agronomy

MDPI

Article

Comparing Efficacy of Different Biostimulants for Hydroponically Grown Lettuce (*Lactuca sativa* L.)

Zheng Wang [1], Rui Yang [1,*], Ying Liang [1,2], Shulan Zhang [3], Zejin Zhang [2], Chaohua Sun [4], Jing Li [5], Zhiyong Qi [1] and Qichang Yang [1,*]

[1] Institute of Urban Agriculture, Chinese Academy of Agricultural Sciences (IUA-CAAS), Chengdu National Agricultural Science and Technology Center (NASC), Chengdu 610213, China; wangzheng@caas.cn (Z.W.); liangashuang@outlook.com (Y.L.); qizhiyong@caas.cn (Z.Q.)
[2] Vegetable Germplasm Innovation and Variety Improvement Key Laboratory of Sichuan Province, Horticulture Research Institute, Sichuan Academy of Agricultural Sciences, Chengdu 610066, China; zhangzj127@163.com
[3] Department of Resources and Environmental Sciences, College of Natural Resources and Environment, Northwest A & F University, Xi'an 712100, China; zhangshulan@nwafu.edu.cn
[4] Boe Houji Technology (Beijing) Co., Beijing 100144, China; sunchaohua@boebrain.cn
[5] Leading Bio-Agricultural Co., Ltd., Qinhuangdao 066004, China; li.jing@leadst.cn
* Correspondence: yangrui01@caas.cn (R.Y.); yangqichang@caas.cn (Q.Y.)

Abstract: Biostimulants can enhance horticultural crop production. However, their application in hydroponically grown lettuce is still limited, and information regarding their relative efficacy is lacking. A greenhouse trial was conducted to address this issue. Five nutrient solution treatments were evaluated on two lettuce cultivars: butterhead and red oak-leaf. The treatments included a half-strength modified Hoagland solution (Hs-H); a full-strength modified Hoagland solution (Fs-H); and Hs-H supplemented with 50 mg L^{-1} fulvic acid (FA), 334 mg L^{-1} seaweed extract (SE), or 5 mL L^{-1} gamma polyglutamic acid (PGA). The results indicated that the shoot biomass observed after biostimulant supplementation was significantly greater than or comparable to that observed with Fs-H. Nutrient solutions supplemented with SE and PGA led to a greater increase in the root biomass than that realized with Hs-H and Fs-H treatments. The Hs-H + FA treatment resulted in the lowest root-to-shoot ratio on a fresh weight basis among all treatments. The nitrate concentration in the shoot was significantly reduced following biostimulant supplementation compared to that realized with Fs-H and Hs-H treatments. Nutrient solutions supplemented with SE and PGA also decreased soluble sugar concentrations compared to that achieved using Hs-H and Fs-H treatments. FA and SE improved nutrient uptake for both cultivars, but PGA had a minimal effect on nutrient uptake. The two cultivars varied in their responses to biostimulant supplementation with regard to biomass, quality traits, and nutrient uptake. This study supports using fulvic acid and seaweed extract, rather than γ-PGA, in hydroponic lettuce production systems.

Keywords: biostimulants; seaweed extract; fulvic acid; γ-PGA; biomass; quality; nutrient uptake

Citation: Wang, Z.; Yang, R.; Liang, Y.; Zhang, S.; Zhang, Z.; Sun, C.; Li, J.; Qi, Z.; Yang, Q. Comparing Efficacy of Different Biostimulants for Hydroponically Grown Lettuce (*Lactuca sativa* L.). *Agronomy* **2022**, *12*, 786. https://doi.org/10.3390/agronomy12040786

Academic Editors: Andrea Ertani and Michela Schiavon

Received: 22 February 2022
Accepted: 21 March 2022
Published: 24 March 2022

1. Introduction

The global population is expected to grow in the next three decades and reach 9.7 billion by 2050 [1]. It will be challenging to manage resources, reduce environmental pollution, and satisfy the growing demand for food under ever-changing climate conditions [2,3]. Increasing productivity through controlled environment agriculture (CEA) is widely considered a sustainable approach to feed the entire world by 2050. Hydroponics is a common cultivation practice for CEA, offering greater yield and higher-quality products while maintaining a lower environmental impact. Generally, hydroponic systems use water and nutrients more efficiently than traditional open-field production systems [4,5].

Among the factors determining crop yield and quality in hydroponic systems, a proper understanding of nutrient solution management is critical. To date, numerous nutrient

solution formulas for hydroponic production systems have been designed, and most of them contain only inorganic ions from soluble salts of elements essential for higher plants [6]. However, it has been reported that the concentration of currently used nutrient solutions can be reduced by up to 50% without adverse effects on biomass and quality of floral crops, such as gerbera [7] and anthurium [8], as well as horticultural crops, such as tomato [9] and zucchini squash [10]. Such results indicate inefficient nutrient use in hydroponic production, possibly because the nutrient composition for the best plant growth and nutrient uptake by plants differs [11,12]. Balancing supply and demand may decrease production costs and reduce the risk of water pollution; however, the best plant growth may not be achieved if a nutrient solution based on the nutrient uptake is used [13]. One way to reduce nutrient use without damaging crop productivity in hydroponic production is to enhance crop uptake of nutrients by using biostimulants [14,15].

Plant biostimulants are "substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality" [16]; the most researched plant biostimulants are humic-like substances, seaweed extracts, and protein hydrolysates [15,17–19]. In terms of application, most plant biostimulants are foliar sprays or applied directly to the soil [20,21]. In contrast, Vernieri et al. [22] considered the combination of hydroponics and biostimulants as a promising environment-friendly production strategy for vegetables grown in greenhouses. However, studies regarding the direct addition of biostimulants to hydroponic solutions are still limited, and their effects on crop production still need verification.

Different biostimulants play different roles in plant growth [23–26]. For example, fulvic acid (FA), the main constituent of humic-like substances with small molecular size [27], can improve root growth by increasing the number and length of lateral roots [28]; shoot weight; and uptake of N, P, K, Ca, Mg, Cu, Fe, and Zn [15]. Seaweed extract (SE) is highly nutritious in terms of essential macro and micronutrients for higher plants and growth-promoting hormones [18]. Gamma poly glutamic acid (γ-PGA) was reported to enhance nutrient uptake, promote nitrogen utilization and metabolism, and alleviate abiotic stress [29–31].

As the most important leafy vegetable and the most consumed salad vegetable worldwide, lettuce is rich in health-promoting compounds [32,33]. The total growing area of lettuce (*Lactuca sativa* L.) is 186 million hectares globally [34]. China has the largest lettuce production worldwide and annual consumption of 15.96 million tons [34]. Hydroponic cultivation is widely used for lettuce production, especially at agricultural facilities. Typical hydroponic nutrient solutions have been modified from the classic Hoagland nutrient solution, which has a high electrical conductivity (EC) [35]. Several studies have modified the composition of the Hoagland nutrient solution for leafy vegetables [36–38], but there is potential for improved lettuce yield and quality [39]. Previous studies have reported positive effects on leafy vegetable growth, nutrient absorption, and qualities following the application of fulvic acid, seaweed extract, and γ-PGA [40]. Despite this, studies directly comparing the effectiveness of various types of biostimulants on hydroponically grown lettuce are limited and warrant further investigation. To address this issue, this study assesses the effect of a traditional hydroponic nutrient solution combined with supplementary biostimulants on the yield, quality, leaf pigment, and nutrient uptake in butterhead and red oak-leaf lettuce.

2. Materials and Methods

2.1. Experimental Design and Growth Conditions

A greenhouse trial was conducted at the Chengdu Academy of Agricultural and Forestry Sciences in Chengdu, Sichuan, China (103.9° N, 30.7° E). The experiment had a split-plot design with three replicates, and both the main plots and subplots were randomly arranged. Two lettuce (*Lactuca sativa* L.) cultivars, butterhead and red oak-leaf, were evaluated as the main-plot factors due to their popularity among growers. The sub-plot factors included five treatments of nutrient solutions: a half-strength modified Hoagland solu-

tion (Hs-H), which also served as the negative control treatment; a full-strength modified Hoagland solution (Fs-H), which also served as the positive control treatment; Hs-H with 50 mg L^{-1} fulvic acid (FA); Hs-H with 334 mg L^{-1} seaweed extract (SE); and Hs-H with 5 mL L^{-1} γ-PGA (PGA). The Fs-H treatment was prepared following the formula presented by Hoagland and Arnon [35]. The nutrient composition of the Hs-H treatment was 4 mM KNO_3, 0.8 mM KH_2PO_4, 0.3 mM K_2HPO_4, 1.5 mM $MgSO_4$, 3 mM $Ca(NO_3)_2$, 0.08 mM Fe-Na EDTA, 60 µM H_3BO_3, 3 µM $ZnSO_4$, 20 µM $MnSO_4$, 0.4 µM $CuSO_4$, and 0.03 µM $(NH_4)_6Mo_7O_{24}$. All biostimulants evaluated in this study were commercially available. The fulvic acid was extracted from leonardite (Zhongnongrunze Biotech Co., Ltd., Chengdu, China), the seaweed extract was derived from *Sargassum* spp. using alkaline extraction, and the γ-PGA was produced by fermentation using proprietary microbial strains and substrates (Leading Bio-agricultural Co., Ltd., Qinhuangdao, China). The nutrient concentrations of the evaluated biostimulants are listed in Table 1. The description, EC, and original pH of all nutrient solution treatments are presented in Table 2. The evaluated biostimulants were added directly to the nutrient solutions. The application rate was extrapolated from the manufacturer's instructions for foliar spray and soil application, as no recommended rate for a hydroponic production system has been defined. To eliminate the effect of different pH values among treatments, the pH of each nutrient solution treatment was adjusted weekly and maintained at pH 5.8 during the entire growing period.

Table 1. Nutrient concentration in evaluated biostimulants.

