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Microbe-Plant Growing Media Interactions Modulate the Effectiveness of Bacterial Amendments on Lettuce Performance Inside a Plant Factory with Artificial Lighting

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Abstract: There is a need for plant growing media that can support a beneficial microbial root environment to ensure that optimal plant growth properties can be achieved. We investigated the effect of five rhizosphere bacterial community inocula (BCI S1–5) that were collected at three open field organic farms and two soilless farms on the performance of lettuce (*Lactuca sativa* L.). The lettuce plants were grown in ten different plant growing media (M1–10) composed of 60% *v/v* peat (black peat or white peat), 20% *v/v* other organics (coir pith or wood fiber), 10% v/v composted materials (composted bark or green waste compost) and 10% *v/v* inorganic materials (perlite or sand), and one commercial plant growing medium inside a plant factory with artificial lighting. Fractional factorial design of experiments analysis revealed that the bacterial community inoculum, plant growing medium composition, and their interaction determine plant performance. The impact of bacterial amendments on the plant phenotype relied on the bacterial source. For example, S3 treatment significantly increased lettuce shoot fresh weight (+57%), lettuce head area (+29%), root fresh weight (+53%), and NO_3-content (+53%), while S1 treatment significantly increased lettuce shoot dry weight (+15%), total phenolic content (+65%), and decreased NO_3-content (−67%). However, the effectiveness of S3 and S1 treatment depended on plant growing medium composition. Principal component analysis revealed that shoot fresh weight, lettuce head area, root fresh weight, and shoot dry weight were the dominant parameters contributing to the variation in the interactions. The dominant treatments were S3-M8, S1-M7, S2-M4, the commercial plant growing medium, S1-M2, and S3-M10. Proper selection of plant growing medium composition is critical for the efficacy of bacterial amendments and achieving optimal plant performance inside a plant factory with artificial lighting.

Keywords: plant growth-promoting rhizobacteria (PGPR); growing media; rhizosphere; lettuce; plant factory; soilless culture; plant quality; plant yield; microbiome; beneficial bacteria

1. Introduction

A growing world population in the course of climate change requires the food supply chain to be revised to secure future universal access to food in a sustainable way [1,2]. In controlled-environment agriculture (CEA), the recent development of state-of-the-art plant factories with artificial lighting (PFAL) allows maximizing plant growth in a resource use efficient way (water, CO_2, fertilizer, energy, etc.) [3]. Plant factories with artificial lighting can tap into new markets that are inaccessible to open-field production and conventional greenhouses by locally producing leafy greens, herbs, medicinal plants, and transplants year-round for local consumption [4].

Plant factories with artificial lighting utilize soilless culture methods [5]. Soilless culture typically requires a plant growing medium that provides a proper physicochemical and biological environment for rooting and plant growth during the seedling stage [6]. Peat, partially degraded *Sphagnum* mosses that accumulated over thousands of years under waterlogged conditions within mires, has been widely used as a plant growing medium because of its low economic cost and good performance [7,8]. However, access to peat will be limited because of sustainability and environmental concerns involving the peat production process [9–11]. Sustainable alternatives are being investigated and a variety of these are on the market (e.g., coir pith, wood fiber, composted materials, biochar, etc.) [6,8,12–16]. Nevertheless, peat will remain an essential plant growing medium constituent, for dilution purposes at any rate as it allows the blending of alternative and circular raw materials [7]. At the same time, because of the expanding world population, the demand for plant growing media is expected to increase drastically [17]. Newly developed peat-reduced plant growing media have to perform equal to or even outperform peat, to ensure universal access to food.

When selecting new plant growing medium materials, environmental factors have become as important as performance and economic cost. However, little attention is given to the microbial properties of these products and their potential to support the amendment of plant growth-promoting rhizobacteria (PGPR). Contrary to plant growing media, soil bacterial communities are widely researched [18]. Soils contain an immense diversity in bacterial communities, enabling various soil ecosystem functions [19]. However, only a minority of bacterial taxa, including Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, encompass the diversity present in soils [19,20]. Plants are in continuous contact with soil bacterial communities through their roots. Via rhizodeposition, plants recruit soil bacteria to the rhizosphere and endosphere that improve the capacity of the plant to adapt to the environment [20–25]. These PGPRs can stimulate germination, enhance growth, improve nutrient acquisition, promote stress resistance, and enable disease suppression [26–29].

Globally, agro-industries are starting to embrace PGPR technology but are confronted with strong variation in efficacy of PGPR application, with no benefits to considerable benefits being reported [30–33]. The underlying factors causing the differential activity are not well known. The development of bacterial amendments mainly focused on single strain PGPR products [34–37]. The complexity of bacterial communities and their interactions with environmental factors and crop specificity is suspected to play an important role in the success of the plant-microbe interaction [26,34,38,39].

Plant growing medium composition may be a determining factor in the successful amendment of microbes in a soilless environment. Rhizosphere bacteria show specific microbial substrate uptake traits that drive the assembly of the rhizosphere bacterial community [21]. In addition to plant root exudate chemistry, plant growing media could provide a source of microbial substrate allowing modulation of the rhizosphere microbiome for improved plant performance [40,41]. The role of plant growing media in beneficial plant-microbe interactions is not well studied [26]. There is evidence that plant growing media have distinct microbial features that can provide stability and resilience to crops in a diverse soilless environment. The complex biological and physicochemical interactions in organic plant growing media influence the rhizosphere microbial communities of the plant [42]. Organic plant growing media have a more diverse and sTable microbial community that decreases the susceptibility of the eggplant *Solanum melongena* to the hairy roots pathogen *Agrobacterium rhizogenes* [43]. Composts maintain a high microbial diversity that is critical to the suppression of soil-borne pathogens and improving plant

performance [44–47]. Biochar amendment to peat growing media and soil may improve plant growth and disease suppressiveness [48–51]. These positive effects of biochar amendment are linked to the activity, diversity, and composition of the rhizosphere microbial community [52,53]. There is evidence that PGPR amendment can improve plant growth and decrease phytopathogen infections in soilless culture [37,54–57]. Though, the role of plant growing medium composition as a potential driver in the success of PGPR amendment is much less clear. Recent research has studied the use of plant growing medium constituents as a carrier material for bacterial inocula [33,40,41,58,59]. For example, Nadeem et al. [41] showed that the combined use of biochar, compost, and the PGPR *Pseudomonas fluorescens* alleviated the negative effect of water deficit on cucumber growth. More research has to be done on the mechanisms of action and the efficiency of using different plant growing medium constituents as a carrier for PGPR consortia.

