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Protein Hydrolysate Combined with Hydroponics Divergently Modifies Growth and Shuffles Pigments and Free Amino Acids of Carrot and Dill Microgreens

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Abstract: Microgreens are the new sophisticated commodity in horticulture that boost the human diet with bioactive metabolites and garnish it with colors and tastes. Microgreens thrive well when cultivated in soilless systems, of which closed-loop soilless systems combined with biostimulant application can provide a sustainable, innovative method of growing microgreens. *Daucus carota* L. and *Anethum graveolens* L. microgreens were grown in greenhouse conditions implementing a floating raft system combined with a protein hydrolysate of leguminous origin as root application (0.3 mL L^{-1} nutrient solution). Growth, colorimetric parameters, macronutrients, chlorophylls, carotenoids, carbohydrates, free amino acids, and soluble proteins were assessed. The use of a protein hydrolysate in the nutrient solution engendered an increase in anthocyanins (+461.7%) and total phenols (+12.4%) in carrot, while in dill, the fresh yield (+13.5%) and ascorbic acid (+17.2%) increased. In both species, soluble proteins and total free amino acids increased by 20.6% and 18.5%, respectively. The floating raft system proved to be promising for microgreens and can ease the application of biostimulants through root application. Future research should also investigate the yield and nutritional parameter responses of other species of microgreens with the aim of large-scale sustainable production.

Keywords: floating raft system; biostimulant; root application; anthocyanins; phenols; reduced sugars; carbohydrates; minerals; pigments

1. Introduction

In recent years, the sophisticated gastronomy market and the chain of horticultural supply have been conquered by a densely rich commodity, the so-called microgreens [1–3]. These immature greens boost the human diet with bioactive health-promoting metabolites and minerals [3,4] and generate a multitude of alluring colors and tastes [2,4]. A range of genotypes are adopted for microgreen cultivation, from commercial to local varieties and covering vast botanical families including Brassicaceae, Lamiaceae, and Apiaceae [2,3]. Microgreens can be grown in loose soil and soilless media [5]. Peat and peat-based materials are among the most adopted, followed by synthetic fibrous substrates (rockwool and polyethylene terephthalate) and natural fiber media (coconut, burlap, jute, cotton, hemp, etc.) [1,5–8]. Growth conditions proved to significantly modulate the qualitative profile of

microgreens [3], of which the substrate material should be given attention [6,8], in addition to the nutrient addition strategies [3].

Innovative methods such as closed-loop hydroponics are economically sustainable for producing leafy vegetables, such as the floating raft system, where the nutrient solution composition can be managed accurately [9]. The authors of the present paper along with Cristofano and coworkers [10] also emphasized the importance of such system in accelerating the growth cycle, raising the nutrient and water use efficiency, reducing the labor cost, and avoiding suboptimal soil reactions. In addition, it can be implemented in urban agriculture projects under economic and social development, and in the reduction in the environmental impact [10].

Floating systems are appropriate for the use of plant biostimulants that act by improving the nutrient use efficiency through increasing the availability of the confined nutrients [10] and boosting plant growth, especially the biostimulants that fall in the category of biotic elicitation methods [11]. As stated by Rouphael and Colla [12], protein hydrolysates (PHs), as biostimulants, contain signaling peptides and free amino acids that can enhance seedling growth and vegetable quality. They are up taken through root absorption and can be converted to the needed plant compounds; in addition, they are highly available for plants, unlike in substrate conditions where microbial competition occurs [10]. Moreover, PHs increase root growth due to hormone-like activities, stimulate nitrogen and carbon metabolism, and modulate the antioxidant systems [13]; PHs change the root architecture from length to lateral root branching, and the root biomass, thus incrementing the root system surface area [14].

Although microgreens are grown hydroponically or semi-hydroponically, especially in peat and peat-based mixes [7], very few works have dealt with floating raft systems or the nutrient film technique (NFT). For instance, Wang and Kniel [15] adopted an NFT system for growing kale and mustard microgreens on hydroponic pads. Bulgari et al. [16] grew rocket, Swiss chard, and basil, and Puccinelli et al. [17] grew basil, both in a hydroponic floating system, adopting polystyrene cell trays filled with vermiculite. In addition, Bulgari et al. [8] grew basil and rocket microgreens in small tanks filled with three different substrates. Thus far, to our knowledge, no study has adopted a floating raft system without an additional substrate for the growth of microgreens, and no study has tested, thus far, the effect of biostimulants, particularly PHs, on the growth and quality of microgreens.

Based on the above-mentioned issues, the current study aimed to verify the possibility of growing microgreens in a floating raft system without any additional substrate, and the potential of adding a PH in the nutrient solution from day one. Therefore, in this study, biometric and colorimetric parameters of microgreens were assessed, in addition to the mineral content, pigments, and primary and secondary metabolites. Such data could be of relevant importance to microgreen growers and to scientists, in order to understand the growth of microgreens in direct contact with a nutrient solution and with biostimulant applications throughout the growth.

2. Materials and Methods

2.1. Growth Conditions, Plant Material, and Experimental Design

In order to evaluate production, bioactive compounds, minerals, carbohydrates, and the free amino acid content, two species of Apiaceae were grown in a floating system as microgreens: carrot (*Daucus carota* L.) and dill (*Anethum graveolens* L.), both purchased from Pagano Costantino & F.III S.R.L (Scafati, Salerno, Italy). The weight of 100 seeds was evaluated in triplicate (89.00 mg and 129.25 mg for carrot and dill, respectively).

The experiment was carried out in autumn 2020 in a glass greenhouse in the Department of Agriculture (DIA), University of Naples Federico II (Portici, Italy; 40°49′ N, 14°15′ E, 72 m above sea level). Both species were primed in water for one day and sown on 20 October 2020, with a density of 6 seeds cm^{-2}. Seeds were manually sown on a perforated plastic tray (total area: 588 cm^2) placed inside a plastic tank (28.5 × 22 × 6 cm) containing 1.1 L of nutrient solution (NS) (Figure 1). A quarter-strength modified Hoagland

NS was prepared with osmotic water (electrical conductivity (EC) of 0.03 dS m^{-1} and pH of 6.2) as follows: 2.0 mM NO_3^-, 0.25 mM S, 0.20 mM P, 0.62 mM K, 0.75 mM Ca, 0.17 mM Mg, 0.25 mM NH_4^+, 20 µM Fe, 9 µM Mn, 0.3 µM Cu, 1.6 µM Zn, 20 µM B, and 0.3 µM Mo, with an EC of 0.4 ± 0.05 dS m^{-1} and pH of 5.8 ± 0.2. During the growth of microgreens, fresh NS was added every other day to the tanks in order to maintain the original volume of 1.1 L. The tanks were arranged in a randomized factorial scheme (2 × 2), which involved a biostimulant application, an untreated control, and two species of microgreens (dill and carrot). Each treatment was replicated three times, with each tray consisting of a single replicate (experimental unit). Dill and carrot microgreens were harvested 22 and 25 days after sowing (DAS), respectively, when the first true leaf appeared.