Biostimulant	C (%)	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	S (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
FA	29.21	0.71	200.34	60,198.85	31,793.12	1054.90	1111.34	10,174.43	83.08	17.78	23.19
SE	27.26	0.86	64.50	83,309.58	35,666.93	242.19	425.13	1556.07	22.99	1.39	6.34
PGA	3.58	1.28	616.10	6137.83	1.88	7.88	105.56	7.60	0.34	0.23	0.49

Table 2. Description, electrical conductivity (EC), and original pH of nutrient solution treatments.

Treatment	Description	EC (mS cm^{-1})	Original pH
Hs-H	Half-strength Hoagland solution	1.28	6.46
Fs-H	Full-strength Hoagland solution	2.74	5.94
FA	Hs-H + 50 mg/L fluvic acid	1.79	6.02
SE	Hs-H + 334 mg/L seaweed extract	1.88	6.92
PGA	Hs-H + 5 ml/L gamma polyglutamic acid	1.96	6.74

Note: To eliminate the effect of the original pH, the pH of each treatment was adjusted weekly and maintained at pH 5.8 throughout the entire growing period.

Seeds of the two lettuce cultivars were germinated on a sponge in deionized (DI) water in a growth chamber with day/night temperatures of 24/22 °C and relative humidity of 60%. Seeds were germinated in darkness for three days before subsequent cultivation under LED lights with a photosynthetic photon flux density of 150 µmol m^{-2} s^{-1} and a photoperiod of 16 h (06:00–22:00). Upon unfolding of the first pair of true leaves (i.e., 10 day after sowing), uniform seedlings were transferred to a deep-floating hydroponic system in the greenhouse and grown for another 45 day under natural sunlight and temperature. The individual plot size was 5 m^2, and the spacing between plants was 0.2 m. The daily maximum and minimum temperatures and daily light integral in the greenhouse are illustrated in Figure S1.

2.2. Harvest and Sample Analysis

Upon harvest, the chlorophyll, flavonoid, and anthocyanin in the second pair of fully expanded leaves were measured in 24 uniform plants within each subplot using a Dualex 4 Scientific optical leaf-clip meter (FORCE-A, Orsay, France). Three plants from each sub-plot were randomly collected, thoroughly mixed to represent a composite sample, and stored in a −80 °C freezer to determine crop quality. Nitrate, soluble sugar, and soluble protein were

analyzed using commercial testing kits that utilized classic and widely used colorimetric methods (Suzhou Grace Biotechnology Co., Ltd., Suzhou, China). For nitrate determination, 0.1 g of fresh leaves was homogenized in 1 mL deionized water, extracted in a water bath at 100 °C for 30 min, cooled to room temperature, and centrifuged at 12,000× *g* for 15 min. Then, 10 µL of the supernatant was thoroughly mixed with 40 µL of 5% salicylic acid solution in concentrated sulfuric acid and 950 µL of 8% sodium hydroxide solution. The absorbance was measured at 410 nm (http://www.geruisi-bio.com/pro/proshow/id/268, (accessed on 20 March 2022) [41]. For soluble sugar determination, fresh leaves (0.1 g) were homogenized in 1.5 mL of 80% ethanol in an ice bath, extracted in a 50 °C water bath for 20 min, and centrifuged at 12,000× *g* for 15 min. Then, 100 µL of the supernatant was thoroughly mixed with 300 µL DI water, 120 µL anthrone reagent, and 1 mL concentrated sulfuric acid and incubated in a 95 °C water bath for 10 min. After cooling to room temperature, the absorbance was measured at 620 nm (http://www.geruisi-bio.com/pro/proshow/id/431, (accessed on 20 March 2022) [42]. For soluble protein determination, 0.1 g of fresh leaves were homogenized in 1 mL 0.1 M phosphatic buffer solution (pH = 6) in an ice bath and centrifuged at 12,000× *g* for 15 min at 4 °C. The supernatant (160 µL) was thoroughly mixed with 800 µL Coomassie Brilliant Blue G250 solution, and the absorbance was measured at 600 nm (http://www.geruisi-bio.com/pro/proshow/id/247, (accessed on 20 March 2022) [43].

Thirty plants from each sub-plot were harvested to determine the fresh weight of the shoots and roots. The shoot and root samples were dried in an air-forced oven at 75 °C for 48 h and weighed. Dried shoot samples were ground into a fine powder using a ball mill and digested with concentrated sulfuric acid and 30% hydrogen peroxide. Nitrogen was determined using the Kjeldahl method, and phosphorus was determined using the molybdenum blue colorimetric method [44]. Sulfur, calcium, magnesium, potassium, copper, manganese, iron, and zinc were analyzed using an Agilent 7700e inductively coupled plasma mass spectrometer (ICP-MS; Agilent Technologies, Inc., Santa Clara, CA, USA). Nutrient content was calculated by multiplying nutrient concentration with dry biomass.

2.3. Data Analysis

A mixed-model methodology was used to analyze the data. Fixed effects included nutrient solution treatment and lettuce cultivar, while random effects included the replicate and replicate × cultivar. Statistical analysis was performed using SAS software (version 9.4; SAS Institute, Cary, NC, USA). To test for differences among fixed effects and their interactions, *p*-values were generated using Tukey's honestly significant difference method as implemented by the PROC GLIMMIX procedure. Principal component analysis (PCA) and heatmap analysis were performed using GraphPad Prism 9 software (GraphPad Software, San Diego, CA, USA).

3. Results

Significant treatment × cultivar interactions ($p < 0.05$) were observed for most of the biomass, quality, and nutrient uptake traits and were thus demonstrated separately by using nutrient solution treatment and cultivar in the following analyses.

3.1. Biomass

Shoot biomass of both cultivars responded to the nutrient solution treatment (Figure 1A,B). The positive control treatment (i.e., Fs-H) significantly increased shoot biomass relative to the negative control treatment (i.e., Hs-H); however, the shoot dry weight of butterhead decreased relatively (Figure 1C,D). The Hs-H and Fs-H treatments did not lead to significantly different root fresh weights (Figure 1E), but the Fs-H treatment led to a significantly higher root dry weight (Figure 1F). The Hs-H and Fs-H treatments afforded similar root-to-shoot (R/S) ratios based on the fresh weight (Figure 1G). However, on a dry weight basis, the Fs-H treatment notably improved the R/S ratio for butterhead but decreased it for red oak-leaf (Figure 1H).

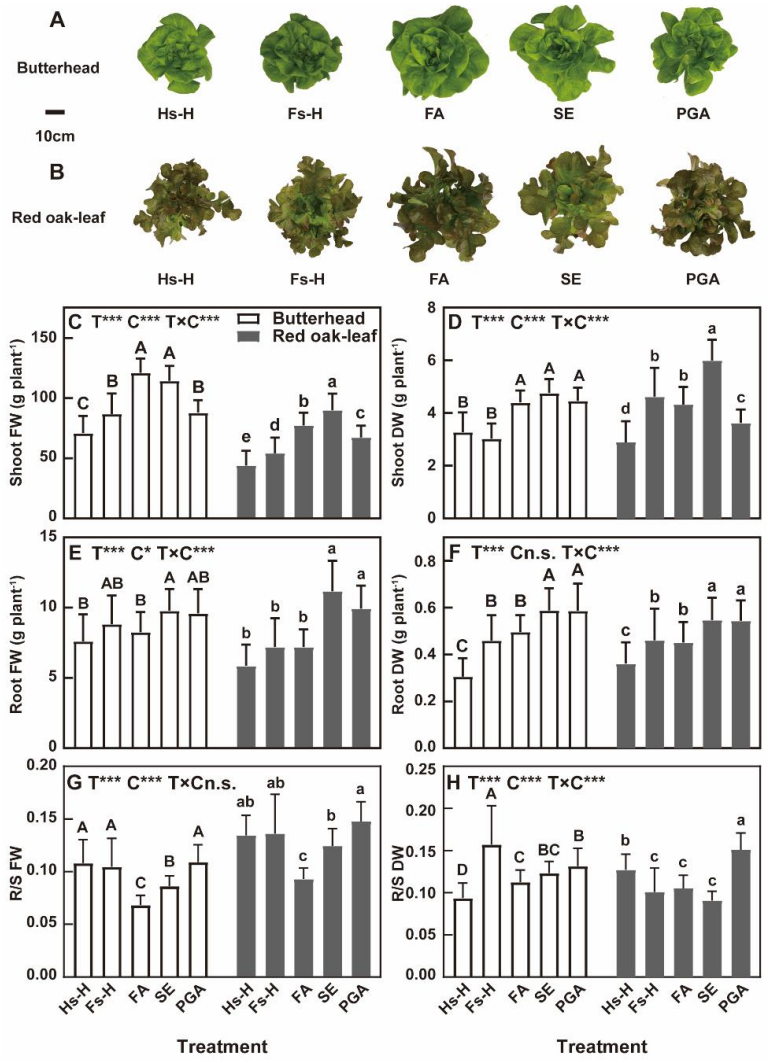

Figure 1. (**A**,**B**) Growth status, (**C**,**D**) fresh weight (FW) and dry weight (DW) of shoot, (**E**,**F**) FW and DW of root, and (**G**,**H**) root-to-shoot (R/S) ratio by nutrient solution treatments and lettuce cultivars. Data represent mean ± standard deviation (SD) (n = 30). Different upper- and lowercase letters indicate significant differences among nutrient solution treatments for butterhead and red oak-leaf, respectively (α = 0.05). T—main effect of treatment; C—main effect of cultivar; T × C—treatment by cultivar interaction. * and *** indicate significant difference at α = 0.05 and 0.001, respectively; n.s. indicates not significant.

