1333
	Magnesium	Potassium	−0.3	0.6833
	Magnesium	Zinc	−0.1	0.95
	Manganese	Phosphorus	0.3	0.6833
	Manganese	Potassium	−0.7	0.2333
	Manganese	Zinc	0.1	0.95
	Phosphorus	Potassium	−0.3	0.6833
	Phosphorus	Zinc	0.6	0.35
	Potassium	Zinc	−0.6	0.35

Table 33. Cont.

Treatment	Variable 1	Variable 2	ρ	p -Value
RA	Calcium	Iron	0.33	0.6833
	Calcium	Magnesium	−0.3	0.6833
	Calcium	Manganese	−0.9	0.0833
	Calcium	Phosphorus	0	1
	Calcium	Potassium	0.4	0.5167
	Calcium	Zinc	−0.1	0.95
	Iron	Magnesium	0.3	0.6833
	Iron	Manganese	−0.1	0.95
	Iron	Phosphorus	−0.7	0.2333
	Iron	Potassium	0.6	0.35
	Iron	Zinc	−0.9	0.0833
	Magnesium	Manganese	0	1
	Magnesium	Phosphorus	−0.8	0.1333
	Magnesium	Potassium	−0.5	0.45
	Magnesium	Zinc	−0.5	0.45
	Manganese	Phosphorus	1	0.95
	Manganese	Potassium	0	1
	Manganese	Zinc	0	1
	Phosphorus	Potassium	−0.1	0.95
	Phosphorus	Zinc	0.9	0.0833
	Potassium	Zinc	−0.5	0.45
F&RA	Calcium	Iron	0	1
	Calcium	Magnesium	0.1818	0.6921
	Calcium	Manganese	−0.6001	0.1730
	Calcium	Phosphorus	−0.2182	0.6381
	Calcium	Potassium	−0.0909	0.8524
	Calcium	Zinc	−0.4001	0.3714
	Iron	Magnesium	0.0357	0.9635
	Iron	Manganese	0.1071	0.8397
	Iron	Phosphorus	0.6429	0.1389
	Iron	Potassium	0.4643	0.3024
	Iron	Zinc	0.25	0.5948
	Magnesium	Manganese	−0.3214	0.4976
	Magnesium	Phosphorus	−0.3571	0.4444
	Magnesium	Potassium	−0.6071	0.1667
	Magnesium	Zinc	−0.1071	0.8397
	Manganese	Phosphorus	0.1429	0.7825
	Manganese	Potassium	0.4286	0.3536
	Manganese	Zinc	0.1071	0.8397
	Phosphorus	Potassium	0.5	0.2667
	Phosphorus	Zinc	0.6071	0.1667
	Potassium	Zinc	0.5357	0.2357

Correlations were calculated using individual observations within each treatment. The results should be interpreted as exploratory due to unequal replication among treatments and the limited sample sizes available for some treatment groups; therefore, correlations should not be interpreted as evidence of a causal relationship. Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer; ρ = Spearman correlation coefficient.

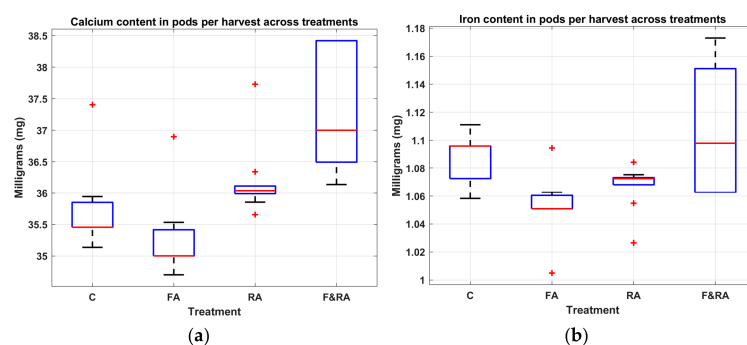


Figure 10. Distribution of calcium content (a) and iron content (b) in pods across treatments represented as boxplots. Values are expressed as $\text{mg } 100 \text{ g}^{-1} \text{ DW}$. The central red line indicates the median, the box represents the interquartile range (IQR), the whiskers (dashed lines) extend to the most extreme values within $1.5 \times \text{IQR}$, and the red '+' symbols denote outliers. Differences among treatments were evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer.