At the start of our work, we hypothesized that plant growing medium composition plays a decisive role in the effectiveness of PGPR amendment inside a complex PFAL environment. Here we report results that show that specific microbe-plant growing medium interactions are the major determinants of performance for *Lactuca sativa* L. (lettuce). Seedlings of lettuce, a leafy green that is abundantly produced in PFALs, were grown in different plant growing media, inoculated with a few selected bacterial communities, and transferred to a PFAL. The different plant growing media were composed by varying five raw material groups: (a) peat (black peat and white peat), (b) other organics (coir pith and wood fiber), (c) composted materials (composted bark and green waste compost), (d) inorganic materials (perlite and sand), and (e) Arabic gum dosed at 1 $kg \cdot m^{-3}$ or 5 $kg \cdot m^{-3}$. Lettuce root-associated bacterial community samples were collected from soil and soilless farms and used as an inoculum. Shoot fresh weight (FW), lettuce head area (LHA), root fresh weight (RW), shoot dry weight (DW), total phenolic content (TPC), NO_3-content, and leaf pigments were quantified.

2. Materials and Methods

2.1. Collection of Root-Associated Bacterial Communities

Lettuce root-associated bacterial community samples (S1–5) were collected at five different locations in Flanders, Belgium during the growing season: three open field organic farms and two soilless farms. An overview of all sampling locations can be found in Table 1. Sampling and extraction were performed following the method described by Barillot et al. [60]. Briefly, 30 plant and root-associated soil samples (20 cm^2 by 30 cm deep) were collected at each location, transported in polyethylene bags, and stored at 4 °C. Bulk soil was removed by manually shaking the roots. The rhizosphere fraction was collected by manually washing the roots in a sterile 0.9% NaCl solution for 10 min. Roots were subsequently washed by hand in sterile 0.9% NaCl + 0.01% Tween 80 for 10 min to obtain the rhizoplane fraction. Both fractions were homogenized on an orbital shaker (125 rpm, 90 min, room temperature). The homogenized samples were centrifuged at low speed (150 g, 10 min, room temperature) to separate soil particles and other debris from the supernatant containing bacteria. The supernatants were centrifuged at high speed (9425 g, 10 min, room temperature) to collect the bacteria in the pellet. The bacterial pellets were resuspended in tryptic soy broth (TSB) + 15% glycerol and stored at −80 °C.

Table 1. Overview of Rhizosphere Sampling Locations.

Sample	Collection Date	Location	Crop	Cultivation Method	Plant Growing Medium
S1	3 October 2017	Wachtebeke, Belgium	*Lactuca sativa* var. crispa (oakleaf)	Organic open field	Sand
S2	17 October 2017	Moerbeke-Waas, Belgium	*Lactuca sativa* var. crispa (oakleaf)	Organic open field	Loamy sand
S3	21 November 2017	Onze-Lieve-Vrouw-Waver, Belgium	*Lactuca sativa* var. crispa (lollo bionda)	Soilless	Black peat
S4	12 December 2017	Ardooie, Belgium	*Lactuca sativa* var. capitata (butterhead)	Soilless	Black peat
S5	5 June 2018	Lochristi, Belgium	*Lactuca sativa* var. crispa (lollo bionda)	Organic open field	Sand

The amount of live bacterial cells present in the rhizosphere and rhizoplane fractions was estimated using flow cytometric analysis to standardize bacterial inoculation in further analysis (see Section 2.3). The samples were diluted and stained with SYBR® Green I combined with propidium iodide (SGPI, 100 × concentrate SYBR® Green I, Invitrogen, and 50 × 20 mM propidium iodide, Invitrogen, in 0.22 µm-filtered dimethyl sulfoxide) for live-dead analysis. Staining was performed as described previously, with incubation for 13 min at 37 °C [61]. Samples were analyzed immediately after incubation on a C6+ flow cytometer (BD Biosciences, Belgium), which was equipped with four fluorescence detectors (530/30 nm, 585/40 nm, >670 nm, and 675/25 nm), two scatter detectors and a 20-mW 488-nm laser. The flow cytometer was operated with Milli-Q (Merck, Darmstadt, Germany) as sheath fluid.

2.2. Plant Growing Media Composition

Ten different experimental plant growing media were composed (M1–10; Table 2). The raw material collection took place at Agaris Belgium NV, Gent, Belgium. All plant growing media have following volumetric composition: 60% *v/v* peat, 20% *v/v* other organics, 10% *v/v* composted materials and 10% *v/v* inorganic materials. For eight plant growing media (M1, M3, M4, M5, M7, M8, M9, and M10), selection of the raw material and the Arabic gum dose was based on a 2^{5-2}_{III} fractional factorial design (Tables S1 and S2). Based on a previous study [62], two more plant growing media were composed: M2 and M6, both showing high microbial activity potential. The peat and coir based commercial plant growing medium (75% peat and 25% coir fibers, Jiffy International AS, Kristiansand, Norway) was used as a control to evaluate the performance of the experimental plant growing media. The physicochemical properties of each plant growing medium were analyzed in triplicate following Verdonck and Gabriels [63], and Gabriels et al. [64]. The data obtained is shown in Table S3.