All three biostimulants significantly improved shoot biomass compared to that achieved with the Hs-H treatment (Figure 1C,D). Biostimulant supplementation also resulted in notably greater or comparable shoot biomass relative to that achieved with the Fs-H treatment; however, the PGA treatment led to a significantly lower shoot dry weight than that achieved using the Fs-H treatment (Figure 1C,D). The SE and PGA treatments produced significantly greater root biomass than that achieved using the Hs-H, Fs-H, and FA treatments. Only the SE treatment notably improved the root fresh weight when compared to that achieved using Hs-H and FA treatments (Figure 1E,F). The R/S ratio on a fresh weight basis following PGA treatment was similar to that realized with Hs-H and Fs-H treatments, but it was significantly greater than that achieved using FA and SE treatments (Figure 1G). An inconsistent trend was observed for the root-to-shoot ratio (R/S ratio) on a dry-weight basis. For butterhead, all three biostimulants resulted in a significantly higher R/S ratio relative to that achieved using the Hs-H treatment but a significantly lower R/S ratio relative to that realized using the Fs-H treatment (Figure 1H). In contrast, the R/S

ratio following PGA treatment was significantly greater than that observed after FA, SE, and both negative and positive control treatments (Figure 1H).

3.2. Crop Quality

The crop quality of lettuce leaves grown in Hs-H and Fs-H nutrient solutions did not significantly differ for either cultivar, except for the fact that Fs-H notably increased soluble protein content compared to that achieved with the Hs-H treatment for red oak-leaf (Figure 2). PGA treatment for butterhead and FA treatment for red oak-leaf significantly decreased nitrate concentrations compared to that achieved using Hs-H and Fs-H treatments (Figure 2A). SE and PGA significantly reduced soluble sugar concentrations relative to that achieved using Hs-H and Fs-H treatments in both cultivars (Figure 2B). Soluble protein was not significantly affected by biostimulant supplementation for butterhead, whereas FA significantly increased soluble protein in red oak-leaf compared to that achieved using Hs-H (Figure 2C). FA and PGA resulted in chlorophyll contents that were not significantly different from those achieved using Hs-H and Fs-H treatments; however, SE induced a notably lower chlorophyll content than that realized using the Fs-H, FA, and PGA treatments (Figure 2D). FA did not significantly change the flavonoid content compared to that achieved using the Hs-H and Fs-H treatments in either cultivar (Figure 2E). SE and PGA significantly reduced flavonoids content in butterhead compared to that realized using Fs-H, whereas PGA significantly increased flavonoids content in red oak-leaf (Figure 2E). The nutrient solution treatments did not significantly affect the anthocyanin content in red oak-leaf (Figure 2F).

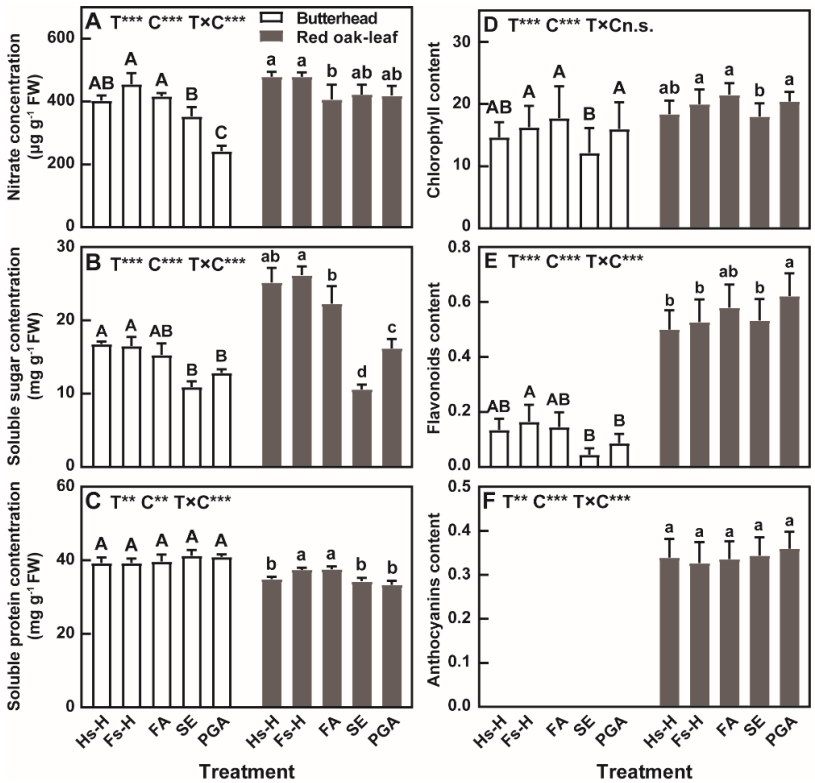

Figure 2. Concentration of (**A**) nitrate, (**B**) soluble sugar, and (**C**) soluble protein, and content of (**D**) chlorophyll, (**E**) flavonoids, and (**F**) anthocyanins in fresh leaves by nutrient solution treatments and lettuce cultivars. Anthocyanin content was below the detection limit for butterhead and is thus not shown here. Data represent mean ± SD (n = 3, (**A**–**C**); n = 24 (**D**–**F**)). Different upper- and lowercase letters indicate significant differences among nutrient solution treatments for butterhead and red oak-leaf, respectively (α = 0.05). T—main effect of treatment; C—main effect of cultivar; T × C—treatment by cultivar interaction. ** and *** indicate significant difference at α = 0.05, 0.01, and 0.001, respectively; n.s. indicates not significant.

3.3. Nutrient Concentration and Content

The heatmap associated with cluster analysis in the current study depicted a clear hierarchy of nutrient uptake (Figures 3 and 4). Butterhead and red oak-leaf responded differently to the nutrient solution treatments. Within those responses, the different biostimulants had inconsistent impacts on the concentration and uptake of different mineral nutrients in the shoot.

A more thorough examination of the data indicated that increasing the nutrient concentration from half-strength (Hs-H) to full-strength (Fs-H) did not increase the nutrient concentration in the shoot proportionately and significantly (Table 3). Fs-H notably reduced Ca concentration in both cultivars (Table 3). FA, SE, and PGA significantly reduced the N concentration in butterhead compared to that achieved with Hs-H and Fs-H treatments. In contrast, for red oak-leaf, the evaluated biostimulants either significantly increased (i.e., FA and SE) or maintained a consistent N concentration (i.e., PGA) relative to that realized with Hs-H and Fs-H treatments (Table 3). All three biostimulants significantly reduced S concentrations in both cultivars compared to that achieved using Fs-H. SE and PGA significantly increased the Mn concentration in both cultivars relative to that achieved using Hs-H and Fs-H treatments, whereas FA did not. FA and PGA significantly increased and decreased Zn concentrations in both cultivars, respectively, compared to that realized using Hs-H and Fs-H treatments (Table 3).

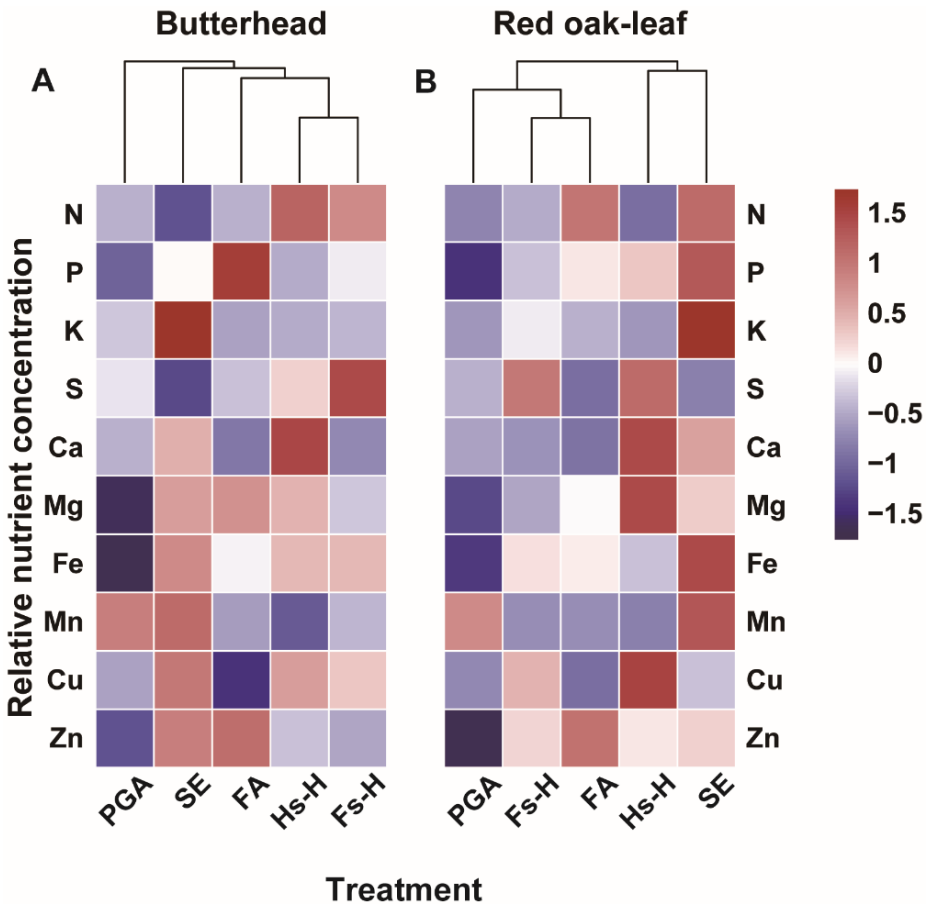

Figure 3. Macro and micronutrient concentration in the shoots of (**A**) butterhead and (**B**) red oak-leaf under different nutrient solution treatments. Nutrient concentration was normalized by the z-score; the red and blue colors indicate upregulation and downregulation, respectively.