Figure 1. Hydroponic system (floating raft system) implemented in this study, showing the perforated plastic trays and the plastic tanks, and the growth of the roots directly in the nutrient solutions (not to scale).

2.2. Biostimulant Application

In this trial, a plant-derived biostimulant with a 5% organic nitrogen content obtained by enzymatic hydrolysis of leguminous biomass (Trainer®®; Hello Nature Italy SRL, Rivoli Veronese, Verona, Italy) was used. Enzymatic hydrolysis was used to release the amino acids and peptides from proteins. The final product contained mostly peptides and amino acids and, to a lesser extent, soluble carbohydrates, mineral elements, and phenolic compounds. Trainer®® has a density of 1.21 kg L^{-1}, a dry matter of 46%, and a pH of 4.0. It contains 310 g kg^{-1} of free amino acids and soluble peptides. As reported by the manufacturer, the biostimulant is composed of amino acids (Ala, Arg, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) and soluble peptides, free from plant hormones [10]. The Trainer®® content of soluble sugars is 90 g kg^{-1} f.w., and its elemental composition is as follows (g kg^{-1} f.w): N (50.0), P (0.9), K (41.1), Ca (10.9), Mg (0.5), Fe (0.024), Zn (0.010), Mn (0.001), B (0.005), and Cu (0.001). N–NO3 and N–NH4 contents are 3.13 and 6.00 mg g^{-1} f.w., respectively. The biostimulant was added to the NS at the dose of 0.3 mL L^{-1} from DAS 1.

2.3. Harvest, Biometric Parameters, and Colorimetric Indices Determination

At the stage of the first true leaf, microgreens of carrot and dill were harvested using sterilized scissors at the tray level. Fresh production was expressed as g m^{-2}. Part of the fresh microgreens was placed in a ventilated oven at 60 °C for 72 h to determine the dry weight (g m^{-2}) and the percentage of dry matter (DM). The dried material was then ground using a Wiley mill (MF10.1 Wiley laboratory mill, IKA®®, StaufenimBreisgau, Baden-Württemberg, Germany) and used for mineral determination. The remaining fresh material was placed immediately in liquid nitrogen and then stored at −80 °C, where a part was freeze dried for further analyses. At the same time, the roots were harvested and placed in a ventilated oven at 60 °C for 72 h to determine the dry weight (dw).

Before harvesting, the canopy color of the microgreens was determined with a Minolta CR-300 colorimeter (Minolta Camera Co. Ltd., Osaka, Japan) at five different points on each tray. Before colorimetric measurements, the colorimeter was calibrated with the Minolta white standard. The measurements were obtained using the CIELAB (Commission Internationale de l'Éclairage) color space parameters, where:

L: brightness (0 = black to 100 = white), a*: greenness (green (−60) to red (+60)), and b∗: yellowness (blue (−60) to yellow (+60)). Chroma (C), which represents the color intensity (chromaticity), was calculated using the following formula: $(a^2 + b^2)1/2$. The hue angle describes the qualitative color attribute in the relative amounts of redness and yellowness, Hue angle $\tan^{-1}$ (b*/a*).

2.4. Mineral Content Determination

Cations (K, Ca, Mg, Na) and anions (NO_3, SO_4, PO_4, Cl) were determined following the protocol described by Formisano et al. [18]. Briefly, 0.25 g of dried and ground microgreen material was extracted in ultrapure water, placed in a water bath at 80 °C, and shaken for 10 min. Then, after centrifuging the extracts (6000 rpm for 10 min), the supernatant was collected, filtered, and processed using an ICS3000 ion chromatograph (Thermo Scientific™ Dionex™, Sunnyvale, CA, USA) coupled to an electrical conductivity detector. Cation separation was performed with methanesulfonic acid (25 mM) using an IonPac CS12A analytical column, an IonPac CG12A precolumn, and a CERS5000 self-regenerating electrolyte suppressor (Thermo Scientific™ Dionex™, Sunnyvale, CA, USA). The separation of anions was performed using potassium hydroxide (5–30 mM) at a flow rate of 1.5 mL min^{-1} using an IonPac ATC-HC trap, an IonPac AG11-HC guard column, an IonPac AG11-HC IC column, and a DRS600 self-regenerating dynamic suppressor (Thermo Scientific™ Dionex™, Sunnyvale, CA, USA). The integration and quantification of minerals and organic acids were performed by comparing the peak areas of the samples with those of the standards. Each treatment was analyzed in triplicate, and the concentrations of anions and cations were expressed as mg g^{-1} dw.

2.5. Total Phenols Determination

According to Folin–Ciocalteu's method [19], to determine total phenols, 0.25 g of freeze-dried plant material was homogenized with 10 mL of 60% methanol and centrifuged for 15 min. An amount of 125 µL of the supernatant was added to 125 µL of Folin–Ciocalteu reagent (phosphotungstic acid + phosphomolybdic acid) in 0.5 mL of distilled water. The absorbance of the resulting mixture was read at 760 nm by UV–Vis spectrophotometry. Total phenols were expressed as mg gallic acid equivalents 100 g^{-1} dw. Each treatment was analyzed in triplicate.

2.6. Pigment and Total Ascorbic Acid Determination

As described by Wellburn [20], for the determination of photosynthetic pigments (chlorophyll a and b, and carotenoids), 0.2 g of fresh microgreen sample was extracted in ammonia acetone and centrifuged for 10 min (2000 rpm). Quantification of chlorophyll a and b and carotenoids was determined by reading the absorbances of the extracts at 647, 664, and 470 nm, respectively, using an ONDA V-10 Plus UV–Vis spectrophotometer (Giorgio Bormac srl, Carpi, Italy). Then, total chlorophyll a and b values were used to calculate total chlorophyll and the chlorophyll a/b ratio.

For the determination of total vitamin C, according to the method described by Kampfenkel et al. [21], 0.4 g of fresh sample was extracted in 2 mL of 6% TCA (trichloroacetic acid) and incubated for 15 min at −20 °C. The extract was then centrifuged for 10 min (4000 rpm), and the absorbance was read at 525 nm.

Total chlorophyll and carotenoids were expressed as mg g^{-1} fw, while total vitamin C was expressed in mg ascorbic acid 100 g^{-1} fw. The chlorophyll/carotenoid ratio was calculated as the total chlorophyll/carotenoids. Each treatment was analyzed in triplicate.

2.7. Anthocyanins, Soluble Carbohydrates, Soluble Proteins, and Amino Acids

Freeze-dried and ground samples of microgreens were used to determine anthocyanins, soluble carbohydrates, proteins, and amino acids.