Table 2. Composition of Plant Growing Media. Each plant growing medium consists of 4 raw material groups at different volume per volume (% v/v): peat (black peat BP or white peat WP), other organics (coir pith CP or wood fiber WF), composted materials (composted bark CB or green waste compost GC) and inorganic materials (perlite P or sand S). Arabic gum was dosed at 1 kg·m^{-3} or 5 kg·m^{-3}.

Plant Growing Medium	Peat (60% *v/v*)	Other Organics (20% *v/v*)	Composted Materials (10% *v/v*)	Inorganic Materials (10% *v/v*)	Arabic Gum (kg·m^{-3})
M1	WP	CP	CB	P	1
M2	WP	WF	CB	P	5
M3	BP	CP	CB	S	5
M4	WP	CP	GC	P	5
M5	WP	WF	CB	S	1
M6	WP	CP	CB	S	5
M7	BP	WF	CB	P	5
M8	BP	CP	GC	S	1
M9	WP	WF	GC	S	5
M10	BP	WF	GC	P	1

2.3. Plant Growth and Inoculation

Sterilized hydroponic mesh pots, with 6.5 cm height, 5 cm bottom diameter, and 7 cm top diameter, were fitted with hydroponic paper (Ellepot, Esbjerg, Denmark), filled with 200 mL of plant growing medium, and watered to saturation. Batavia lettuce seeds (Enza Zaden, Enkhuizen, The Netherlands) were sown in ten pots of each plant growing medium (Table 2). The seeds were wetted by spraying water. The pots were placed in a sterilized tray inside a growth chamber (Urban Crop Solutions, Beveren-Leie, Belgium) with a temperature of 22–23 °C, relative humidity of 60–70%, and 800 ppm CO_2-fertilization. LED light fixtures (Urban Crop Solutions, Beveren-Leie, Belgium) provided an 18 h light regime at 220 $\mu mol.m^{-2} \cdot s^{-1}$. For the following two weeks, the pots were irrigated by hand with tap water when necessary. Two weeks after sowing six pots with uniform lettuce seedlings were selected per plant growing medium and placed in a sterilized tray fitted with an overflow drain for automated irrigation. In each tray, the six selected plants from a single plant growing medium were positioned at a distance of 18.6 cm in length and 22.2 cm in width from each other.

At this point, the bacterial community inocula (BCI S1–5) were applied to all experimental plant growing media at the base of the plant. Based on the live bacterial cell counts, determined with flow cytometric analysis (see Section 2.1), equal volumes of the collected rhizosphere and rhizoplane fractions were mixed and diluted with TSB. Application of 1 mL of inoculum provided a dose of 3.2×10^9 CFU per L plant growing medium. As a positive control treatment (PGPR), *Bacillus* sp. with plant growth-promoting properties was added as an inoculum to each plant growing medium at a dose of 3.2×10^9 CFU per L plant growing medium. As a negative control treatment (C), 1 mL of sterile TSB solution was added to every plant growing medium. Unlike the experimental plant growing media, the commercial plant growing medium was only treated with 1mL of sterile TSB solution. After inoculation, the trays were placed inside a PFAL (Urban Crop Solutions, Beveren-Leie, Belgium) for three weeks under the growing conditions as mentioned above. During these three weeks, all plants were irrigated automatically four times a day with the following nutrient solution: 14 mM NO_3^-, 2 mM PO_4^{3-}, 7 mM K^+, 4 mM Ca^{2+}, 2 mM Mg^{2+}, 635 µM SO_4^{2-}, 72 µM Fe^{2+}, 18 µM Mn^{2+}, 2 µM Zn^{2+}, 46 µM B, 0.8 µM Cu^{2+}, 1 µM Mo^{2-}, and 356 µM Si.

The experiment was split into five batches. Each batch consisted of all ten experimental plant growing media treated with one bacterial community inoculum, two randomly selected experimental plant growing media treated with the positive control, and two randomly selected experimental plant growing media treated with the negative control. The commercial plant growing medium was added to the last batch.

2.4. Plant Sample Analysis

2.4.1. Plant Sample Processing

The plants were harvested three weeks after inoculation. During harvest, top view images were taken to determine the lettuce head area (LHA) by image processing in ImageJ [65]. The harvested plants were transported in polyethylene bags to avoid excessive transpiration and stored at 4 °C until further processing. Within 24 h, the plant samples were cut at the base to separate root and shoot. Shoot fresh weight (FW) was determined by weighing the lettuce head immediately after cutting. After weighing, a section (weighing approximately 10 g) of the whole lettuce head, containing young and mature leaves, was cut out. This subsample was ground using an IKA A11 liquid nitrogen mixer (IKA, Staufen, Germany) and stored at −80 °C until further analysis. To determine the shoot dry weight (DW), the remaining shoot was placed in a paper bag and dried at 70 °C for 72 h. The difference in weight before and after drying was used to calculate the shoot dry weight of the sample. Next, the dried subsample was ground with a coffee mill (Proficook PC-KSW 1021, Clatronic International GmbH, Kempen, Germany) and stored until further analysis. If the total shoot weight was too low for obtaining both fresh and dry subsamples, priority was given to the fresh subsample. This was the case for the following treatments: S1-M6, S2-M3, PGPR-M3, PGPR-M6, and PGPR-M9.

The roots and plant growing medium of the sample were used to isolate the root-associated bacterial community following the procedure described in Section 2.1. After the second washing step, plant roots were weighed to determine root fresh weight (RW).