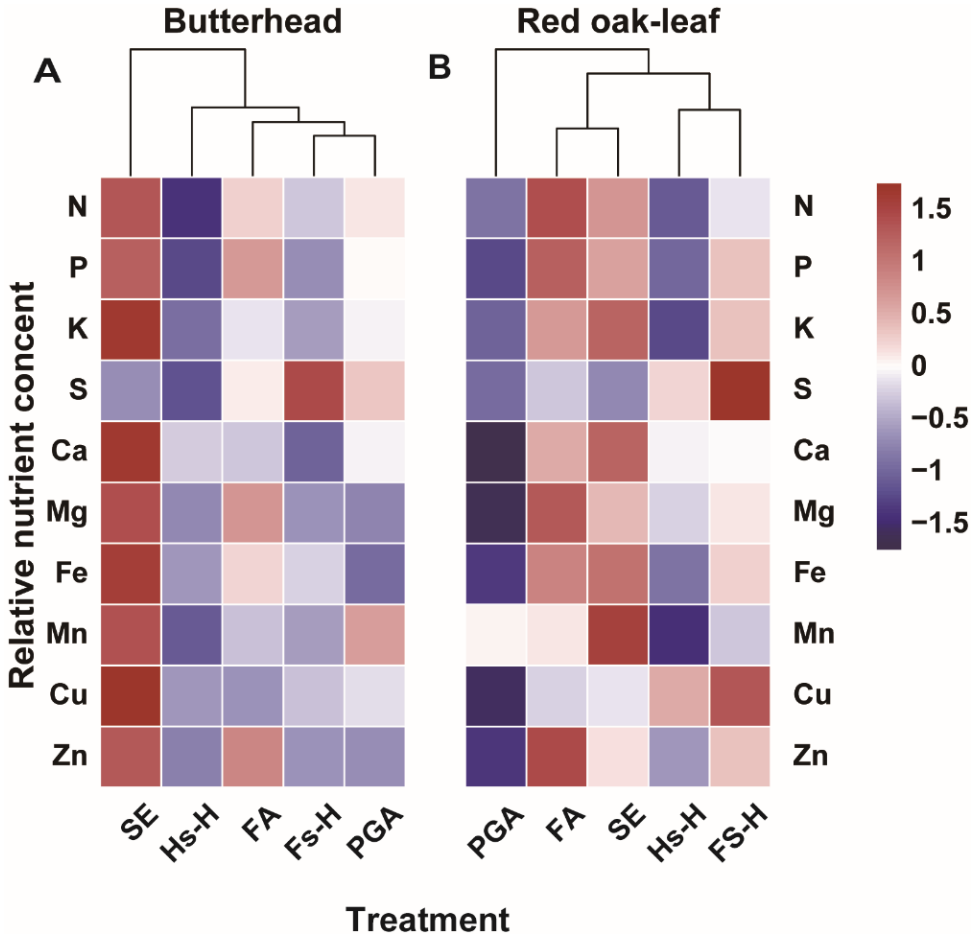

Figure 4. Macro and micronutrient content in the shoot of (**A**) butterhead and (**B**) red oak-leaf under different nutrient solution treatments. Nutrient concentration was normalized by the z-score; the red and blue colors indicate upregulation and downregulation, respectively.

The effect of nutrient solution treatment on nutrient content in the shoot was inconsistent with that of the nutrient concentration. For example, significant differences in N content among treatments did not occur for butterhead (Table 4), despite significantly lower N concentrations following biostimulant supplementation (Table 3). For red oak-leaf, Fs-H resulted in a significantly higher N content relative to that achieved with Hs-H (Table 4), although the N concentration was similar after Hs-H and Fs-H treatments (Table 3). In butterhead, FA enhanced the Mn and Zn contents compared to those achieved using the Hs-H treatment; however, in red oak-leaf, all but S, Ca, Mg, and Cu contents were enhanced. Only the Zn content was significantly elevated by FA when compared to that achieved using the Fs-H treatment (Table 4). For both cultivars, SE significantly improved or tended to improve the content of most nutrients compared to that realized using Hs-H; however, PGA had a minimal effect on nutrient content (Table 4).

Table 3. Macro and micronutrient concentration in the shoot of butterhead and red oak-leaf lettuce. Data represent mean ± SD (n = 3). Means within each column followed by different uppercase and lowercase letters are significantly different for butterhead and red oak-leaf (α = 0.05).

Treatment	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	S (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
					Butterhead					
Hs-H	33.1 ± 1.21 A	9.9 ± 0.54 A	79.2 ± 1.55 B	2.45 ± 0.06 AB	1.55 ± 0.04 A	3.55 ± 0.03 A	81.8 ± 4.75 A	48.6 ± 0.29 C	7.48 ± 0.32 AB	54.1 ± 2.58 B
Fs-H	31.7 ± 1.22 A	10.1 ± 0.09 A	80.3 ± 2.21 B	3.09 ± 0.16 A	1.14 ± 0.06 B	3.20 ± 0.07 AB	81.9 ± 6.93 A	64.6 ± 2.42 B	7.16 ± 0.50 AB	51.3 ± 0.81 B
FA	27.3 ± 3.46 B	10.8 ± 0.19 A	78.4 ± 3.91 B	2.15 ± 0.21 B	1.12 ± 0.01 B	3.69 ± 0.17 A	75.2 ± 4.94 A	60.6 ± 2.29 B	5.33 ± 0.43 B	74.4 ± 1.19 A
SE	24.7 ± 0.41 B	10.1 ± 0.05 A	107 ± 2.91 A	1.64 ± 0.04 B	1.37 ± 0.05 AB	3.64 ± 0.12 A	86.8 ± 0.15 A	101 ± 2.72 A	7.86 ± 0.19 A	71.9 ± 3.37 A
PGA	27.2 ± 0.74 B	9.7 ± 0.40 A	81.4 ± 1.65 B	2.25 ± 0.01 B	1.19 ± 0.02 B	2.58 ± 0.31 B	54.1 ± 2.61 B	96.0 ± 0.68 A	6.23 ± 0.28 B	41.9 ± 2.37 C
					Red oak-leaf					
Hs-H	24.5 ± 0.44 b	12.6 ± 0.92 ab	81.7 ± 2.79 c	3.88 ± 0.43 a	2.46 ± 0.14 a	4.22 ± 0.11 a	58.4 ± 2.93 b	53.5 ± 0.29 c	8.55 ± 0.74 a	68.8 ± 1.68 b
Fs-H	27.9 ± 1.11 b	12.0 ± 0.41 b	89.8 ± 0.89 b	3.76 ± 0.21 a	1.65 ± 0.06 c	3.02 ± 0.11 bc	68.7 ± 1.20 b	55.2 ± 1.26 c	6.69 ± 0.84 b	70.9 ± 4.00 b
FA	39.5 ± 1.14 a	12.4 ± 0.52 ab	84.5 ± 4.27 b	1.96 ± 0.06 b	1.57 ± 0.11 c	3.33 ± 0.26 b	67.0 ± 2.60 b	55.0 ± 5.35 c	4.21 ±0.10 c	86.3 ± 5.89 a
SE	40.3 ± 0.49 a	13.6 ± 0.21 a	118 ± 1.94 a	2.09 ± 0.00 b	2.14 ± 0.06 b	3.53 ± 0.09 b	96.4 ± 1.00 a	95.6 ± 1.55 a	5.30 ± 0.36 bc	71.1 ± 3.09 b
PGA	25.9 ± 1.72 b	10.8 ± 0.33 b	82.0 ± 2.29 b	2.42 ± 0.13 b	1.69 ± 0.10 c	2.60 ± 0.27 c	36.7 ± 3.47 c	85.3 ± 1.06 b	4.57 ± 0.48 c	35.3 ± 3.69 c

Table 4. Macro and micronutrient content in the shoot of butterhead and red oak-leaf lettuce. Data represent mean ± SD (n = 3). Means within each column followed by different uppercase and lowercase letters are significantly different for butterhead and red oak-leaf (α = 0.05).

Treatment	N (mg plant^{-1})	P (mg plant^{-1})	K (mg plant^{-1})	S (mg plant^{-1})	Ca (mg plant^{-1})	Mg (mg plant^{-1})	Fe (µg plant^{-1})	Mn (µg plant^{-1})	Cu (µg plant^{-1})	Zn (µg plant^{-1})
					Butterhead					
Hs-H	107 ± 15.8 A	31.9 ± 4.20 B	255 ± 25.2 B	7.89 ± 0.92 B	4.99 ± 0.59 A	11.5 ± 1.36 B	265 ± 43.0 B	157 ± 17.4 C	24.0 ± 2.22 B	174 ± 12.6 B
Fs-H	116 ± 7.10 A	36.7 ± 2.76 AB	293 ± 27.2 B	11.3 ± 1.37 A	4.15 ± 0.23 A	11.7 ± 0.71 B	298 ± 27.2 B	236 ± 17.2 BC	26.2 ± 3.30 B	187 ± 16.0 B
FA	120 ± 3.76 A	47.9 ± 4.39 AB	348 ± 26.9 B	9.53 ± 1.06 AB	4.97 ± 0.50 A	16.4 ± 1.43 AB	336 ± 47.3 AB	271 ± 38.9 B	24.0 ± 4.48 B	332 ± 36.2 A
SE	128 ± 10.9 A	52.4 ± 3.56 A	554 ± 50.2 A	8.54 ± 0.78 AB	7.11 ± 0.60 A	18.9 ± 1.50 A	451 ± 32.4 A	525 ± 25.9 A	40.8 ± 3.26 A	375 ± 42.2 A
PGA	119 ± 6.28 A	42.3 ± 4.36 AB	356 ± 29.2 B	9.84 ± 0.61 AB	5.21 ± 0.40 A	11.4 ± 2.05 B	236 ± 7.36 B	420 ± 28.6 A	27.3 ± 2.98 B	184 ± 22.1 B
Hs-H	81.7 ± 10.7 c	42.3 ± 7.26 b	273 ± 39.2 b	13.1 ± 2.89 b	8.27 ± 1.47 ab	14.1 ± 1.62 ab	195 ± 28.7 b	179 ± 22.6 c	28.5 ± 4.57 ab	230 ± 31.6 bc
Fs-H	139 ± 12.4 b	59.8±7.18 ab	450±51.7 a	18.9±2.78 a	8.32±1.32 ab	15.2±2.28 ab	344±39.2 a	277±40.1 bc	33.2±4.04 a	353±24.9 b
FA	22 6± 18.9 a	71.1 ± 6.18 a	485 ± 51.6 a	11.2 ± 0.80 b	9.02 ± 1.13 ab	19.2 ± 2.53 a	384 ± 29.6 a	315 ± 18.7 b	24.1 ± 1.70 ab	496 ± 61.9 a
SE	186 ± 22.8 a	63.0 ± 7.81 a	545 ± 64.2 a	9.64 ± 1.06 b	9.87 ± 0.84 a	16.3 ± 1.52 a	446 ± 52.9 a	442 ± 51.4 a	24.7 ± 4.48 ab	330 ± 50.8 b
PGA	93.6 ± 2.81 c	39.2 ± 2.78 b	297 ± 19.5 b	8.75 ± 0.59 b	6.14 ± 0.63 b	9.44 ± 1.36 b	133 ± 16.7 b	309 ± 13.9 b	16.6 ± 2.27 b	128 ± 18.2 c