Total anthocyanin analysis was performed according to the protocol described by Rouphael et al. [22] by extracting 0.2 g of sample in 180 µL of ethanol (40% *v/v*). The extract was then incubated on ice for 20 min and centrifuged (14,000 rpm; 10 min). The pellet was re-extracted by the same procedure, and the two extracts were combined and placed in a polypropylene microplate with 75 µL of 25 mM potassium chloride (pH 1.0) or 75 µL of 400 mM sodium acetate (pH 4.5). Using a Synergy HT spectrophotometer (BioTEK Instruments, Bad Friedrichshall, Stuttgart Germany), absorbance was read at 520 and 700 nm. The anthocyanin content was expressed as µg cyanidin-3-glucoside g^{-1}dw.

The extraction of soluble carbohydrates and starch was performed according to the protocol described by Carillo et al. [23]. Briefly, soluble sugars were quantified from ethanol extraction of microgreen samples, while starch content was determined from the pellet of ethanolic extract after hydrolysis to glucose, by an enzymatic assay coupled with pyridine nucleotide reduction. The increase in absorbance at 340 nm was recorded using an FLX-Xenius spectrophotometer (SAFAS, Munich City, Munich, Germany). Soluble sugars and starch were expressed as mg g^{-1} dw.

Soluble proteins were determined according to the protocol described by Ciriello et al. 2021 [9]. An aliquot of 0.2 g of sample was extracted in 1 mL of 200 mM Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl) (pH 7.5) containing 500 mM magnesium chloride ($MgCl_2$). The extract was centrifuged, the supernatant was taken, and the protein content was measured using protein assay dye reagent concentrate (Bio-Rad, Milan, Italy). The primary amino acids of microgreens were determined in ethanolic extracts (60% *v/v*) by high-performance liquid chromatography (HPLC) after precolumn derivatization with *o*-phthaldialdehyde (OPA) according to the method described by Woodrow et al. [24]. Soluble proteins and amino acids were expressed as mg g^{-1} dw and µmol g^{-1} dw, respectively.

2.8. Statistical Analysis

Experimental data were subjected to bifactorial analysis of variance (two-way ANOVA) (species of microgreens (S) × biostimulant (B)) using IBM SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA). The main effects of S and B were compared according to Student's *t*-test. For the S × B interaction, significant statistical differences were determined using the Duncan multiple range test at the level of $p < 0.05$. Principal component analysis (PCA) was performed using Minitab 18.1 statistical software (Minitab LLC, State College, PA, USA) according to Ciarmiello et al. [25].

3. Results

3.1. Yield and Color Parameters

The yield of carrot and dill microgreens was dictated by the species × biostimulant interaction, where carrot was not significantly influenced by the addition of the protein hydrolysates in the nutrient solutions, while the dill yield significantly increased by 13.5% (Figure 2A). Carrot and dill microgreens in a floating system were characterized by a yield of 768.6 and 814.7 g fw m^{-2} on average, respectively. A similar trend was noted for the microgreen dry biomass; only the dill dry weight significantly increased (13.5%), whereas carrot was not influenced by the PH presence (Figure 2B). As for DM%, an interaction of both factors was clear, since only the carrot DM% significantly increased with PH application (by 11.28%), while dill was steady at 11.95% (Figure 2C).

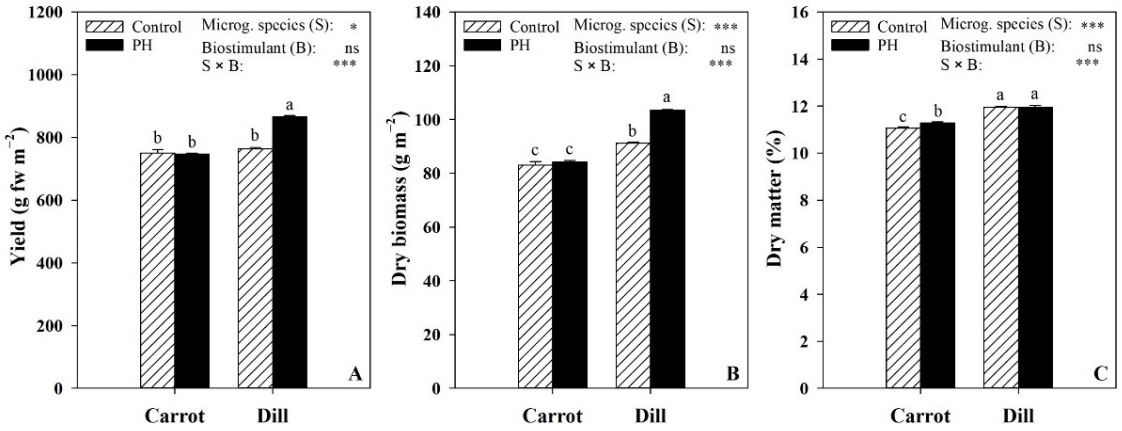

Figure 2. Fresh yield (**A**), dry biomass (**B**), and dry matter (**C**) of carrot and dill microgreens, as influenced by protein hydrolysate addition in the nutrient solution. Different letters above bars indicate significant mean differences according to Duncan's multiple range tests ($p = 0.05$). Vertical bars indicate ± SE (standard error) of means. ns, *, and *** indicate non-significant, or significant at $p \leq 0.05$ and 0.001, respectively.

All the color parameters of the carrot and dill microgreen canopies were influenced by the interaction of species × biostimulant. The brightness (L*) of the carrot canopy was not affected by the addition of the PH in the NS, whereas dill exhibited a decrease in L* (Table 1). The opposite trend was noted for the greenness parameter, where only carrot showed a darker green (+13.2%). On the other hand, carrot b* and chroma significantly increased with PH application, concomitantly with a decrease in the hue angle, whereas the opposite trend was noticed for the dill microgreen canopy (Table 1).

Table 1. Canopy colorimetric indices of carrot and dill microgreens as dictated by the application of a protein hydrolysate in the nutrient solution.

Treatments		L*	a*	b*	Chroma	Hue Angle
Microgreen species						
Carrot		33.90 ± 0.18	−10.75 ± 0.32	33.98 ± 1.72	35.64 ± 1.73	107.6 ± 0.40
Dill		37.88 ± 0.60	−8.39 ± 0.06	27.44 ± 0.52	28.70 ± 0.52	106.9 ± 0.26
Biostimulant						
Control		36.37 ± 1.18	−9.29 ± 0.37	29.38 ± 0.41	30.82 ± 0.50	107.4 ± 0.48
PH		35.41 ± 0.70	−9.84 ± 0.70	32.04 ± 2.58	33.52 ± 2.67	107.1 ± 0.19
Microgreen species	Biostimulant					
Carrot	Control	33.79 ± 0.13 c	−10.08 ± 0.23 b	30.18 ± 0.44 b	31.82 ± 0.49 b	108.5 ± 0.16 a
	PH	34.01 ± 0.36 c	−11.41 ± 0.15 c	37.78 ± 0.45 a	39.47 ± 0.43 a	106.8 ± 0.26 c
Dill	Control	38.94 ± 0.55 a	−8.49 ± 0.03 a	28.57 ± 0.08 c	29.82 ± 0.09 c	106.4 ± 0.21 c
	PH	36.82 ± 0.59 b	−8.28 ± 0.05 a	26.30 ± 0.27 d	27.58 ± 0.27 d	107.5 ± 0.09 b
Source of variance				(*p*-value)		
Microgreen species		***	***	**	**	ns
Biostimulant		ns	ns	ns	ns	ns
Microgreen species × Biostimulant		*	***	***	***	***

Non-significant (ns). *, **, and *** indicate significant at $p \leq 0.05$, 0.01, and 0.001, respectively. All data are expressed as mean ± SE (standard error), n = 3. Microgreen species and biostimulant means were compared by Student's *t*-test. Microgreen species and biostimulant interaction was compared by Duncan's multiple range test ($p = 0.05$). Different letters within each column indicate significant differences ($p = 0.05$). PH: protein hydrolysate.