2.4.2. Total Phenolic Content

Total phenolic content (TPC) was ascertained following the Folin–Ciocalteu method [66]. Colorimetric TPC measurements of fresh subsample extracts in 80% methanol were carried out with a Tecan infinite plate reader (Tecan Group Ltd., Männedorf, Switzerland) at a wavelength of 765 nm. Total phenolic content was expressed as mg gallic acid equivalents (GAE) per 100 g FW.

2.4.3. Nitrate Content

The nitrate (NO_3) concentration was determined colorimetrically with salicylic acid as described by Cataldo et al. [67]. Oven-dried subsamples were used. Measurements were performed by a Tecan infinite plate reader at a wavelength of 410 nm.

2.4.4. Chlorophylls and Carotenoids

Chlorophyll a (Chl_a) a, chlorophyll b (Chl_b), and carotenoids were quantified by UV-VIS spectroscopy of a whole-pigment extract of the fresh subsamples in 80% acetone [68]. Absorption at 470 nm, 648.8 nm, and 663.2 nm wavelengths, and zero absorption at 750 nm were measured with a Tecan infinite plate reader. The amount of Chl_a, Chl_b, and carotenoids (C_{x+c}) were calculated in $\mu g.mL^{-1}$ with the following equations:

$$Chl_a = 12.25 \times A_{663.2} - 2.79 \times A_{646.8} \quad (1)$$

$$Chl_b = 21.5 \times A_{646.8} - 5.1 \times A_{663.2} \quad (2)$$

$$C_{x+c} = (1000 \times A_{470} - 1.82 \times Chl_a - 85.02 \times Chl_b)/198 \quad (3)$$

2.5. Statistical Analysis

Before subjecting the plant performance data to statistical analysis, any data points further than 1.5 times the interquartile range from the mean were considered as outliers, and were removed from the dataset. Analyses of differences between BCI means and principal component analysis (PCA) were carried out using R 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). All statistical analyses were performed at the 95% confidence level. Differences between BCI means were analyzed per plant growing medium. Quantile-quantile plots were used to check for normality of the data (*stats* package). Levene's test was used to determine the homogeneity of variance across groups (*car* package). In case the assumptions of normality and homoscedasticity were met, one-way ANOVA was used to determine significant differences between BCI means (*stats* package). As a post hoc test, a linear model was created using the *stats* package. Following, the estimated marginal means were calculated, using the Tukey's honest significance test for separation of the means at the $P < 0.05$ level (*emmeans* package). Finally, a compact letter display was created using the *multcomp* package. In case the assumptions of normality and homoscedasticity were not met, the Kruskal–Wallis test was used to compare BCI means (*stats* package). Dunn's test with Bonferroni correction was used as a post hoc method to separate the means (*FSA* package). A compact letter display of the comparison of means was produced using the *rcompanion* package.

Principal component analysis was used to determine which BCIs and plant growing media contribute most to the variation in data, and to which key performance parameters they are associated to. The data was standardized by scaling to unit variance before analysis (*stats* package). A quality of representation (cos^2) correlation circle, a contribution plot of the variables, and a contribution plot of the samples were generated using the *factoextra* package.

A 1/4 fractional factorial statistical design of experiments (DOE; 2^{5-2}_{III}) was used to simultaneously evaluate the effect of the plant growing medium raw material groups (five control factors having a high +1 and a low −1 factor level) and their interactions on the plant performance parameters. The fractional factorial design was established and analyzed in Minitab 17 (Minitab Inc., State College, Pennsylvania, United States) using main effects plots, ANOVA, and response optimization. The design was extended with an additional control factor to determine the effect of inoculation. The levels of the inoculation control factor were: negative control treatment (C) as low factor level and inoculum treatment (S1–5) as high factor level. An overview of all control factors and the final fractional factorial design can be found in Tables S1 and S2. Following decisions were made to deal with aliasing effects. (a) Three-factor and higher-order interactions are extremely rare and were omitted. (b) When aliasing occurred between the main effect and two-factor interactions, the main effect was assumed significant. (c) Aliasing between two-factor interactions was resolved by following the heredity principle: an interaction effect is likely significant when the main effects involved are also significant [69].

3. Results

3.1. Effect of Bacterial Community Inoculum and Plant Growing Medium on Shoot Fresh Weight

Both BCI ($P < 0.001$) and plant growing medium ($P < 0.001$) significantly altered FW. Lettuce FW varied from 6.03 g (S2-M3) to 74.85 g (S3-M8). Significant differences in FW were observed between BCIs in each plant growing medium (Figure 1). Bacterial community inoculum S3 significantly ($P < 0.05$) increased FW in multiple plant growing media (M5, M7, M8, M9, and M10) compared to C. For example, FW was 17.78 g in C-M7 compared to 50.83 g in S3-M7, which is more than a 2.5-fold increase. S3-M8 (74.85 g) and S3-M10 (64.63 g) were the only BCI and plant growing medium combinations that had significantly ($P < 0.05$) higher FW than the commercial plant growing medium (48.65 g). On average, inoculating the plant growing media with BCI S3 increased FW with 57% ($P < 0.001$; Figure 2f). Response optimization showed that, excluding BCI S3, the addition of a BCI was not vital to reaching maximal FW (Table S4). Moreover, BCI S3 treatment was the largest contributor to FW, compared to the plant growing medium raw material groups (Figure S1). The positive effects of S3 on FW do not occur in each plant growing medium, underlining the importance of plant growing medium composition on the effectiveness of BCI treatment.