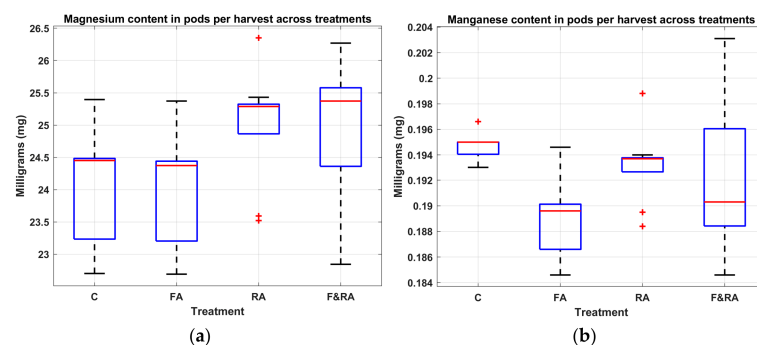


Figure 11. Distribution of magnesium content (a) and manganese content (b) in pods across treatments represented as boxplots. Values are expressed as $\text{mg } 100 \text{ g}^{-1} \text{ DW}$. The central red line indicates the median, the box represents the interquartile range (IQR), the whiskers (dashed lines) extend to the most extreme values within $1.5 \times \text{IQR}$, and the red '+' symbols denote outliers. Differences among treatments were evaluated using the Kruskal–Wallis test ($\alpha = 0.05$). Abbreviations: C = Control treatment (Steiner nutrient solution only); FA = Foliar application of *Ascophyllum nodosum* biofertilizer; RA = Root application of *Ascophyllum nodosum* biofertilizer; F&RA = Combined foliar and root application of *Ascophyllum nodosum* biofertilizer.

4. Discussion

The evaluation of the phenological evolution, productivity and nutritional quality of *Phaseolus vulgaris* L. var. *Opus* under aeroponic conditions showed that treatment-associated responses were not uniform across variables. Overall, the *A. nodosum*-based biofertilizer had limited effects on structural growth and yield-related traits, while more evident responses were observed in physiological parameters and nutritional composition. These results suggest a partial decoupling between yield formation and compositional responses, where changes in nutritional quality do not necessarily translate into increased biomass or yield.

4.1. Product Evaluation

Product evaluation is essential for understanding how treatments influence both the qualitative and quantitative attributes of yield. In this study, variables such as the number of grains per pod and pod weight were analyzed as indicators of reproductive efficiency and commercial quality.

The results (Table 1; Figures 1 and 2) showed that the number of grains per pod was the only morphological variable significantly affected by treatments ($p < 0.05$), while pod weight did not show statistically significant differences among treatments. Nevertheless, descriptive tendencies indicated that RA and F&RA treatments were associated with a higher number of grains, whereas pod weight tended to be lower.

This pattern suggests a potential trade-off between grain number and individual grain size, which has been widely reported in leguminous crops. Such a trade-off may reflect differences in resource allocation during reproductive development, where an increase in the number of grains may limit the accumulation of biomass per grain. However, since these mechanisms were not directly measured, this interpretation should be considered as a plausible physiological explanation rather than a directly demonstrated mechanism.

Furthermore, the interpretation of relationships between morphological traits and grain number should consider the scale of analysis. Correlation results based on averaged treatment values showed limited statistical significance, whereas analyses using individual observations revealed stronger and more consistent relationships among morphological variables. This suggests that data aggregation may obscure underlying biological patterns by reducing variability and statistical power, potentially masking treatment effects. Therefore, the lack of significant correlations in some cases does not necessarily imply the absence of biological relationships, but rather reflects methodological limitations associated with sample size and data structure.