3.2. Microgreen Pigments and Total Ascorbic Acid

All microgreen pigments and the related parameters were only dominated by the main effect of the PH application. Total chlorophylls and carotenoids incremented with the presence of the PH in the NS, by 43.9% and 16.8%, respectively (Table 2). In addition, the chlorophylls/carotenoids ratio also increased by 23.5% with the same treatment, whereas the chlorophyll a/b ratio decreased with the presence of the PH (−11.1%).

Table 2. Pigments (total chlorophylls and carotenoids), chlorophyll a/b ratio, chlorophylls/carotenoids ratio, anthocyanins, total ascorbic acid, and total phenols of carrot and dill microgreens as dictated by the application of a protein hydrolysate in the nutrient solution.

Treatments		Total Chlorophylls (mg g^{-1} fw)	Carotenoids (mg g^{-1} fw)	Chlorophyll a/b Ratio	Chlorophylls/Carotenoids Ratio	Anthocyanins (mg 100 g^{-1} fw)	Total Ascorbic Acid (mg AA 100 g^{-1} fw)	Total Phenols (mg gallic a. eq. 100 g^{-1} dw)
Microgreen species								
Carrot		1.146 ± 0.101	0.316 ± 0.011	1.94 ± 0.044	3.60 ± 0.20	10.35 ± 3.26	70.35 ± 3.81	4.11 ± 0.13
Dill		1.032 ± 0.083	0.316 ± 0.013	1.99 ± 0.059	3.24 ± 0.14	26.92 ± 2.31	137.8 ± 5.24	3.69 ± 0.19
Biostimulant								
Control		0.893 ± 0.036	0.291 ± 0.008	2.08 ± 0.022	3.06 ± 0.06	17.40 ± 6.41	102.3 ± 11.28	3.99 ± 0.08
PH		1.285 ± 0.042	0.340 ± 0.001	1.85 ± 0.010	3.78 ± 0.13	19.87 ± 1.27	105.9 ± 19.21	3.81 ± 0.25
Microgreen species	Biostimulant							
Carrot	Control	0.927 ± 0.041	0.292 ± 0.007	2.03 ± 0.015	3.18 ± 0.06	3.13 ± 0.65 d	77.68 ± 4.31 c	3.87 ± 0.14 b
	PH	1.365 ± 0.040	0.340 ± 0.002	1.84 ± 0.014	4.02 ± 0.12	17.58 ± 0.71 c	63.01 ± 0.57 d	4.35 ± 0.07 a
Dill	Control	0.859 ± 0.059	0.291 ± 0.017	2.12 ± 0.017	2.95 ± 0.04	31.67 ± 1.29 a	126.9 ± 3.48 b	4.10 ± 0.01 ab
	PH	1.205 ± 0.030	0.340 ± 0.001	1.86 ± 0.014	3.54 ± 0.10	22.16 ± 1.53 b	148.8 ± 2.34 a	3.27 ± 0.11 c
Source of variance					(p-value)			
Microgreen species		ns	ns	ns	ns	**	***	ns
Biostimulant		***	***	***	***	ns	ns	ns
Microgreen species × Biostimulant		ns	ns	ns	ns	***	***	***

Non-significant (ns). ** and *** indicate significant at $p \leq 0.01$ and 0.001, respectively. All data are expressed as mean ± SE (standard error), n = 3. Microgreen species and biostimulant means were compared by Student's *t*-test. Microgreen species and biostimulant interaction was compared by Duncan's multiple range test ($p = 0.05$). Different letters within each column indicate significant differences ($p = 0.05$). AA: ascorbic acid; gallic a. eq.: gallic acid equivalent; fw: fresh weight; dw: dry weight; PH: protein hydrolysate.

The total ascorbic acid content of the cultivated microgreens was influenced by the interaction of species × biostimulant. PH application in the NS caused a decrease of 18.9% in carrot microgreens compared to an increase of 17.3% in dill microgreens. On average, dill was characterized by a higher content of total ascorbic acid (2-fold) in comparison to carrot microgreens (70.35 mg AA 100 g^{-1} fw) (Table 2).

3.3. Microgreen Anthocyanins and Total Phenols

As listed in Table 2, the anthocyanin content incremented markedly by around 5.6-fold in carrot microgreens when the PH was applied, while it decreased by 30.0% in dill microgreens. On average, dill microgreens were 2.6-fold richer in anthocyanins in comparison to carrot. The variation in anthocyanins is in line with the total phenols in both microgreen species, where it increased by 12.4% in carrot and decreased by 20.2% in dill microgreens when the biostimulant was applied (Table 2).

3.4. Nitrate and Mineral Contents

Table 3 reports the nitrate and macronutrient contents of the cultivated microgreen species. Only nitrate, calcium, and magnesium were influenced by the interaction of the species and the biostimulant application. The carrot microgreen nitrate content was not influenced by the biostimulant application in the NS, whereas the dill nitrate content increased by 65.3% when the PH was added to the NS. Nonetheless, dill, on average, was characterized by a lower nitrate content (252.4 mg kg^{-1} fw) in comparison to carrot microgreens (602.7 mg kg^{-1} fw). As for calcium, it increased in both species in the presence of the PH, but in different percentages, with carrot registering 9.6% and dill 20.5%, whereas magnesium was modulated diversely in both species with the biostimulant application but not significantly different from the control treatment. On the other hand, phosphorous and sulfur were only dictated by the main effect of the biostimulant, where the former decreased when the PH was added, and the latter increased. Moreover, potassium and sodium contents were only dictated by the species main effect, where dill was rich in both minerals. In general, both microgreen species were high in K, followed by Ca and, ultimately, Mg, S, and P.

Table 3. Nitrate and mineral contents (P, K, Ca, Mg, S, and Na) of carrot and dill microgreens as dictated by the application of a protein hydrolysate in the nutrient solution.