3.4. Principal Component Analysis

PCA was performed to explore relationships among growth traits, quality traits, pigment contents, and macro and micronutrients and their responses to nutrient solution and lettuce cultivar treatments. The PCA results are presented as biplots, including variables leading to each selected trait component being combined with treatment component scores. The association between the traits represented by the first and second principal components is shown in Figure 5. These two primary components accounted for approximately 57% of the total variation. The first principal component (x-axis) mainly includes biomass, soluble protein, and concentrations of Fe and Mn on the positive side of the scale, and crop quality traits (including chlorophyll, anthocyanins, flavonoids, soluble sugar, nitrate, and concentrations of Ca, S, and P) on the negative side of the scale. The second principal component (y-axis) mainly includes the Cu concentration and the R/S ratio (on a dry weight basis) on the positive side and the N, K, and Zn concentrations on the negative side. These results indicate that the Ca, S, and P concentrations and most crop qualities were negatively correlated with shoot biomass. In contrast, the Fe, Mn, and K concentrations were positively correlated with biomass. The vector for the concentration of Mg was shorter than that for other traits, indicating that only a small proportion of variability was explained by principal components 1 and 2.

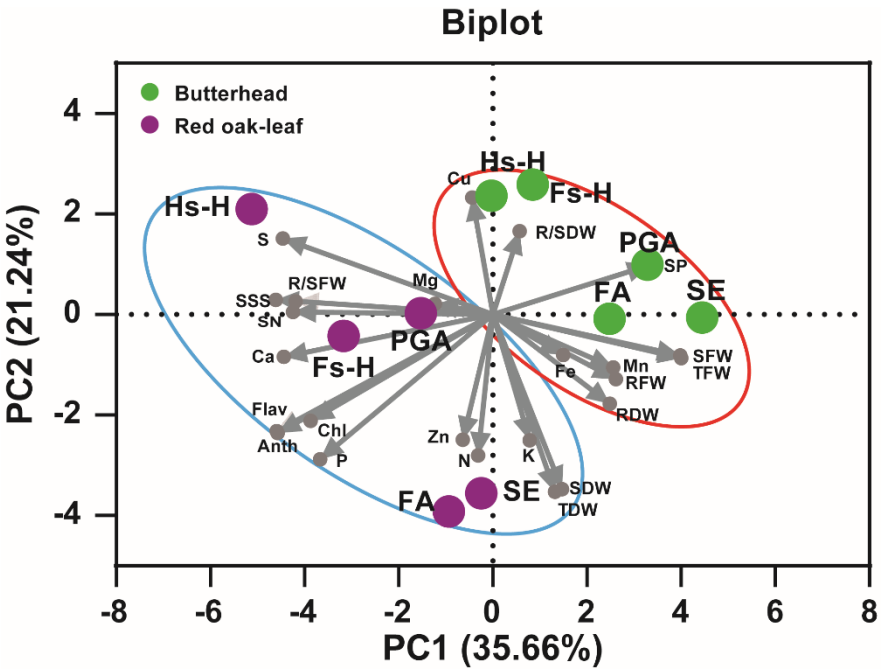

Figure 5. Principal component pattern for correlations of yield, quality, and nutrient concentration traits in butterhead and red oak-leaf. SFW—shoot fresh weight; SDW—shoot dry weight; RFW—root fresh weight; RDW—root dry weight; TFW—total fresh weight; TDW—total dry weight; R/SFW —ratio of root to shoot in fresh weight; R/SDW—ratio of root to shoot in dry weight; SN—shoot nitrate concentration; SSS—shoot soluble sugar concentration; SP—soluble protein; Chl—leaf chlorophyll content; Flav—leaf flavonoids content; Anth—leaf anthocyanins content; N, P, K, S, Ca, Mg, Fe, Mn, Cu, and Zn—their respective concentrations in the shoot.

4. Discussion

4.1. Biomass

Hs-H, which served as the negative control treatment in this study, is widely used in lettuce production. However, our study found that Fs-H produced ~23% greater shoot fresh biomass than that realized using Hs-H, indicating that a higher EC of nutrient solution (i.e., higher nutrient concentration) may increase the yield of hydroponically produced lettuce.

Previous studies have reached the same conclusion, but an interaction between the EC of the nutrient solution and air temperature may exist and needs to be considered when determining the optimal EC for lettuce production in a hydroponic system [45,46].

In the current study, Hs-H combined with each biostimulant (i.e., FA, SE, and PGA) elevated lettuce yield to a level comparable to or higher than that of the Fs-H treatment, which is in agreement with previous studies [15,47,48]. As fertilizer prices have been increasing and may continue to increase in the future, biostimulant supplementation in the currently adopted hydroponic solutions may be a more sustainable approach to increase crop production. Additionally, we compared the efficacy of different categories of biostimulants on plant growth. SE and FA improved shoot biomass more efficiently than PGA did in both cultivars. SE and FA were equally effective in butterhead with regard to the shoot biomass, which is consistent with a previous study reporting no significant difference in shoot FW between *Ascophyllum nodosum* extract and humic acid treatments [48]. In contrast, SE was more effective than FA in improving shoot FW of red oak-leaf, indicating that different cultivars differed in their response to exogenous biostimulants.

Root biomass responded differently to biostimulant treatments compared to shoot biomass. FA exhibited a lower performance relative to that of SE and PGA with regard to enhancing root FW; consequently, the lowest and highest R/S was observed for FA and PGA, respectively. A similar result was reported in a study evaluating the impact of different types of leonardite-derived humic acid on 60-day old corn seedlings [49]; this study indicated that fulvic acid (i.e., low molecular weight humic acid) enhanced shoot and root biomass, but a decrease in the R/S ratio from 1.3 to 0.84 was observed. A meta-analysis also revealed that a brown coal-derived humic substance had no significant effect on root growth [50]. Moreover, Wang et al. [51] found that the addition of PGA significantly improved the shoot and root biomass concentrations and R/S ratio of cucumber seedlings. Taken together, these results indicate that the effect of FA on lettuce growth is more evident in shoots than in roots, and this trend is reversed for PGA.

4.2. Crop Quality

Biostimulants affect biomass production and improve crop quality by altering plant physiology and metabolism [24,40]. Excessive accumulation of nitrates in vegetables is a common issue that poses a potential threat to human health [52]. Our studies demonstrated a decreasing trend in nitrate concentration following the addition of biostimulants relative to that achieved using Hs-H (Figure 2A). This is especially true for the PGA treatment, which decreased the nitrate concentration in the shoot by ~50% in butterhead lettuce. A similar conclusion was reached in a study evaluating hydroponically cultivated maize seedlings [53]. Their results indicated that adding protein hydrolysates could increase plant shoot and root growth while decreasing nitrate concentration by >85%. In addition, Di Mola et al. [54] found that the foliar application of legume-derived protein hydrolysates and seaweed extract can increase lettuce yield and quality. However, PGA resulted in significantly lower leaf nitrate content than SE content under various mineral N rates (i.e., 0–30 kg N kg^{-1}). Protein hydrolysates offer abundant organic nitrogen, including polypeptides and amino acids [55]. Compared to mineral fertilizers that directly provide nitrate, organic fertilizers gradually release nitrogen, limiting the excessive accumulation of nitrate in vegetables [52]. The beneficial effects of seaweed extract on the quality of horticultural crops such as tomato, mint, basil, grape, and olive have been previously reported [21]. However, studies on lettuce are limited. Soluble sugar, soluble protein, flavonoids, anthocyanins, and chlorophyll content were the common traits used to evaluate the quality of lettuce. These components modulate the plant photosynthesis process and nitrate assimilation and play important roles in human health. Flavonoids also play an important role in flavor (i.e., bitterness) [56–58]. The current study found that the SE treatment significantly decreased leaf soluble sugar concentration compared to that of the negative control treatment in both butterhead and red oak-leaf cultivars (Figure 2B,E). PGA slightly reduced flavonoid content in butterhead but significantly increased it in red

oak-leaf. Similar inconsistent effects on quality traits (i.e., soluble sugars and carotenoids) following the application of protein hydrolysates and amino acids have been reported in two carrot cultivars [59]. Such changes in physiochemical quality traits are possibly due to SE and PGA being able to alter carbon metabolism [47,60].