The application route of the biofertilizer may have influenced these patterns. Root application (RA) and combined application (F&RA) may be associated with a more sustained nutrient supply, potentially supporting reproductive development. In contrast, FA and control treatments showed fewer grains but slightly higher individual mass, suggesting differences in grain-filling dynamics.

These findings are consistent with previous studies reporting that *A. nodosum* extracts can increase fruit or seed number (*Lycopersicon esculentum* Mill and *Solanum lycopersicum* L., respectively) without significantly altering individual weight [32,33]. From an agronomic perspective, greater uniformity in grain formation is directly related to yield stability and the commercial quality of the product, especially in sustainable systems such as aeroponics.

Previous research has reported both positive and inconsistent responses depending on crop type, dosage, and environmental conditions. For example, [34] observed effects on growth and biomass in *Vigna aconitifolia* (Jacq.), while [35] reported approximately a 13% decrease in yield and number of fruits in cucumber (*Cucumis sativus* L.). Similarly [36] observed increases in Alphonse Lavallée grape (*Vitis vinifera* L.), although emphasizing that responses depend strongly on species, dosage, and environmental conditions.

4.2. Vegetative State

The analysis of vegetative variables revealed that structural traits such as plant height, number of flowers, and number of pods per plant did not differ significantly among treatments (Table 7), suggesting a relatively homogeneous phenotypic response under the evaluated conditions. This suggests that the biofertilizer did not substantially alter plant architecture.

In contrast, leaf and root temperatures showed statistically significant differences ($p < 0.01$), indicating that treatments were associated with differences in physiological responses related to transpiration, water uptake, and thermal regulation [37–40]. These findings highlight that physiological variables may be more sensitive indicators of treatment effects than structural traits.

Although no significant differences were observed in the total number of pods, production dynamics over time (Figure 4) revealed distinct patterns. RA and F&RA treatments

showed a tendency toward more sustained production in later harvests, extending the production cycle compared to C and FA. This suggests that treatment effects may be more accurately captured through temporal production dynamics rather than cumulative metrics alone.

These observations are consistent with studies reporting that biostimulants enhance physiological processes such as photosynthesis, chlorophyll synthesis, and stress tolerance [41,42]. However, other studies indicate that these effects are not always consistent and depend on multiple factors, including formulation, dosage, and environmental conditions [43–45]. This indicates that improvements in yield or productivity metrics are not consistently achieved across systems. While the results of this study show a positive response under the evaluated system, the literature indicates that such effects are not universal.

Temperature also played a critical role and likely interacted with treatment effects, contributing to variability in phenological and productive responses. The wide thermal variation observed during the experiment (17 °C to 40 °C at midday) likely contributed to variability in flower, plant height, and development, as reported in previous studies [46]. Temperatures outside the optimum range can generate variability in flowers and pod formation [47,48]. These thermal fluctuations may explain part of the variability observed in structural variables.

These responses may be associated with temperature-driven changes in transpiration rates and metabolic activity, which can influence resource allocation and reproductive development.

4.3. Agricultural Performance and Production Dynamics

Performance is a key indicator of the agronomic success of any biotechnological treatment, as it reflects the interaction between plant physiology, nutrient availability, and environmental conditions within the production system [49]. In this context, the number of pods per plant (a primary yield component) showed a statistically uniform response across treatments ($p > 0.05$), indicating no statistically significant differences under the evaluated aeroponic conditions. This lack of significant differences may be attributed to the controlled environment and homogeneous nutrient availability, which may have buffered treatment-related variability and masked potential differences in yield expression.

While other biotechnological approaches, such as nanoparticles or microbial consortia, have demonstrated significant increases in the performance of *Phaseolus vulgaris* [28,50], the results obtained in this study suggest that macroalgae-based biofertilizers may primarily influence physiological and functional processes rather than directly enhancing structural yield components.