Treatments		Nitrate (mg kg^{-1} fw)	P (mg g^{-1} dw)	K (mg g^{-1} dw)	Ca (mg g^{-1} dw)	Mg (mg g^{-1} dw)	S (mg g^{-1} dw)	Na (mg g^{-1} dw)
Microgreen species								
Carrot		602.7 ± 7.21	2.51 ± 0.10	10.18 ± 0.32	7.77 ± 0.16	2.58 ± 0.14	2.91 ± 0.25	0.50 ± 0.02
Dill		252.4 ± 28.12	2.70 ± 0.08	14.87 ± 0.36	7.33 ± 0.33	3.80 ± 0.21	2.28 ± 0.19	1.10 ± 0.03
Biostimulant								
Control		402.3 ± 94.99	2.80 ± 0.05	13.10 ± 1.11	7.03 ± 0.19	3.21 ± 0.43	2.12 ± 0.12	0.82 ± 0.13
PH		452.7 ± 61.97	2.41 ± 0.06	11.96 ± 1.03	8.06 ± 0.10	3.17 ± 0.18	3.07 ± 0.18	0.78 ± 0.14
Microgreen species	Biostimulant							
Carrot	Control	614.4 ± 10.85 a	2.72 ± 0.02	10.66 ± 0.45	7.41 ± 0.02 b	2.31 ± 0.03 c	2.37 ± 0.08	0.53 ± 0.02
	PH	591.0 ± 2.35 a	2.29 ± 0.02	9.71 ± 0.29	8.12 ± 0.08 a	2.85 ± 0.14 bc	3.45 ± 0.10	0.46 ± 0.01
Dill	Control	190.3 ± 4.58 c	2.87 ± 0.07	15.53 ± 0.25	6.65 ± 0.16 c	4.11 ± 0.30 a	1.88 ± 0.11	1.11 ± 0.04
	PH	314.5 ± 8.69 b	2.53 ± 0.04	14.22 ± 0.39	8.01 ± 0.21 a	3.48 ± 0.19 ab	2.69 ± 0.11	1.09 ± 0.04
Source of variance					(p-value)			
Microgreen species		***	ns	***	ns	***	ns	***
Biostimulant		ns	***	ns	***	ns	**	ns
Microgreen species × Biostimulant		***	ns	ns	*	*	ns	ns

Non-significant (ns). *, **, and *** indicate significant at $p \leq 0.05$, 0.01, and 0.001, respectively. All data are expressed as mean ± SE (standard error), n = 3. Microgreen species and biostimulant means were compared by Student's t-test. Microgreen species and biostimulant interaction was compared by Duncan's multiple range test ($p = 0.05$). Different letters within each column indicate significant differences ($p = 0.05$). fw: fresh weight; dw: dry weight; PH: protein hydrolysate.

3.5. Starch and Reduced Sugar Contents

As listed in Table 4, no interaction between both factors was registered for the sugar content. All the analyzed sugars were dictated by the main effect of the species, and no changes were noted when the biostimulant was applied. Dill microgreens were characterized by a significant higher starch (54.16 mg g^{-1} dw), fructose (36.79 mg g^{-1} dw), and sucrose (3.58 mg g^{-1} dw) content, whereas carrot was characterized by a significant higher content of glucose (118.8 mg g^{-1} dw).

Table 4. Starch and reduced sugars (glucose, fructose, and sucrose) of carrot and dill microgreens as dictated by the application of a protein hydrolysate in the nutrient solution.

Treatments		Starch (mg g^{-1} dw)	Glucose (mg g^{-1} dw)	Fructose (mg g^{-1} dw)	Sucrose (mg g^{-1} dw)
Microgreen species					
Carrot		31.97 ± 2.05	118.8 ± 1.76	21.96 ± 0.28	1.88 ± 0.08
Dill		54.16 ± 2.63	100.4 ± 1.35	36.79 ± 0.83	3.58 ± 0.13
Biostimulant					
Control		47.23 ± 5.23	106.6 ± 3.87	30.42 ± 3.60	2.58 ± 0.39
PH		38.90 ± 5.10	112.6 ± 4.48	28.33 ± 3.06	2.89 ± 0.39
Microgreen species	Biostimulant				
Carrot	Control	36.28 ± 0.95	115.2 ± 0.45	22.40 ± 0.34	1.75 ± 0.12
	PH	27.66 ± 1.27	122.4 ± 1.46	21.52 ± 0.28	2.02 ± 0.02
Dill	Control	58.18 ± 4.02	98.04 ± 1.14	38.44 ± 0.55	3.41 ± 0.22
	PH	50.15 ± 1.50	102.8 ± 1.44	35.13 ± 0.63	3.76 ± 0.10
Source of variance			(p-value)		
Microgreen species		***	***	***	***
Biostimulant		ns	ns	ns	ns
Microgreen species × Biostimulant		ns	ns	ns	ns

Non-significant (ns). *** indicates significant at $p \leq 0.001$. All data are expressed as mean ± SE (standard error), n = 3. Microgreen species and biostimulant means were compared by Student's t-test. Microgreen species and biostimulant interaction was compared by Duncan's multiple range test ($p = 0.05$). dw: dry weight; PH: protein hydrolysate.

3.6. Soluble Protein and Amino Acid Contents

The application of the PH in the NS had different effects on the diverse amino acids tested in the dill and carrot microgreens (Table 5). The arginine, asparagine, glutamine, glutamic acid, phenylalanine, and valine contents were influenced by the interaction of species × biostimulant. Some amino acids increased in one species when the PH was applied, while they did not change significantly in the other species, or they increased intensively in one of the species. For instance, asn increased in both species when the PH was applied (by 58.9% in carrot and 54.4% in dill), whereas gln increased only in dill microgreens (by 32.1%). Moreover, the essential amino acids and the branched-chain amino acids increased only in carrot microgreens with PH application, whereas they remained statistically unchanged in dill. On the other hand, soluble proteins and total amino acids were both influenced by the main effect of the biostimulant, both increasing by 20.6% and 18.5%, respectively, when the PH was added to the NS. In addition, carrot microgreens were characterized by a higher total amino acid content on average when compared to dill. Other amino acids were also significantly influenced by the biostimulant, such as isoleucine, lysine, proline, and threonine. Finally, aspartic acid, glycine, histidine, and leucine were only dictated by the species effect, being significantly higher in carrot microgreens.

Table 5. Soluble proteins (mg g^{-1} dw) and amino acids (μmol g^{-1} dw) of carrot and dill microgreens as dictated by the application of a protein hydrolysate in the nutrient solution.