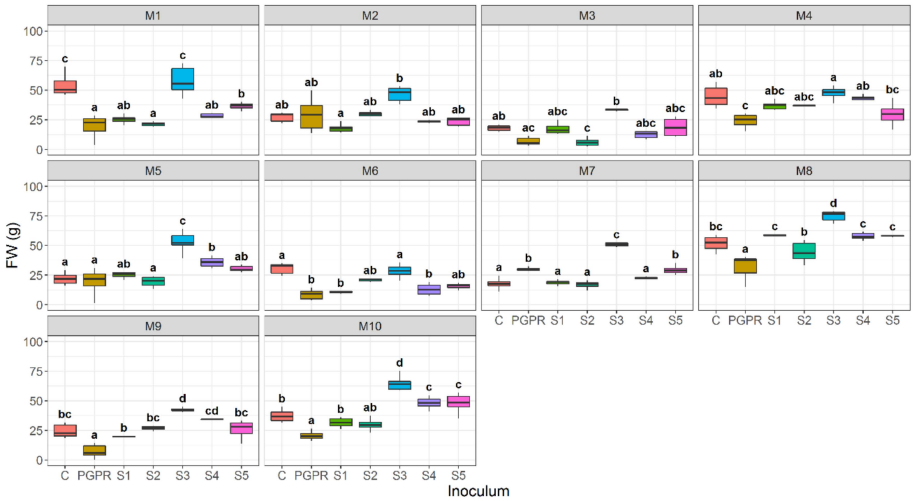

Figure 1. Boxplot of shoot fresh weight (FW; g) grouped per plant growing medium. Letters show comparison of BCI means per plant growing medium at the 95% confidence level. S indicates the bacterial community inoculum, M indicates the plant growing medium, C indicates the negative control treatment without addition of inoculum, and PGPR indicates the positive control treatment with a *Bacillus* sp. inoculum. Number of plants ≥ 3.

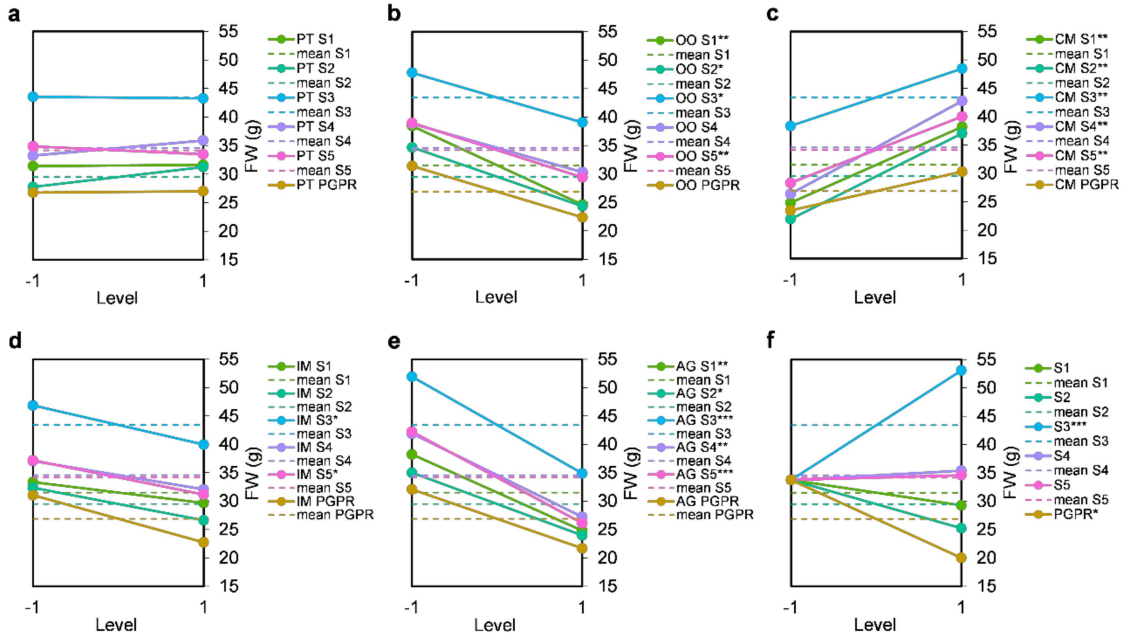

Figure 2. Main effects of plant growing medium constituents on shoot fresh weight (FW; g) under different bacterial community inoculum treatments (S1–5 and positive control PGPR). (**a**) Peat (PT; −1 = black peat and 1 = white peat); (**b**) Other organics (OO; −1 = coir pith and 1 = wood fiber); (**c**) Composted materials (CM; −1 = composted bark and 1 = green waste compost); (**d**) Inorganic materials (IM; −1 = perlite and 1 = sand); (**e**) Arabic gum (AG; −1 = 1 kg.m^{-3} and 1 = 5 kg.m^{-3}); (**f**) Bacterial inoculum (BCI; −1 = C and 1 = S1–5 or PGPR). Dashed lines indicate mean levels of FW for each bacterial treatment. Asterisks indicate level of significance: $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

Surprisingly, the positive control treatment PGPR significantly decreased FW in several plant growing media (M1, M4, M6, M8, M9, and M10) compared to negative control C (Figure 1). For example, treating M9 with PGPR decreased FW with 70% compared to C. On average, the positive control treatment significantly decreased FW with 41% ($P < 0.05$; Figure 2f).

A significant ($P < 0.001$) interaction between plant growing medium and BCI was observed. Indeed, DOE analysis revealed a significant ($P < 0.05$) interaction effect between BCI S3 and the type of other organics (Figure S2). In the absence of S3, FW of lettuce grown in plant growing media containing coir pith (42.15 g) was higher than plant growing media with wood fiber (25.37 g). When treated with S3, lettuce FW increased and the difference in FW between the OO raw materials was no longer visible (coir pith: 53.42 g and wood fiber: 52.72 g). Treatment with BCI S3 negates the advantage of using coir pith over wood fiber.