The evaluation of agricultural yield revealed that treatment effects were more complex than suggested by inferential statistics alone, indicating the importance of complementary descriptive and temporal analyses. Although no statistically significant differences were detected among treatments for yield per unit area ($p > 0.05$), consistent tendencies were observed in both production dynamics (Figure 4) and cumulative yield (Table 12).

In particular, the RA treatment showed the highest mean yield per plant (Table 13), despite sharing similar overall yield patterns with other treatments. This suggests that individual plant performance may reflect treatment-associated variability that is not fully captured by aggregate yield metrics.

An important factor influencing these results is the difference in harvest duration among treatments. RA and F&RA were subjected to a longer production cycle, with two additional harvests compared to C and FA. This extended harvesting period may be associated with the cumulative yield observed, particularly in RA, which combined prolonged production with higher per-plant productivity.

Interestingly, despite having the same extended harvest period, the F&RA treatment exhibited the lowest mean yield per plant. This suggests that increased production time does not necessarily translate into higher productivity, which may reflect differences in physiological efficiency among treatments. Such patterns may be associated with trade-offs in resource allocation, where prolonged physiological activity does not always result in proportional increases in reproductive output.

These findings are supported by the production patterns shown in Figure 4, where RA and F&RA exhibited more sustained yield over time, in contrast to the earlier decline observed in C and FA. However, the relatively higher efficiency observed in RA suggests that both production persistence and plant-level efficiency appear to be important factors in determining yield outcomes.

Additionally, differences in sample size among treatments ($n = 30$ for FA and F&RA; $n = 5$ for C and RA) should be considered when interpreting these results, as they may influence variability and the detection of statistical differences. This highlights the importance of complementing inferential analysis with descriptive statistics and effect size estimation, particularly in unbalanced experimental designs.

Overall, these results indicate that yield responses cannot be fully explained by a single metric and require an integrated analysis that includes production dynamics, efficiency, and temporal patterns. This supports the idea that biofertilizer effects may be more strongly associated with physiological regulation and production dynamics than with direct increases in total yield.

4.4. Nutritional Quality of Green Beans

The analysis of nutritional quality revealed that biofertilizer treatments were associated with significant differences in the chemical composition of green beans. In contrast to the limited effects observed in structural and yield variables, nutritional parameters showed a strong and consistent response across treatments.

From a nutritional and functional perspective, variables such as carbohydrates, sugars, fiber, fat, protein, and water are key indicators of crop quality, as they determine both the energetic value and the nutraceutical potential of the final product [51,52]. In this context, the statistical differences observed among treatments suggest that the application of macroalgae-based biofertilizers may selectively influence the biochemical composition of the crop.

In agreement with the statistical analysis presented in Table 15, all evaluated macronutrients showed statistically significant differences among treatments. These findings suggest that biofertilizer application was associated with changes in nutritional composition under the evaluated aeroponic conditions.

All nutritional parameters, except moisture content, were expressed on a dry weight basis (DW), which allows for standardized comparisons among treatments by minimizing the influence of variable water content. This approach strengthens the interpretation that observed differences reflect true changes in nutrient accumulation and metabolic activity rather than dilution effects.

4.5. Macronutrients

All macronutrients showed statistically significant differences among treatments ($p < 0.01$), indicating that nutrient accumulation was highly responsive to biofertilizer application. Treatments FA, RA, and F&RA consistently increased carbohydrates, sugars, fiber, fat, and protein, while reducing water content (Table 16). These results suggest that biofertilizer application may be associated with changes in nutrient accumulation and compositional profiles, which is consistent with previous studies reporting that seaweed-based

biostimulants, particularly those derived from *A. nodosum*, can influence mineral assimilation, antioxidant-related responses, and plant metabolism [42,53]. However, several studies have shown that the response to seaweed-based biostimulants is highly variable and depends on environmental conditions, crop genotype, and application method, leading to inconsistent effects on nutrient accumulation [54–56].