Compounds	Microgreen Species			Biostimulant			Microgreen Species × Biostimulant				ANOVA
							Carrot		Dill		
	Carrot	Dill	*t*-Test	Control	PH	*t*-Test	Control	PH	Control	PH	
Soluble proteins	43.05 ± 2.35	43.24 ± 1.42	ns	39.11 ± 0.89	47.18 ± 0.51	***	38.01 ± 1.48	48.10 ± 0.24	40.21 ± 0.71	46.26 ± 0.65	ns
Ala	4.28 ± 0.16	3.50 ± 0.33	ns	3.81 ± 0.30	3.96 ± 0.32	ns	4.27 ± 0.10	4.28 ± 0.34	3.35 ± 0.47	3.65 ± 0.55	ns
Arg	1.80 ± 0.12	1.67 ± 0.07	ns	1.70 ± 0.07	1.77 ± 0.13	ns	1.59 ± 0.08 b	2.01 ± 0.14 a	1.82 ± 0.05 ab	1.53 ± 0.06 b	**
Asn	38.63 ± 3.97	23.74 ± 2.32	**	24.25 ± 2.59	38.11 ± 4.17	*	29.84 ± 1.18 b	47.41 ± 0.29 a	18.66 ± 0.91 c	28.81 ± 0.60 b	***
Asp	5.84 ± 0.48	3.66 ± 0.37	**	5.52 ± 0.64	3.97 ± 0.44	ns	6.78 ± 0.44	4.89 ± 0.27	4.27 ± 0.50	3.05 ± 0.23	ns
GABA	14.34 ± 0.38	15.24 ± 0.47	ns	14.40 ± 0.43	15.19 ± 0.44	ns	13.83 ± 0.61	14.86 ± 0.28	14.96 ± 0.46	15.52 ± 0.89	ns
Gln	27.95 ± 0.55	31.10 ± 2.23	ns	27.83 ± 0.60	31.22 ± 2.18	ns	28.86 ± 0.36 b	27.04 ± 0.74 b	26.80 ± 0.78 b	35.40 ± 2.39 a	*
Glu	4.56 ± 0.33	1.26 ± 0.12	***	3.18 ± 0.91	2.64 ± 0.60	ns	5.16 ± 0.40 a	3.96 ± 0.16 b	1.20 ± 0.19 c	1.32 ± 0.19 c	*
Gly	1.99 ± 0.11	1.27 ± 0.11	***	1.66 ± 0.16	1.60 ± 0.23	ns	1.88 ± 0.20	2.09 ± 0.11	1.43 ± 0.17	1.11 ± 0.07	ns
His	1.49 ± 0.06	0.87 ± 0.03	***	1.17 ± 0.13	1.20 ± 0.17	ns	1.44 ± 0.03	1.54 ± 0.13	0.89 ± 0.05	0.85 ± 0.04	ns
Ile	2.18 ± 0.19	1.78 ± 0.15	ns	1.62 ± 0.09	2.34 ± 0.13	***	1.81 ± 0.07	2.55 ± 0.20	1.43 ± 0.03	2.12 ± 0.03	ns
Leu	1.66 ± 0.07	1.30 ± 0.10	*	1.33 ± 0.08	1.63 ± 0.12	ns	1.50 ± 0.02	1.82 ± 0.03	1.15 ± 0.00	1.44 ± 0.18	ns
Lys	0.529 ± 0.037	0.597 ± 0.045	ns	0.499 ± 0.021	0.627 ± 0.043	*	0.453 ± 0.003	0.605 ± 0.035	0.544 ± 0.012	0.649 ± 0.086	ns
MEA	2.62 ± 0.13	2.47 ± 0.26	ns	2.55 ± 0.23	2.54 ± 0.19	ns	2.69 ± 0.19	2.55 ± 0.21	2.40 ± 0.45	2.53 ± 0.36	ns
Met	0.254 ± 0.010	0.241 ± 0.016	ns	0.262 ± 0.007	0.233 ± 0.016	ns	0.261 ± 0.015	0.248 ± 0.016	0.262 ± 0.003	0.219 ± 0.030	ns
Orn	1.10 ± 0.05	0.85 ± 0.15	ns	1.12 ± 0.11	0.83 ± 0.11	ns	1.13 ± 0.06	1.06 ± 0.08	1.10 ± 0.23	0.60 ± 0.05	ns
Phe	0.89 ± 0.08	0.52 ± 0.04	**	0.60 ± 0.06	0.81 ± 0.11	ns	0.72 ± 0.05 b	1.06 ± 0.02 a	0.47 ± 0.01 c	0.57 ± 0.06 c	*
Pro	3.33 ± 0.27	3.79 ± 0.33	ns	3.06 ± 0.16	4.05 ± 0.28	*	2.88 ± 0.11	3.79 ± 0.39	3.25 ± 0.29	4.32 ± 0.41	ns
Ser	4.54 ± 0.32	3.43 ± 0.38	*	4.05 ± 0.52	3.93 ± 0.31	ns	5.10 ± 0.30	3.99 ± 0.33	3.00 ± 0.39	3.86 ± 0.61	ns
Thr	0.836 ± 0.08	0.647 ± 0.08	ns	0.590 ± 0.05	0.893 ± 0.06	**	0.699 ± 0.02	0.974 ± 0.11	0.481 ± 0.02	0.813 ± 0.01	ns
Trp	0.557 ± 0.04	0.571 ± 0.04	ns	0.528 ± 0.02	0.599 ± 0.04	ns	0.499 ± 0.03	0.614 ± 0.06	0.557 ± 0.02	0.584 ± 0.08	ns
Tyr	2.04 ± 0.11	1.81 ± 0.21	ns	1.76 ± 0.15	2.09 ± 0.17	ns	1.96 ± 0.17	2.11 ± 0.17	1.55 ± 0.21	2.07 ± 0.34	ns
Val	3.72 ± 0.34	2.99 ± 0.15	ns	3.10 ± 0.08	3.61 ± 0.40	ns	2.96 ± 0.08 bc	4.48 ± 0.03 a	3.23 ± 0.08 b	2.75 ± 0.23 c	***
Essential AA	13.92 ± 0.91	11.18 ± 0.21	*	11.39 ± 0.31	13.71 ± 1.00	*	11.94 ± 0.36 b	15.90 ± 0.33 a	10.84 ± 0.22 c	11.52 ± 0.23 bc	**
BCAAs	7.56 ± 0.59	6.06 ± 0.13	*	6.04 ± 0.13	7.58 ± 0.58	*	6.27 ± 0.16 b	8.85 ± 0.23 a	5.82 ± 0.11 b	6.31 ± 0.11 b	***
Total AA	125.1 ± 4.14	103.3 ± 4.82	**	104.6 ± 5.50	123.9 ± 4.54	*	116.3 ± 2.70	133.9 ± 0.72	92.82 ± 2.44	113.8 ± 0.77	ns

Non-significant (ns). *, **, and *** indicate significant at $p \leq 0.05$, 0.01, and 0.001, respectively. All data are expressed as mean ± SE (standard error), n = 3. Microgreen species and biostimulant means were compared by Student's *t*-test. Microgreen species and biostimulant interaction was compared by Duncan's multiple range test ($p = 0.05$). Different letters within each column indicate significant differences ($p = 0.05$). dw: dry weight; PH: protein hydrolysate.