Design of experiments analysis showed significant differences in FW between plant growing media, following similar trends in each BCI treatment (Figure 2). Use of coir pith increased ($P < 0.05$ in S1, S2, S3, and S5) FW compared to the use of wood fiber (+37% averaged over S1–5). Plant growing media containing green waste compost showed significantly ($P < 0.01$ in S1–5) higher FW compared to plant growing media comprising composted bark (+47% averaged over S1–5). Application of perlite instead of sand as inorganic material showed a positive trend ($P < 0.05$ in S3 and S5) in FW (+20% averaged over S1–5). The type of peat (black peat or white peat) did not significantly affect FW. Increasing the dose of Arabic gum significantly ($P < 0.05$ in S1–5) lowered FW (−35% averaged over S1–5). For the majority of the BCI treatments (S1, S2, S3, and S5) the use of coir pith, green waste compost, and a low dose of Arabic gum in the plant growing medium was required to reach maximal FW (Table S4). Additionally, the use of perlite was needed in BCI treatments S3 and S5.

A significant ($P < 0.05$ in S3 and S5) interaction effect occurred between the type of other organics and the dose of Arabic gum (Figures S2 and S3). Under a low dose of Arabic gum (1 kg·m^{-3}), the use

of coir pith increased FW (59.64 g in S3) compared to wood fiber (44.22 g in S3). By increasing the amount of Arabic gum in the plant growing medium (5 $kg.m^{-3}$) FW dropped and the difference in FW between coir pith (35.92 g in S3) and wood fiber (33.87 g in S3) was lost.

3.2. Effect of Bacterial Community Inoculum and Plant Growing Medium on Lettuce Head Area

Lettuce head area varied significantly depending on BCI ($P < 0.001$), plant growing medium ($P < 0.001$), and BCI-plant growing medium interaction ($P < 0.001$). The BCI-plant growing medium combination S3-M8 (457.24 cm^2) exhibited the highest LHA, while S2-M3 (86.91 cm^2) showed the lowest LHA. Bacterial community inoculum treatment resulted in significant differences in LHA in each plant growing medium (Figure S4). Treatment with BCI S3 significantly ($P < 0.05$) increased LHA compared to C in the plant growing media M3, M5, M7, and M8. For example, the treatment of plant growing medium M3 with BCI S3 (305 cm^2) resulted in a more than 1.5-fold increase in LHA compared to the M3 control treatment (170 cm^2). The LHA of S3-M8 (457.24 cm^2), the highest LHA of all treatments, did not differ significantly from the LHA of the commercial plant growing medium (429.35 cm^2). The average increase in LHA under BCI S3 treatment was 29% ($P < 0.01$; Figure S5f). Response optimization showed that treatment with BCI S3 was necessary to obtain maximal LHA (Table S5). Also, S3 treatment was the largest contributor to LHA, compared to the plant growing medium raw material groups (Figure S6).

Bacterial community inoculum S2 treatment significantly ($P < 0.05$) decreased LHA compared to Fifurdecreased LHA with 51% compared to C (358 cm^2). On average, BCI S2 significantly ($P < 0.01$) decreased LHA with 33% (Figure S5f). Response optimization towards maximal LHA is reached after the removal of BCI S2 (Table S5). Additionally, BCI S2 was the largest contributor to change in LHA (absolute), compared to the plant growing medium raw material groups (Figure S7).

As also noted in lettuce FW analysis, treatment with the positive control PGPR unexpectedly decreased ($P < 0.05$) LHA compared to negative control C in M1, M3, M4, M6, M8, and M10. For example, compared to C (335 cm^2), LHA decreased by 43% when M10 was treated with PGPR (189 cm^2). On average, the application of PGPR showed a strong downward trend in LHA (−26%; Figure S5f).

Plant growing medium composition significantly affected LHA (Figure S5). The use of green waste compost resulted in significantly ($P < 0.01$ in S1–5) higher LHA compared to composted bark (+35% averaged over S1–5). Application of coir pith over wood fiber showed a positive trend (+16% averaged over S1–5) but was only significant ($P < 0.01$) under BCI S1 treatment (+28% under S1). The type of peat and inorganic material did not significantly affect LHA, though utilization of perlite resulted in a positive shift in LHA compared to sand (+13% averaged over S1–5). A high dose of Arabic gum significantly ($P < 0.05$ in S1–5) lowered LHA (−22% averaged over S1–5). For all BCI treatments (S1–5) the use of green waste compost and a low dose of Arabic gum in the plant growing medium were required to reach maximal LHA (Table S5). Also, the use of coir pith was needed under BCI treatment S1.

The treatment with BCI S1 showed a significant ($P < 0.05$) interaction effect between the type of other organics and composted materials (Figure S8). When plant growing media contained green waste compost, the application of coir pith increased LHA (382.68 cm^2) compared to wood fiber (265.09 cm^2). Changing the type of compost in the plant growing media to composted bark resulted in a decline in LHA, and the difference in LHA between coir pith (243.11 cm^2) and wood fiber (223.99 cm^2) vanished.

3.3. Effect of Bacterial Community Inoculum and Plant Growing Medium on Root Fresh Weight

Treatment of the plant growing media with BCI S3 significantly ($P < 0.05$) increased RW (+53%), while both S2 and S4 significantly ($P < 0.05$) decreased RW (−53% and −18% respectively; Figure S9). Indeed, optimization of RW response towards maximum showed that the application of BCI S3 maximizes RW, while BCI S2 and S4 have to be removed to reach maximum RW (Table S6). Both BCI S2 and S3 treatment were the largest contributors to RW, compared to the plant growing medium raw

material groups (Figures S10 and S11). The application of the positive control PGPR biostimulant resulted in a strong downward trend in RW (−49%; Figure S9).