The increase in carbohydrates, sugars, and fiber, particularly in RA and F&RA, may indicate treatment-associated changes in carbon allocation, as suggested by the positive correlations observed among these variables (Table 23). Similar responses have been reported in crops treated with *A. nodosum* extracts, where changes in photosynthetic performance and carbon partitioning have been associated with accumulation of structural and non-structural carbohydrates [14,57,58]. These responses may be linked to hormone-like compounds present in *A. nodosum* extracts, such as auxins and cytokinins, which are known to influence nutrient uptake pathways, carbon partitioning, and metabolic regulation [13,59]. Additionally, previous studies have suggested that physiological responses associated with osmotic regulation and nutrient transport may contribute to differences in nutrient accumulation [42,58]. In contrast, other reports indicate that carbohydrate accumulation is not always significantly affected, particularly under non-limiting nutrient conditions or when baseline photosynthetic activity is already optimal [60,61].

Protein content also increased significantly, especially in FA and F&RA, suggesting possible changes in nitrogen-related metabolism. This interpretation is consistent with studies reporting that seaweed extracts may influence nitrogen assimilation pathways, amino acid biosynthesis, and nutrient uptake [59]. Nevertheless, some studies have reported minimal or no changes in protein content following biofertilizer application, suggesting that nitrogen availability in the growth system may modulate this response [62,63].

The reduction in water content may reflect a concentration effect when expressed on a fresh-weight basis, where increased accumulation of organic compounds reduces relative moisture content. However, since most nutritional parameters in this study were expressed on a dry weight basis (DW), the observed increases in macronutrients cannot be attributed solely to concentration effects, but rather suggest treatment-associated changes in nutrient accumulation rather than dilution effects alone. This distinction is particularly relevant in controlled cultivation systems, including hydroponic and aeroponic production, where improved nutrient availability can promote dry matter accumulation and increase nutritional density [64]. Conversely, other studies have reported increased water content in fruits following the application of seaweed extracts, often reflected in higher fresh weight and improved fruit turgidity, which has been associated with enhanced osmotic adjustment and water retention capacity mediated by bioactive compounds [65,66].

Following these observations, the overall nutritional patterns suggest that biofertilizer application may be associated with shifts in nutrient allocation and metabolic activity in green bean plants. In particular, the higher accumulation of macronutrients such as carbohydrates, sugar, fiber, and protein in RA and F&RA treatments may reflect a tendency toward increased synthesis of structural and storage compounds.

The concurrent reduction in water content across treatments relative to the control is consistent with an increase in dry matter concentration rather than a decrease in plant water status. This interpretation is further supported by Cliff's delta results for water content (Table 22), where δ values consistently indicate an inverse relationship between treatments and the control, reflecting a systematic shift in distribution rather than sporadic variation. Overall, these responses point to a potential modulation of metabolic processes associated with carbon assimilation and biomass partitioning, although the underlying mechanisms remain to be elucidated.

Importantly, the consistency of large Cliff's delta (δ) values across comparisons suggests that treatment effects are reflected in distributional shifts rather than isolated differences in central tendency. This indicates that the observed differences are not driven solely by mean variation, but rather by a systematic separation between treatment and control distributions. Given that Cliff's delta is a non-parametric measure, these results also provide robustness against potential deviations from normality, supporting their use as complementary indicators of distributional differences, rather than definitive evidence of biological separation.

Overall, these results indicate that the macronutrient composition of *Phaseolus vulgaris* grown under aeroponic conditions was associated with *A. nodosum*-based biofertilizer application, highlighting its potential influence on crop nutritional quality.

4.6. Micronutrients

In contrast to macronutrients, micronutrient responses were more selective. Only calcium, iron, and manganese showed statistically significant differences among treatments (Table 24), while magnesium, phosphorus, potassium, and zinc did not show statistically significant variation. This pattern suggests a differential sensitivity of micronutrient accumulation to biofertilizer application under the evaluated aeroponic conditions.