3.7. Principal Component Analysis (PCA)

A principal component analysis was performed on all analyzed dill and carrot microgreens data in relation to the biostimulant vs. control treatment, and the loading plot and scores are reported in Figure 3. The variables in the first three principal components (PCs) were highly correlated, with eigenvalues greater than 1, thus explaining 100% of the total variance, with PC1, PC2, and PC3 accounting for 64.8%, 20.5%, and 14.7%, respectively. PC1 was positively correlated with glucose, nitrate, asn, total amino acids, essential amino acids, b*, BCAAs, and glu, while it was negatively correlated with starch, a*, K, Na, fructose, TAA, and L*. Moreover, PC2 was positively correlated with carotenoids, total chlorophylls, Ca, yield, and gln, while it was negatively correlated with P and TP. The dill and carrot microgreen cultivars under different treatments were well separated and uniformly clustered with respect to PC1 and PC2. In fact, both the species and treatment factors examined in this study were relevant in the PCA clustering along PC1 and PC2, respectively. In particular, carrot microgreen treatments were distributed on the positive side of PC1, in the upper and lower right quadrants, while dill microgreen treatments were distributed on the negative side of PC1, in the upper and lower left quadrants. Moreover, the control treatment was distributed on the negative side of PC2, while the PH treatments were on the positive side of PC2 (Figure 3). Interestingly, the dill microgreens under PH treatment showed the highest yield and dry biomass, whereas the carrot microgreens were correlated with total amino acids, asn, BCAAs, and essential amino acids (Figure 3).

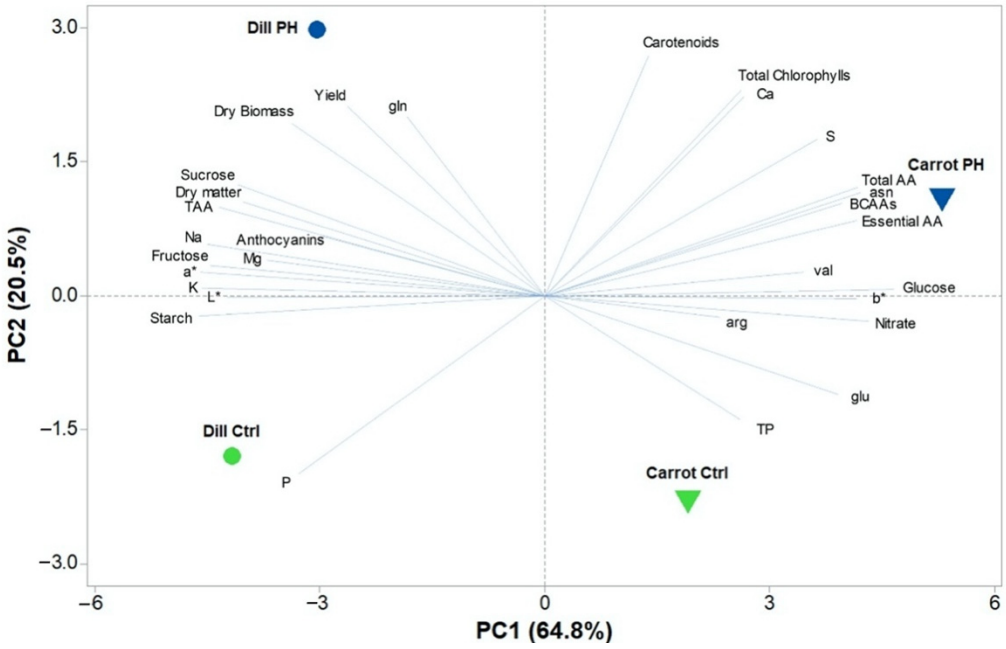

Figure 3. Principal component loading plot and scores of principal component analysis (PCA) of biometric traits, leaf colorimetry, minerals, carbohydrates, amino acids, and bioactive compounds of carrot and dill microgreens, as influenced by protein hydrolysate addition in the nutrient solution.

4. Discussion

The aim of our work was to assess the feasibility of growing microgreens in a soilless system without a substrate (floating system) by discriminating the effect of a protein hydrolysate-based biostimulant added to the nutrient solution.

In this study, regardless of the biostimulant application, the fresh production of carrot (748.5 g fw m^{-2}) and dill (814.7 g fw m^{-2}) microgreens was inconsistent with the results of the most common and used species planted in coco peat and peat-based substrates [26,27]. Ghoora et al. [26] evaluated the production performance of ten microgreen species characterized by a wide range of production (1.12–4.93 kg fw m^{-2}), indicating that the genetic aspect is a strong discriminator for fresh production. In the above study, the minor pro-

ductive species was fennel (*Foeniculum vulgare* L.), belonging to the Apiaceae family. Our research confirms the strong family-dependent productive response for microgreens. Coriander that was assessed in diverse soilless substrates exhibited a similar fresh weight in capillary mat conditions (800 g fw m^{-2}) [6], when compared to carrot and dill from the same botanical family. In the same study by Kyriacou et al. [6], all the tested species manifested around 2.2-fold more fresh weight when a peat-based substrate was adopted compared to the other soilless substrates (capillary mat, coconut fiber, Agave fiber, and cellulose sponge). Furthermore, it should be noted that the production values reported by Ghoora et al. [26] and Kyriacou et al. [27] refer to microgreens harvested at the two true leaf stage, as opposed to our study in which the harvest was carried out at the one true leaf stage. However, the lower yields obtained cannot be solely attributed to the above conditions but also to the different growth conditions under which the microgreens were grown. In fact, all studies reported in the literature evaluated the productivity of microgreens under controlled conditions (growth chambers). In contrast, the greenhouse system used in our study cannot provide stable conditions of temperature, humidity, light intensity, and quality that certainly affect productivity [1,28–30]. The variable and inconstant environmental conditions that occur in the greenhouse could be the key to explaining the high percentage of dry matter compared to the results reported by Kyriacou et al. [27] on 13 different species of microgreens, and by El-Nakhel et al. [31] on parsley (*Petroselinum crispum* (Mill.) Fuss.). Although the achievement of a high fresh yield is a crucial factor for growing microgreens, the absence of a substrate (as in our work) is also an advantage since the substrates ordinarily used are expensive and nonrenewable [8].

Under suboptimal growth conditions, the imperative to maximize production has driven the horticultural industry towards the use of biostimulants [32]. However, there have not been studies in the literature that evaluated the effectiveness of biostimulants in the production and quality performance of microgreens. Our results show that the use of a protein hydrolysate-based biostimulant (Trainer®®) in the nutrient solution increased both the fresh yield and the shoot dry weight in dill (Figure 2A,B). This increase in yield can probably be attributed to the large number of positive effects caused by the bioactive molecules in Trainer®® and not to simple nitrogen and carbon supplementation [13,14,33]. Specifically, this improvement could be related to the presence of peptides eliciting hormone-like signals capable of modulating plant growth and development [34]. The different responses of carrot production, which did not benefit from the application of the biostimulant, highlight that, even in microgreens, the response to a biostimulant is strongly species-dependent [35]. However, it should be considered that species' sensitivity to biostimulant activity is also a function of the mode and application dose [35].