4.3. Nutrient Uptake

Although extensive studies indicate that different categories of biostimulants could promote the uptake of plant macro and micronutrients by stimulating root growth [23,24,40,50], inconsistencies in their effectiveness may exist. For example, the effect of applied biostimulants on shoot nitrogen concentration and content showed an opposite trend between a butterhead and red oak-leaf, indicating an apparent genotypic effect on nitrogen accumulation. Typical leonardite-derived humic substances contain only 1–2% nitrogen, which may not be an adequate contributor to the nitrogen pool and may not be readily available for plant uptake [61]. In the current study, root biomass did not notably improve following the FA treatment. However, FA treatment still significantly enhanced N uptake in red oak-leaf, suggesting that other mechanisms may be involved in FA-mediated enhancement in N assimilation in certain cultivars. In addition, Xu et al. [31] reported that γ-PGA was involved in nitrogen metabolism, improving nitrogen reduction and assimilation. However, this study showed that the application of PGA did not significantly improve N uptake, even though the N content in PGA was greater than that in FA and SE (Table 2). A relatively low utilization rate (6–25%) of amino acids that are added to the cultivation medium by plants, mainly due to microorganism competition and hydrolysis of amino acids, may explain this phenomenon [62]. Taken together, the impact of biostimulants on nutrient uptake varies depending on plant variety, substrate conditions, and application rates [63]. Our study evaluated only one application rate for each biostimulant according to the recommended foliar spray or soil application rate. Thus, the optimal rate for hydroponic cultivation needs to be determined in the future.

Higher Zn and Mn uptake was detected in the FA and SE treatments in this study. One possible reason is that the FA and SE treatments contained a high concentration of the respective micronutrients, possibly in a form readily available for plant absorption (Table 2). Meanwhile, an increase in root growth, particularly the lateral root, could enhance the capability of plants to acquire nutrients [24,40,47,60]. In contrast, the Fe concentrations in FA and SE were also abundant. However, an increase in Fe uptake was not observed, possibly because Fe exists in forms that are not readily available to plants or for the competitive uptake of other cations. In this study, the two evaluated cultivars responded differently to added biostimulants with respect to nutrient concentration and uptake, indicating that the role of genotype should not be ignored when comparing the efficacy of different biostimulants.

4.4. Mode of Action

Currently, determining the mode of action of biostimulants is difficult because of the complex nature of the components. Standardization is challenging because the source material may vary in composition and quality [20]. The underlying mechanism affecting lettuce growth may differ among seaweed extracts, fulvic acid, and γ-PGA. For example, extensive studies have reported that *Ascophyllum nodosum*-based commercial seaweed extract could enhance the fresh matter production of different crops. Craigie [64] suggested that the higher content of plant hormones in seaweed extract (including abscisic acid, gibberellic acid, auxins, brassinosteroids, and cytokinins) is responsible for improved plant growth [60], especially root growth [65]. However, experiments conducted by Mondal et al. [66] demonstrated that *K. alvarezii* extracts, from which gibberellins and auxins were removed by solvent extraction, maintained their activity (such as increasing maize (*Zea mays* L.) biomass production and grain yield). This suggests that gibberellins and auxins may not be the active ingredients in the seaweed extract. Fulvic acid is the major constituent of humic substances with low molecular weight and has been reported to have

the greatest stimulatory effect on plant growth, physiological metabolism, and assimilate accumulation. Unlike seaweed extract, phytohormones in fulvic acid were detected in many studies [67–69]; however, it has been well established that humic substances are positively correlated with plant shoot and root growth, physiological performance, and nitrogen metabolism in higher plants [23,24,40,50], as other molecules in fulvic acid may exhibit auxin-like effects.

Biostimulants may play a more critical role when plants are subjected to abiotic stress [21]. In the current study, the shoot FW after the SE treatment was enhanced two-fold when compared to that observed after the Hs-H treatment in both butterhead and red oak-leaf varieties. This may be explained by enhanced tolerance to cold environments irradiated with weak light after SE application. The present study was performed in Chengdu, China, during winter, which is characterized by low temperatures and lack of sunlight irradiation, as illustrated in Figure S1 [70]. During the study period, the daily mean temperature (approximately 10 °C) was significantly lower than the optimum temperature for lettuce growth (20–24 °C) [71,72]. Studies on *Arabidopsis thaliana* have indicated that the lipophilic components extracted from the brown seaweed *Ascophyllum nodosum* could alleviate cold stress by elevating synthesis and accumulation of osmoprotectants and modifying cellular fatty acid composition [73].

It is generally accepted that biostimulants can enhance crop production. However, a lack of a yield increase or a negative impact on yield has also been reported in many crops such as lettuce [74], tomato [75], and wheat [76,77]. Therefore, one should proceed with caution when deciding whether to use biostimulant directly or the type and application rate of a particular biostimulant [54,78]. For example, the effectiveness of humic substances is coordinated by the application rate, properties, environmental conditions, and plant type [50]. Humic substances could be divided into different categories based on the extracted sources, and brown-coal-extracted humic substances are common commercial products [50]. However, humic substances extracted from brown coal did not notably improve root biomass, whereas those extracted from peat and manure promoted root growth by 12–40% [50].

5. Conclusions

Compared to the half-strength nutrient solution treatment, the full-strength nutrient solution further improved biomass; however, it did not increase crop quality traits or nutrient uptake. The supplemental fulvic acid, seaweed extract, and γ-PGA solutions improved shoot and root biomass by different magnitudes when compared to that achieved using the half-strength nutrient solution. FA resulted in the lowest R/S ratio, while SE resulted in the lowest soluble sugar and chlorophyll contents among all treatments. The addition of biostimulants tended to reduce nitrate accumulation in the leaves. Crop responses to biostimulants with regard to nutrient concentration varied according to cultivar and mineral nutrient contents.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12040786/s1, Figure S1: Daily maximum and minimum temperatures and daily light integral during the period of lettuce growth.

Author Contributions: Conceptualization, Z.W. and R.Y.; data curation, Z.W., R.Y., Z.Z. and Y.L.; formal analysis, Y.L. and Z.Z.; investigation and methodology, S.Z. and C.S.; resources, J.L.; funding acquisition and validation, Z.Q.; supervision, Q.Y.; writing—original draft, Z.W., R.Y. and Y.L.; writing—review and editing, Z.W., R.Y. and Q.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key Research and Development Program, Ministry of Science and Technology of China (No. 2020YFE0203600), Sichuan Science and Technology Program (No. 2021YJ0500 and 2021JDRC0120), Central Public-Interest Scientific Institution Basal Research Fund (No. Y2021XK04 and Y2021XK02), the Agricultural Science and Technology Innovation Program of Chinese Academy of Agricultural Sciences (No. ASTIP-CAAS, 34-IUA-03 and

34-IUA-04), the Local Financial Project of the National Agricultural Science and Technology Center (No. NASC2021ST02 and NASC2020AR09), and the Key Research and Development Program of Chengdu (No. 2021-YF09-00108-SN).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We acknowledge Tao Huang, Hui Jiang and Jiamin Wang for their assistance with the experimental setup, sample collection, and analysis.