The observed increases in calcium and iron may be associated with enhanced nutrient availability or uptake efficiency. Since mineral concentrations were expressed on a dry weight basis (DW), these differences reflect actual changes in nutrient accumulation rather than dilution or concentration effects related to water content. These responses are consistent with the presence of bioactive compounds in improving nutrient mobility and absorption, as previously reported [42,53].

Calcium is an essential element for cell wall integrity, fruit firmness, and resistance to mechanical or physiological stress, while iron is involved in essential metabolic processes including chlorophyll synthesis and electron transport. Therefore, the higher levels observed in these elements may be associated with physiological processes linked to plant development and metabolic activity, although these relationships were not directly measured in this study.

From a nutritional perspective, the observed differences in calcium, iron, and manganese may be relevant because mineral composition is an important component of the overall nutritional quality of legumes and green beans [9]. Although the magnitude of the observed changes does not necessarily imply a substantial contribution to daily dietary requirements, the results suggest that *A. nodosum*-based biofertilizer application may influence the mineral composition of aeroponically grown green beans. Therefore, these responses may be of interest for future biofortification-oriented research and the development of value-added horticultural products [8,9].

The post hoc analyses provided additional resolution regarding the micronutrient responses observed among treatments. For calcium, the combined foliar and root application treatment (F&RA) showed significantly higher concentrations than both the control and foliar-only treatments. For iron, a significant difference was detected only between F&RA and the control treatment, whereas manganese differed significantly only between the control and foliar application treatments. These findings indicate that the observed treatment effects were restricted to specific micronutrients and treatment combinations. Therefore, the responses associated with the *Ascochyta nodosum*-based biofertilizer should be interpreted as nutrient-specific rather than indicative of a generalized increase across all evaluated micronutrients.

In contrast, the absence of statistically significant differences in magnesium, phosphorus, potassium, and zinc suggests that their uptake remained relatively stable across

treatments. This may reflect a homogeneous nutrient distribution within the aeroponic system or limited responsiveness of these elements to the applied biofertilizer under the experimental conditions.

From a non-parametric perspective, effect size analysis complemented the interpretation of these observations. Calcium, iron, and manganese showed moderate to large Cliff's delta (δ) values across several pairwise comparisons, whereas the remaining micronutrients exhibited smaller and less consistent δ values, suggesting greater overlap in their distributions. However, these effect sizes should be interpreted cautiously because their magnitude may be influenced by sample size, tied observations, and within-group variability.

The selective micronutrient responses observed in this study are consistent with previous reports indicating that *A. nodosum*-based biostimulants may influence plant physiological processes associated with nutrient acquisition and utilization. However, the specific mechanisms underlying these responses were not directly investigated and therefore remain to be clarified [13,42,59].

Although some associations between micronutrients and productive variables were observed, these should be interpreted cautiously. For instance, higher calcium and iron levels may be associated with patterns of vegetative stability and productive persistence observed in certain treatments; however, these relationships were not directly evaluated and should not be interpreted as causal.

Studies such as [67] on green beans (*Phaseolus vulgaris* L. cv. *Perona*) highlight that nutritional quality should be considered alongside post-harvest handling and storage practices to preserve vitamins and minerals. Similarly, [26] reported that liquid biofertilizers may enhance growth and nutrient absorption in hydroponic systems. In this context, the observed changes in micronutrient composition may be associated not only with treatment effect during cultivation, but also with broader implications for post-harvest quality and nutrient retention, particularly in controlled-environment systems.

Overall, these findings indicate that micronutrient responses to biofertilizer application were selective rather than uniform. Calcium, iron, and manganese appeared to be the most responsive elements, while the remaining micronutrients remained relatively stable. These selective responses may reflect differences in nutrient acquisition or utilization; however, further studies are required to elucidate the underlying physiological mechanisms.