The use of the biostimulant led to a change in colorimetric parameters in both tested species (Table 1), changing the perception of color (chroma), a key aspect for the acceptability and marketability of microgreens [36]. The increase in chroma in carrot and its decrease in dill due to biostimulant application could be related to the change in the anthocyanin content, which showed the same trend as chroma (Table 3). In addition to their role in plant coloration, anthocyanins bring benefits to human health by reducing the risk of chronic diseases, as documented in several clinical studies [37,38].

Interestingly, the biostimulant application in carrot increased total anthocyanins and phenols (Table 2) but did not lead to any increase in yield. It seems that the biostimulant specifically activated the secondary metabolism in carrot, improving its nutraceutical characteristics. In contrast, in dill, the use of Trainer®® promoted the biosynthesis of ascorbic acid, an essential micronutrient for the human body with a strong antioxidant power, and an enhancer of the most effective absorption of nonheme iron [39,40]. The biostimulant application in dill resulted in a higher accumulation of ascorbic acid (148.8 mg 100 g^{-1} fw) than that recorded by Xiao et al. [41] in 25 species of microgreens. Independent of the biostimulatory effect, the ascorbic acid content of dill and carrot was 62% and 1092% higher than their mature edible counterparts, respectively [42]. These results confirm that microgreens provide a source of ascorbic acid at the same level as citrus fruits, potentially

falling under foods with "high vitamin C" according to Annex II of the 2006 EU Regulation 1924/2006 [26,43]. Another key qualitative parameter that defines the healthiness of food is the nitrate content. The recognized detrimental effects on human health combined with the awareness that approximately 80% of total nitrate intake is due to fresh vegetable consumption further emphasize the relevance of this antinutritional compound content [44,45]. As with the mature counterparts, the nitrate levels of microgreens vary greatly among different species, as observed by Kyriacou et al. [27] and Bulgari et al. [8].

Regardless of the application of a biostimulant, carrot (602.7 mg kg^{-1} fw) and dill (252.4 mg kg^{-1} fw) accumulated much lower nitrates than those reported in the literature. Although data on nitrate content are currently still limited [46], especially for species belonging to the Apiaceae family, such low values could be attributable to the different growing system (floating system) that does not involve the use of a substrate, as well as the uncontrolled growth conditions compared to the ones used in comparable experiments [8,27,47]. For dill, the application of the biostimulant increased the nitrate content, but this value (314.5 mg kg^{-1} fw) was approximately half of the lowest value (687.4 mg kg^{-1} fw) recorded by Bulgari et al. [8], emphasizing that food security for this species of microgreen is not undermined at all. The increase in the nitrate content as a result of the application of the biostimulant that was observed only in dill could contribute to a better understanding of the relative increase in yield, which can be related to a probable remodulation of root growth that would have improved mineral uptake and thus nutrient acquisition [48,49].

Indeed, the biostimulant improved the uptake of nitrate, and this was evident in dill in which its content was highly increased, while in carrot, the nitrate levels were already high in the control and remained stable after PH treatment. However, the reductive assimilation of nitrate to ammonia and its incorporation into amino acids were also improved by the biostimulant given the strong increase in asn and proteins in the treated plants. The fact that the content of asparagine in carrot after treatment reached more than a third of the total free amino acid content could be a symptom of excess accumulation of ammonium. When this ion concentration becomes very high in cytosolic compartments because it is not promptly incorporated in amino acids, it has the ability to cross membranes in the neutral form, previously reacting with OH^- ($NH_4^+ + OH^- \rightarrow NH_3 + H_2O$), and then reconverting to NH_4^+ after a reaction with protons (H^+). This determines a strong dissipation of the membrane potential and proton motive force, impairing membrane transport and function [24,50]. Therefore, the synthesis of asn, the amide with the highest N-to-C ratio, may play a role in ammonia detoxification when the uptake of nitrate and its conversion to ammonium exceed the cellular needs, but because it is energetically expensive, this may affect the capacity of the biostimulant to improve growth in carrot.

The biostimulant root application improved the accumulation of calcium in both species, a mineral critical to human skeletal health, since insufficient intake increases the risk of osteoporosis in older age [51]. In agreement with the report of de la Fuente et al. [52], the most abundant element found on average in the two microgreens was potassium, followed by calcium, magnesium, phosphorus, and sulfur, minerals essential in the human diet for their recognized homeostatic and metabolic functions [53]. Although the influence of genotype on mineral concentrations in microgreens is more than established, the lower potassium values found in our experiment are more than established compared to what was recorded by Kyriacou et al. [27], which could help explain the differences in production, considering the critical physiological role played by potassium [54].

Based on these results, lower potassium concentrations would allow microgreen species grown under these specific conditions to be labeled with the nutritional claim of "reduced K" [43] and therefore recommended for patients with impaired kidney function [55]. Furthermore, the low sodium content recorded, especially in carrot (0.50 mg g^{-1}dw), is another critical nutritional aspect, as low-sodium foods reduce the incidence of hypertension and stroke [56]. In most microgreens, the content of starch, as well as soluble sugars (i.e., sucrose, fructose, and glucose), is generally low [8], especially compared to

the typical values of counterpart mature vegetables [57]. In any case, the differences in the carbohydrate profile of the two species of microgreens tested in our work confirm what was previously observed by Paradiso et al. [58], who compared the carbohydrate content of six different genotypes of microgreens. Taking into account the importance of sugars, especially in post-harvest (shelf life), as essential compounds for the maintenance of cellular metabolism, the higher levels of glucose, fructose, and sucrose compared to the average results obtained by Xiao et al. [59] could confer a better shelf life.

5. Conclusions

The remodeling of the nutritional architecture of plant-based foods is a valuable resource in which new categories of functional foods (microgreens) take the lead. In recent decades, the interest in microgreens has increased due to their outstanding nutritional properties. The results achieved prove the feasibility of producing microgreens in soilless systems without any substrate, reducing the waste of nonrenewable resources and the overall cost. The two tested species (dill and carrot) belonging to the Apiaceae family stood out positively for their low nitrate content (average 427.5 mg kg^{-1} fresh weight). The use of a protein hydrolysate (Trainer®®) in the nutrient solution led to an increase in anthocyanins (+461.7%) and total phenols (+12.4%) in carrot, while in dill, the fresh yield (+13.5%) and ascorbic acid (+17.2%) increased. In both species, Trainer®® increased soluble proteins and total free amino acids by 20.6% and 18.5%, respectively. In light of the encouraging results achieved using our cultivation system, future research should also investigate the yield and nutritional parameter responses of other microgreen species with the aim of large-scale sustainable production, in addition to depicting the adequate application dose for each species.

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