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

References

1. United Nations, Department of Economic and Social Affairs. World Population Prospects. 2019. Available online: https://reliefweb.int/sites/reliefweb.int/files/resources/WPP2019_Highlights.pdf (accessed on 20 March 2022).
2. Godfray, H.C.; Beddington, J.R.; Crute, I.R.; Haddad, L.; Lawrence, D.; Muir, J.F.; Pretty, J.; Robinson, S.; Thomas, S.M.; Toulmin, C. Food security: The challenge of feeding 9 billion people. *Science* **2010**, *327*, 812–818. [CrossRef] [PubMed]
3. Wang, X.; Dou, Z.; Shi, X.; Zou, C.; Liu, D.; Wang, Z.; Guan, X.; Sun, Y.; Wu, G.; Zhang, B.; et al. Innovative management programme reduces environmental impacts in Chinese vegetable production. *Nat. Food* **2021**, *2*, 47–53. [CrossRef]
4. Barbosa, G.L.; Gadelha, F.D.; Kublik, N.; Proctor, A.; Reichelm, L.; Weissinger, E.; Wohlleb, G.M.; Halden, R.U. Comparison of land, water, and energy requirements of lettuce grown using hydroponic vs. conventional agricultural methods. *Int. J. Environ. Res. Public. Health* **2015**, *12*, 6879–6891. [CrossRef] [PubMed]
5. Putra, P.A.; Yuliando, H. Soilless Culture System to Support Water Use Efficiency and Product Quality: A Review. *Agric. Agric. Sci. Procedia* **2015**, *3*, 283–288. [CrossRef]
6. Jones, J.B. *Complete Guide for Growing Plants Hydroponically*; CRC Press–Taylor & Francis Group: Boca Raton, FL, USA, 2014.
7. Zheng, Y.; Graham, T.H.; Richard, S.; Dixon, M. Can Low Nutrient Strategies Be Used for Pot Gerbera Production in Closed-Loop Subirrigation? *Acta Hortic.* **2005**, *691*, 365–372. [CrossRef]
8. Dufour, L.; Guérin, V. Nutrient solution effects on the development and yield of *Anthurium andreanum* Lind. in tropical soilless conditions. *Sci. Hortic.* **2005**, *105*, 269–282. [CrossRef]
9. Siddiqi, M.Y.; Kronzucker, H.J.; Britto, D.T.; Glass, A.D.M. Growth of a tomato crop at reduced nutrient concentrations as a strategy to limit eutrophication. *J. Plant Nutr.* **1998**, *21*, 1879–1895. [CrossRef]
10. Rouphael, Y.; Colla, G. The Influence of Drip Irrigation or Subirrigation on Zucchini Squash Grown in Closed-loop Substrate Culture with High and Low Nutrient Solution Concentrations. *HortScience* **2009**, *44*, 306–311. [CrossRef]
11. Sonneveld, C.; Voogt, W. Nutrient solutions for soilless cultures. In *Plant Nutrition of Greenhouse Crops*; Springer: Dordrecht, The Netherlands, 2009; pp. 257–275.
12. Walters, K.J.; Currey, C.J. Effects of Nutrient Solution Concentration and Daily Light Integral on Growth and Nutrient Concentration of Several Basil Species in Hydroponic Production. *HortScience* **2018**, *53*, 1319–1325. [CrossRef]
13. Wada, T. Theory and Technology to Control the Nutrient Solution of Hydroponics. In *Plant Factory Using Artificial Light*; Anpo, M., Fukuda, H., Wada, T., Eds.; Elsevier: Amsterdam, The Netherland, 2019; pp. 5–14.
14. Shahein, M.M.; Afifi, M.M.; Algharib, A.M. Assessing the effect of humic substances extracted from compost and biogas manure on yield and quality of lettuce (*Lactuca sativa* L.). *J. Mater. Environ. Sci.* **2014**, *14*, 996–1009.
15. Guilayn, F.; Benbrahim, M.; Rouez, M.; Crest, M.; Patureau, D.; Jimenez, J. Humic-like substances extracted from different digestates: First trials of lettuce biostimulation in hydroponic culture. *Waste Manag.* **2020**, *104*, 239–245. [CrossRef] [PubMed]
16. European Biostimulants Industry Council EBIC and Biostimulants in Brief. 2012. Available online: https://www.biostimulants.eu/ (accessed on 20 March 2022).
17. Ertani, A.; Pizzeghello, D.; Altissimo, A.; Nardi, S. Use of meat hydrolyzate derived from tanning residues as plant biostimulant for hydroponically grown maize. *J. Plant Nutr. Soil Sci.* **2013**, *176*, 287–295. [CrossRef]
18. Nanda, S.; Kumar, G.; Hussain, S. Utilization of seaweed-based biostimulants in improving plant and soil health: Current updates and future prospective. *Int. J. Environ. Sci. Technol.* **2021**. [CrossRef]
19. Smoleń, S.; Ledwożyw-Smoleń, I.; Sady, W. The role of exogenous humic and fulvic acids in iodine biofortification in spinach (*Spinacia oleracea* L.). *Plant Soil* **2015**, *402*, 129–143. [CrossRef]
20. Du Jardin, P.; Xu, L.; Geelen, D. Agricultural functions and action mechanisms of plant biostimulants (PBs): An introduction. In *The Chemical Biology of Plant Biostimulants*; Geelen, D., Xu, L., Eds.; Wiley: Hoboken, NJ, USA, 2020; pp. 14–16.
21. Wozniak, E.; Blaszczak, A.; Wiatrak, P.; Canady, M. Biostimulant Mode of Action: Impact of biostimulant on whole-plant level. In *The Chemical Biology of Plant Biostimulants*; Geelen, D., Xu, L., Eds.; Wiley: Hoboken, NJ, USA, 2020; pp. 205–227.
22. Vernieri, P.; Borghesi, E.; Ferrante, A.; Magnani, G. Application of biostimulants in floating system for improving rocket quality. *Int. J. Environ. Sci. Technol.* **2005**, *3*, 86–88.

23. Bulgari, R.; Cocetta, G.; Trivellini, A.; Vernieri, P.; Ferrante, A. Biostimulants and crop responses: A review. *Biol. Agric. Hortic.* **2014**, *31*, 1–17. [CrossRef]
24. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41. [CrossRef]
25. Du Jardin, P. Plant biostimulants: Definition, concept, main categories and regulation. *Sci. Hortic.* **2015**, *196*, 3–14. [CrossRef]
26. Yakhin, O.I.; Lubyanov, A.A.; Yakhin, I.A.; Brown, P.H. Biostimulants in plant science: A global perspective. *Front. Plant Sci.* **2017**, *7*, 2049. [CrossRef]
27. Varanini, Z.; Pinton, R. Direct versus indirect effects of soil humic substances on plant growth and nutrition. In *The Rhizosphere*; Pinton, R., Varanini, Z., Nannipieri, P., Eds.; Marcel Dekker, Inc.: Basel, Switzerland, 2001; pp. 141–158.
28. Dobbss, L.B.; Medici, L.O.; Peres, L.E.P.; Pino-Nunes, L.E.; Rumjanek, V.M.; Façanha, A.R.; Canellas, L.P. Changes in root development of Arabidopsis promoted by organic matter from oxisols. *Ann. Appl. Biol.* **2007**, *151*, 199–211. [CrossRef]
29. Lei, P.; Xu, Z.; Ding, Y.; Tang, B.; Zhang, Y.; Li, H.; Feng, X.; Xu, H. Effect of Poly(gamma-glutamic acid) on the Physiological Responses and Calcium Signaling of Rape Seedlings (*Brassica napus* L.) under Cold Stress. *J. Agric. Food Chem.* **2015**, *63*, 10399–10406. [CrossRef] [PubMed]
30. Lei, P.; Xu, Z.; Liang, J.; Luo, X.; Zhang, Y.; Feng, X.; Xu, H. Poly(γ-glutamic acid) enhanced tolerance to salt stress by promoting proline accumulation in *Brassica napus* L. *Plant Growth Regul.* **2015**, *78*, 233–241. [CrossRef]
31. Xu, Z.; Lei, P.; Feng, X.; Xu, X.; Liang, J.; Chi, B.; Xu, H. Calcium involved in the poly(gamma-glutamic acid)-mediated promotion of Chinese cabbage nitrogen metabolism. *Plant Physiol. Biochem.* **2014**, *80*, 144–152. [CrossRef] [PubMed]
32. Yang, X.; Cui, X.; Zhao, L.; Guo, D.; Feng, L.; Wei, S.; Zhao, C.; Huang, D. Exogenous glycine nitrogen enhances ac-cumulation of glycosylated flavonoids and antioxidant activity in lettuce (*Lactuca sativa* L.). *Front. Plant Sci.* **2017**, *8*, 2098. [CrossRef] [PubMed]
33. Yang, X.; Wei, S.; Liu, B.; Guo, D.; Zheng, B.; Feng, L.; Liu, Y.; Tomas-Barberan, F.A.; Luo, L.; Huang, D. A novel integrated non-targeted metabolomic analysis reveals significant metabolite variations between different lettuce (*Lactuca sativa.* L) varieties. *Hortic. Res.* **2018**, *5*, 33. [CrossRef]
34. FAO. FAOSTAT Database: Agriculture Production. Food and Agriculture Organization of the United Nations: Rome, Italy, 2019. Available online: https://www.oecd.org/gov/regulatory-policy/FAO_Full-Report.pdf (accessed on 20 March 2022).
35. Hoagland, D.R.; Arnon, D.I. The water culture method for growing plants without soil. *Calif. Agric. Exp. Stn.* **1950**, *347*, 39.
36. Djidonou, D.; Leskovar, D.I. Seasonal Changes in Growth, Nitrogen Nutrition, and Yield of Hydroponic Lettuce. *HortScience* **2019**, *54*, 76–85. [CrossRef]
37. Gillespie, D.P.; Papio, G.; Kubota, C. High Nutrient Concentrations of Hydroponic Solution Can Improve Growth and Nutrient Uptake of Spinach (*Spinacia oleracea* L.) Grown in Acidic Nutrient Solution. *HortScience* **2021**, *56*, 687–694. [CrossRef]
38. ÖZtekİN, G.B.; UludaĞ, T.; TÜZel, Y. Growing spinach (*Spinacia oleracea* L.) in a floating system with different concentrations of nutrient solution. *Appl. Ecol. Environ. Res.* **2018**, *16*, 3333–3350. [CrossRef]
39. Manzocco, L.; Foschia, M.; Tomasi, N.; Maifreni, M.; Dalla Costa, L.; Marino, M.; Cortella, G.; Cesco, S. Influence of hydroponic and soil cultivation on quality and shelf life of ready-to-eat lamb's lettuce (*Valerianella locusta* L. Laterr). *J. Sci. Food Agric.* **2011**, *91*, 1373–1380. [CrossRef]
40. Cristofano, F.; El-Nakhel, C.; Rouphael, Y. Biostimulant substances for sustainable agriculture: Origin, operating mechanisms and effects on cucurbits, leafy greens, and nightshade vegetables species. *Biomolecules* **2021**, *11*, 1103. [CrossRef] [PubMed]
41. Cataldo, D.A.; Maroon, M.; Schrader, L.E.; Youngs, V.L. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun. Soil. Sci. Plan.* **1975**, *6*, 71–80. [CrossRef]
42. Ruuska, S.A.; Rebetzke, G.J.; van Herwaarden, A.F.; Richards, R.A.; Fettell, N.A.; Tabe, L.; Jenkins, C.L.D. Genotypic variation in water-soluble carbohydrate accumulation in wheat. *Funct. Plant Biol.* **2006**, *33*, 799–809. [CrossRef] [PubMed]
43. Sedmak, J.J.; Grossberg, S.E. A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue G250. *Anal. Biochem.* **1977**, *79*, 544–552. [CrossRef]
44. Kitson, R.; Mellon, M. Colorimetric determination of phosphorus as molybdivanadophosphoric acid. *Ind. Eng. Chem.* **1944**, *16*, 379–383. [CrossRef]
45. Gent, M.P.N. Factors Affecting Relative Growth Rate of Lettuce and Spinach in Hydroponics in a Greenhouse. *HortScience* **2017**, *52*, 1742–1747. [CrossRef]
46. Yang, T.; Samarakoon, U.; Altland, J.; Ling, P. Photosynthesis, Biomass Production, Nutritional Quality, and Flavor-Related Phytochemical Properties of Hydroponic-Grown Arugula (*Eruca sativa* Mill.) 'Standard' under Different Electrical Conductivities of Nutrient Solution. *Agronomy* **2021**, *11*, 1340. [CrossRef]
47. Colla, G.; Hoagland, L.; Ruzzi, M.; Cardarelli, M.; Bonini, P.; Canaguier, R.; Rouphael, Y. Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. *Front. Plant Sci.* **2017**, *8*, 2202. [CrossRef]
48. Sandepogu, M.; Shukla, P.S.; Asiedu, S.; Yurgel, S.; Prithiviraj, B. Combination of *Ascophyllum nodosum* extract and humic acid improve early growth and reduces post-harvest loss of lettuce and spinach. *Agriculture* **2019**, *9*, 240. [CrossRef]
49. Sun, Q.; Ding, W.; Yang, Y.; Sun, J.; Ding, Q. Humic Acids Derived from Leonardite-Affected Growth and Nutrient Uptake of Corn Seedlings. *Commun. Soil Sci. Plan.* **2016**, *47*, 1275–1282. [CrossRef]
50. Rose, M.T.; Patti, A.F.; Little, K.R.; Brown, A.L.; Jackson, W.R.; Cavagnaro, T.R. A Meta-Analysis and Review of Plant-Growth Response to Humic Substances: Practical Implications for Agriculture. *Adv. Agron.* **2014**, *124*, 37–89.