4.7. Integrated Interpretation

The integrated analysis of agronomic, physiological, and nutritional parameters reveals that the application of *A. nodosum*-based biofertilizer primarily modulates plant responses at the biochemical and physiological levels rather than inducing major structural or yield-related changes. While most morphological and productivity variables remained statistically stable, both macro- and micronutrient composition showed significant and consistent modifications across treatments.

Since nutritional parameters were expressed mainly on a dry weight basis (DW), the observed differences are less likely to be explained solely by variation in water content.

The differential response observed between structural stability and compositional variability suggests a partial decoupling between yield formation and metabolic processes, where improvements in nutritional quality do not necessarily translate into increased biomass or yield. This pattern highlights the importance of incorporating biochemical and nutritional indicators when evaluating the effectiveness of biofertilizers, particularly in controlled systems where environmental variability is minimized.

In this context, Cliff's delta (δ) values provided complementary evidence of distributional differences among treatments; however, these estimates were interpreted cautiously and were not considered definitive evidence of biological separation.

Overall, these findings support the potential of macroalgae-based biofertilizers as a sustainable strategy to enhance the nutritional value and functional quality of crops, even in the absence of significant changes in traditional agronomic performance indicators.

Although *Ascophyllum nodosum*-based biofertilizers are widely considered sustainable agricultural inputs, the long-term sustainability of these products also depends on the responsible sourcing of the algal biomass. In Mexico, particularly along the Baja California coast, macroalgae represent an important marine resource with recognized agricultural and biotechnological potential, and their exploitation is subject to management and regulatory frameworks intended to promote sustainable use [68]. However, increasing demand for seaweed-derived agricultural products highlights the importance of responsible biomass sourcing, traceability, and resource conservation. Although *Ascophyllum nodosum* is primarily harvested from natural populations in the North Atlantic, sustainable harvesting practices such as biomass monitoring, harvest quotas, rotational harvesting, and recovery periods have been proposed to maintain long-term resource availability. Future research should also evaluate the potential of locally available Mexican macroalgae as alternative biostimulant sources, which may contribute to reducing dependence on imported biomass while promoting regional bioeconomy development and marine resource sustainability [69,70].

This is particularly relevant for controlled-environment agriculture, where improving quality without increasing resource input represents a key sustainability objective. In this context, the ability to enhance crop composition independently of yield may provide opportunities to optimize both nutritional value and production efficiency in sustainable production systems.

These findings should be interpreted considering certain limitations, including unequal sample sizes among treatments, differences in harvest frequency, and the controlled nature of the aeroponic system, which may limit extrapolation to field conditions. Additionally, correlation analyses should be considered exploratory due to the limited number of observations and should not be interpreted as evidence of a causal relationship.

5. Conclusions

- Application of the *Ascophyllum nodosum*-based biofertilizer did not produce statistically significant improvements in most growth- and yield-related variables of *Phaseolus vulgaris* L. var *Opus* cultivated under aeroponic conditions.
- Significant differences were detected in physiological parameters, particularly leaf and root temperature, suggesting that physiological responses were more sensitive to treatment application than to structural growth variables.
- Nutritional quality was more responsive than agronomic performance. Significant changes were observed in all evaluated macronutrients, including carbohydrates, sugars, fiber, fat, protein, and water content.
- Among micronutrients, calcium, iron, and manganese showed significant treatment effects, whereas magnesium, phosphorus, potassium, and zinc exhibited limited or inconsistent responses.
- The combined foliar and root application (F&RA) was associated with some of the largest changes in nutrient composition, particularly for calcium and iron.
- Overall, the results indicate that *Ascophyllum nodosum*-based biofertilizer application was associated with greater changes in the nutritional composition of green beans than in growth- or yield-related variables under controlled aeroponic conditions.
- Future studies should employ balanced replication and evaluate long-term productivity under equivalent harvest durations to further clarify treatment effects.

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Abbreviations

The following abbreviations are used in this manuscript:

L	Linnaeus
var	Variety
cv	Cultivated variety
LD	Linear dichroism
ppm	Parts Per Million
DW	Dry weight
FW	Fresh weigh

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