Contrary to FW and LHA, the type of peat significantly ($P < 0.05$ in S1 and S4) affected RW (Figure S9). Application of white peat increased lettuce RW compared to black peat (+41% averaged over S1–5). For the remaining plant growing medium raw material groups, similar effects on RW were observed compared to FW and LHA (see Sections 3.1 and 3.2). However, the discerned trends were only marginally significant under certain BCI treatments. The interaction between the type of other organics and composted materials significantly ($P < 0.05$) affected RW under BCI S1 and S4 treatment (Figures S12 and S13). This interaction effect was also detected in the LHA DOE analysis (see Section 3.2). Bacterial community inoculum treatment S4 showed significant ($P < 0.001$) interaction with several plant growing medium raw material groups (peat, other organics, and inorganic materials): when the plant growing media were inoculated with S4, the observed differences in RW between the raw material group levels vanished.

3.4. *Effect of Bacterial Community Inoculum and Plant Growing Medium on Shoot Dry Weight*

Shoot dry weight was significantly affected by BCI ($P < 0.001$), plant growing medium ($P < 0.01$), and their interaction ($P < 0.001$). Shoot dry weight varied from 4.25% DW (PGPR-M10) to 7.39% DW (S1-M7). Figure 3 shows the effect of BCI treatment on lettuce DW in each plant growing medium. Compared to C, DW rose significantly ($P < 0.05$) after treatment with BCI S1 in several plant growing media (M1, M2, M4, M7, M8, and M10). For example, the treatment of plant growing media M1 and M7 with BCI S1 (S1-M1: 7.05% DW; S1-M7: 7.39% DW) resulted in a 1.3-fold increase in DW compared to C (C-M1: 5.48% DW; C-M7: 5.80% DW). Only S1-M7 (7.39% DW) and S1-M10 (7.23% DW) showed significantly ($P < 0.05$) higher DW compared to the commercial plant growing medium (6.35% DW). On average, treatment of the plant growing media with BCI S1 significantly ($P < 0.05$) increased DW (+15%; Figure S14) and BCI S1 treatment was required to optimize DW response towards maximum (Table S7). Moreover, BCI S1 treatment was the largest contributor to DW, compared to the plant growing medium raw material groups (Figure S15).

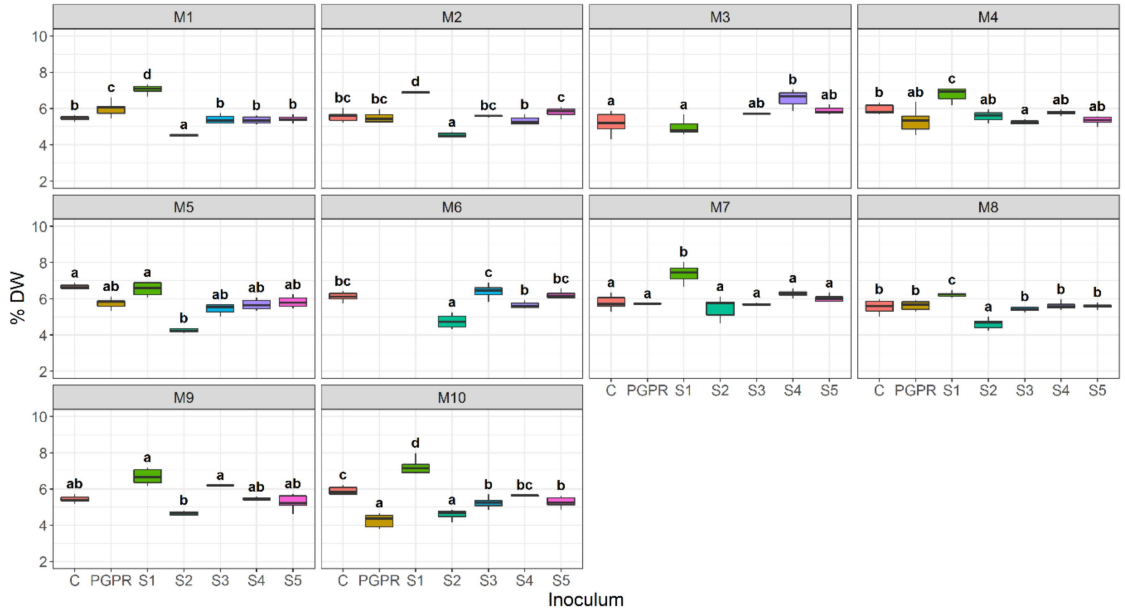

Figure 3. Boxplot of shoot dry weight (%DW) grouped per plant growing medium. Letters show comparison of BCI means per plant growing medium at the 95% confidence level. S indicates the bacterial community inoculum, M indicates the plant growing medium, C indicates the negative control treatment without addition of inoculum, and PGPR indicates the positive control treatment with a Bacillus sp. inoculum. Number of plants ≥ 3.

Bacterial community inoculum S2 significantly ($P < 0.05$) lowered DW compared to C in M1, M2, M5, M6, M8, and M10 (Figure 3). For instance, treating M2 with S2 (4.54% DW) decreased DW with 18.5% compared to C (5.57% DW). The average decrease in lettuce DW caused by BCI S2 treatment was 16% ($P < 0.01$) and S2 treatment was the largest contributor to DW compared to the plant growing medium raw material groups (Figure S16). Application of the positive control PGPR biostimulant resulted in a significant ($P < 0.01$) decline in DW (−5.3% on average; Figure S14). No significant effects of the plant growing medium raw material groups on lettuce DW were observed (Figure S14).