51. Wang, Q.; Chen, S.; Zhang, J.; Sun, M.; Liu, Z.; Yu, Z. Co-producing lipopeptides and poly-gamma-glutamic acid by solid-state fermentation of Bacillus subtilis using soybean and sweet potato residues and its biocontrol and fertilizer synergistic effects. *Bioresour. Technol.* **2008**, *99*, 3318–3323. [CrossRef] [PubMed]
52. Bian, Z.; Wang, Y.; Zhang, X.; Li, T.; Grundy, S.; Yang, Q.; Cheng, R. A Review of Environment Effects on Nitrate Accumulation in Leafy Vegetables Grown in Controlled Environments. *Foods* **2020**, *9*, 732. [CrossRef] [PubMed]
53. Ertani, A.; Cavani, L.; Pizzeghello, D.; Brandellero, E.; Altissimo, A.; Ciavatta, C.; Nardi, S. Biostimulant activity of two protein hydrolyzates in the growth and nitrogen metabolism of maize seedlings. *J. Soil Sci. Plant Nut.* **2009**, *172*, 237–244. [CrossRef]
54. Di Mola, I.; Cozzolino, E.; Ottaiano, L.; Giordano, M.; Rouphael, Y.; Colla, G.; Mori, M. Effect of vegetal- and seaweed extract-based biostimulants on agronomical and leaf quality traits of plastic tunnel-grown baby lettuce under four regimes of nitrogen fertilization. *Agronomy* **2019**, *9*, 571. [CrossRef]
55. Cavani, L.; Margon, A.; Sciubba, L.; Ciavatta, C.; Marzadori, C. What we talk about when we talk about protein hydrolyzate-based biostimulants. *AIMS Agric. Food* **2017**, *2*, 221–232. [CrossRef]
56. Lee, D.; Nishizawa, M.; Shimizu, Y.; Saeki, H. Anti-inflammatory effects of dulse (*Palmaria palmata*) resulting from the simultaneous water-extraction of phycobiliproteins and chlorophyll a. *Food Res. Int.* **2017**, *100*, 514–521. [CrossRef]
57. Viršilė, A.; Brazaitytė, A.; Vaštakaitė-Kairienė, V.; Jankauskienė, J.; Miliauskienė, J.; Samuolienė, G.; Novičkovas, A.; Duchovskis, P. Nitrate, nitrite, protein, amino acid contents, and photosynthetic and growth characteristics of tatsoi cultivated under various photon flux densities and spectral light compositions. *Sci. Hortic.* **2019**, *258*, 108781. [CrossRef]
58. Zhang, X.; Bian, Z.; Yuan, X.; Chen, X.; Lu, C. A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. *Trends Food Sci. Tech.* **2020**, *99*, 203–216. [CrossRef]
59. Grabowska, A.; Kunicki, E.; Sekara, A.; Kalisz, A.; Wojciechowska, R. The effect of cultivar and biostimulant treatment on the carrot yield and its quality. *Veg. Crops Res. Bull.* **2012**, *77*, 37–48. [CrossRef]
60. Ali, O.; Ramsubhag, A.; Jayaraman, J. Biostimulant properties of seaweed extracts in plants: Implications towards sustainable crop production. *Plants* **2021**, *10*, 531. [CrossRef] [PubMed]
61. Valdrighi, M.M.; Pera, A.; Agnolucci, M.; Frassinetti, S.; Lunardi, D.; Vallini, G. Effects of compost-derived humic acids on vegetable biomass production and microbial growth within a plant (*Cichorium intybus*)-soil system: A comparative study. *Agric. Ecosyst. Environ.* **1996**, *58*, 133–144. [CrossRef]
62. Colla, G.; Nardi, S.; Cardarelli, M.; Ertani, A.; Lucini, L.; Canaguier, R.; Rouphael, Y. Protein hydrolysates as biostimu-lants in horticulture. *Sci. Hortic.* **2015**, *196*, 28–38. [CrossRef]
63. Moe, L.A. Amino acids in the rhizosphere: From plants to microbes. *Am. J. Bot.* **2013**, *100*, 1692–1705. [CrossRef] [PubMed]
64. Craigie, J.S. Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.* **2010**, *23*, 371–393. [CrossRef]
65. Ertani, A.; Francioso, O.; Tinti, A.; Schiavon, M.; Pizzeghello, D.; Nardi, S. Evaluation of seaweed extracts from *Laminaria* and *Ascophyllum nodosum* spp. as biostimulants in *Zea mays* L. using a combination of chemical, biochemical and morphological approaches. *Front. Plant Sci.* **2018**, *9*, 428. [CrossRef]
66. Mondal, D.; Ghosh, A.; Prasad, K.; Singh, S.; Bhatt, N.; Zodape, S.T.; Chaudhary, J.P.; Chaudhari, J.; Chatterjee, P.B.; Seth, A.; et al. Elimination of gibberellin from Kappaphycus alvarezii seaweed sap foliar spray enhances corn stover production without compromising the grain yield advantage. *Plant Growth Regul.* **2015**, *75*, 657–666. [CrossRef]
67. Aguirre, E.; Lemenager, D.; Bacaicoa, E.; Fuentes, M.; Baigorri, R.; Zamarreno, A.M.; Garcia-Mina, J.M. The root application of a purified leonardite humic acid modifies the transcriptional regulation of the main physiological root responses to Fe deficiency in Fe-sufficient cucumber plants. *Plant Physiol. Biochem.* **2009**, *47*, 215–223. [CrossRef]
68. Azcona, I.; Pascual, I.; Aguirreolea, J.; Fuentes, M.; García-Mina, J.M.; Sánchez-Díaz, M. Growth and development of pepper are affected by humic substances derived from composted sludge. *J. Soil Sci. Plant Nut.* **2011**, *174*, 916–924. [CrossRef]
69. Chen, Y.; Magen, H.; Riov, J. Humic substances originating from rapidly decomposing organic matter: Properties and effects on plant growth. In *Humic Substances in the Global Environment and Implication on Human Health*; Senesi, N., Tiano, E.M., Eds.; Elsevier: Amsterdam, The Netherland, 1994; pp. 427–433.
70. Yang, X.; Qin, W.; Wang, L.; Zhang, M.; Niu, Z. Long-term variations of surface solar radiation in China from routine meteorological observations. *Atmos. Res.* **2021**, *260*, 105715. [CrossRef]
71. Gent, M.P.N. Effect of temperature on composition of hydroponic lettuce. *Acta Hortic.* **2016**, *1123*, 95–100. [CrossRef]
72. Miller, A.; Langenhoven, P.; Nemali, K. Maximizing Productivity of Greenhouse-grown Hydroponic Lettuce during Winter. *HortScience* **2020**, *55*, 1963–1969. [CrossRef]
73. Nair, P.; Kandasamy, S.; Zhang, J.; Ji, X.; Kirby, C.; Benkel, B.; Hodges, M.D.; Critchley, A.T.; Hiltz, D.; Prithiviraj, B. Transcriptional and metabolomic analysis of *Ascophyllum nodosum* mediated freezing tolerance in *Arabidopsis thaliana*. *BMC Genom.* **2012**, *13*, 643. [CrossRef] [PubMed]
74. Colla, G.; Svecová, E.; Cardarelli, M.; Rouphael, Y.; Reynaud, H.; Canaguier, R.; Planques, B. Effectiveness of a Plant-Derived Protein Hydrolysate to Improve Crop Performances under Different Growing Conditions. *Acta Hortic.* **2013**, *1009*, 175–179. [CrossRef]
75. Koukounaras, A.; Tsouvaltzis, P.; Siomos, A.S. Effect of root and foliar application of amino acids on the growth and yield of greenhouse tomato in different fertilization levels. *J. Food Agric. Environ.* **2013**, *11*, 644–648.
76. Dunstone, R.L.; Richards, R.A.; Rawson, H.M. Variable responses of stomatal conductance, growth, and yield to fulvic acid applications to wheat. *Aust. J. Agric. Res.* **1988**, *39*, 547–553. [CrossRef]

77. Michalak, I.; Chojnacka, K.; Dmytryk, A.; Wilk, R.; Gramza, M.; Roj, E. Evaluation of Supercritical Extracts of Algae as Biostimulants of Plant Growth in Field Trials. *Front. Plant Sci.* **2016**, *7*, 1591. [CrossRef]
78. Cristofano, F.; El-Nakhel, C.; Pannico, A.; Giordano, M.; Colla, G.; Rouphael, Y. Foliar and Root Applications of Vegetal-Derived Protein Hydrolysates Differentially Enhance the Yield and Qualitative Attributes of Two Lettuce Cultivars Grown in Floating System. *Agronomy* **2021**, *11*, 1194. [CrossRef]