3.5. Effect of Bacterial Community Inoculum and Plant Growing Medium on Total Phenolic Content

Bacterial community inoculum treatment ($P < 0.001$), plant growing medium ($P < 0.001$), and their interaction ($P < 0.001$) impacted the TPC of lettuce. Total phenolic content levels were located between 91.50 mg GAE/100 g FW (S1-M2) and 12.73 mg GAE/100 g FW (S5-M1). Significant changes in TPC were detected between BCIs in each plant growing medium (Figure S17). Bacterial community inoculum S1 significantly ($P < 0.05$) increased TPC, compared to C, in several plant growing media (M1, M3, M5, and M10). For example, treating M1 with S1 (81.73 mg GAE/100 g FW) increased TPC with 210% compared to C-M1 (26.32 mg GAE/100 g FW). Compared to the commercial plant growing medium (43.13 mg GAE/100 g FW), TPC of lettuce grown in M1, M2, M5, and M7 was significantly ($P < 0.05$) higher when inoculated with S1. On average, BCI treatment S1 ($P < 0.001$) and S4 ($P < 0.01$) significantly increased TPC (+65% and +26% respectively), while BCI S5 significantly ($P < 0.05$) decreased TPC (−15%) (Figure S18). Response optimization of TPC towards maximum required the addition of S1 and S4 (Table S8). Furthermore, BCI S1 and S4 treatment were the largest contributors to TPC, compared to the plant growing medium raw material groups (Figures S19 and S20).

Design of experiments analysis (Figure S18) showed a significant effect ($P < 0.05$) of the OO raw material group on lettuce TPC under BCI S1, S3, and S5: the use of wood fiber increased TPC compared to coir pith (+26.5% averaged over S1–5). Remarkably, between BCI S1 and S5, an opposite effect of the CM raw material group on TPC was observed. Application of green waste compost over composted bark showed a negative trend in TPC (−35%) under S1 treatment, while TPC increased (+34.5%) under S5. These differences in TPC are caused by a significant ($P < 0.01$) interaction effect that occurred between the type of CM and inoculation with BCI S1 or S5 (Figures S21 and S22). In the C treatment, we observed no difference in TPC between lettuce grown in plant growing media containing either composted bark (33.88 mg GAE/100 g FW) or green waste compost (32.62 mg GAE/100 g FW). Treating BCI S1 to plant growing media containing composted bark resulted in a sharp increase in TPC (73.19 mg GAE/100 g FW), while lettuce TPC (36.65 mg GAE/100 g FW) in green waste compost plant growing media did not differ from C. Contrary to this, BCI S5 treatment of plant growing media containing composted bark resulted in a decrease in TPC (18.65 mg GAE/100 g FW), while TPC (38.04 mg GAE/100 g FW) in green waste compost plant growing media did not differ from C.

The interaction between BCI S4 and the OO raw materials group showed a significant effect ($P < 0.05$) on TPC (Figure S23). In the C treatment, TPC of lettuce grown in plant growing media containing wood fiber (39.16 mg GAE/100 g FW) was higher than in coir pith plant growing media (27.37 mg GAE/100 g FW). Inoculation with S4 increased lettuce TPC in coir pith plant growing media (43.19 mg GAE/100 g FW) but did not affect wood fiber plant growing media (40.47 mg GAE/100 g FW), whereby the difference in lettuce TPC between coir pith and wood fiber plant growing media was nullified.

3.6. Effect of Bacterial Community Inoculum and Plant Growing Medium on NO_3-Content

NO_3-content was significantly impacted by BCI source ($P < 0.001$), plant growing medium ($P < 0.001$), and BCI-plant growing medium interaction ($P < 0.001$). NO_3-content of all samples was well below the EU regulation limit (4000 mg/kg FW; EU 1258/2011), varying from 213 mg/kg FW (S1-M3) to 1952 mg/kg FW (S3-M8). Significant differences in lettuce NO_3-content between BCIs are shown in Figure S24. Bacterial community inoculum S3 treatment significantly ($P < 0.05$) increased

NO_3-content in M4, M5, M8, and M10 compared to C. For instance, NO_3-content of S3-M5 (1567 mg/kg FW) was close to 5-fold higher than C-M5 (322 mg/kg FW). On average, S3 significantly ($P < 0.05$) raised NO_3-content (+53%; Figure S25). Compared to C, NO_3-content significantly ($P < 0.05$) decreased in multiple plant growing media when treated with BCI S1 (M1, M3, M7, M8, and M10). For example, treating M1 with S1 (253 mg/kg FW) decreased NO_3-content with 84% compared to C-M1 (1626 mg/kg FW). Treatment of the plant growing media with BCI S1 significantly ($P < 0.01$) lowered NO_3-content with 67% on average (Figure S25). Both BCI S1 and S3 treatment were the largest contributors to NO_3-content, compared to the plant growing medium raw material groups (Figures S26 and S27). We observed no BCI-plant growing medium combinations with a significantly lower NO_3-content than the commercial plant growing medium (644 mg/kg FW). Contrary, multiple treatments (C-M1, C-M7, PGPR-M2, PGPR-M4, S2-M4, S2-M8, S3-M1, S3-M2, S3-M4, S3-M5, S3-M7, S3-M8, S3-M10, S4-M8, and S4-M10) showed significantly ($P < 0.05$) higher NO_3-content than the commercial plant growing medium.

Design of experiments analysis revealed that the plant growing medium raw material groups had no significant effect on NO_3-content (Figure S25). Treatment with BCI S5 showed a significant ($P < 0.05$) interaction effect between the type of other organics and the Arabic gum dose (Figure S28). NO_3-content of plant growing media containing coir pith was higher than wood fiber plant growing media under a low dose of Arabic gum. Contrary, an opposite shift in NO_3-content was observed under a high dose of Arabic gum. Application of BCI S1 was required to minimize NO_3-content, while BCI S3 should not be applied when minimizing NO_3-content (Table S9).

3.7. Effect of Bacterial Community Inoculum and Plant Growing Medium on Leaf Pigments

Chlorophyll a+b was significantly affected by BCI ($P < 0.001$), plant growing medium ($P < 0.01$), and their interaction ($P < 0.001$). Chlorophyll a + b varied from 11.65 mg/100 g FW (S5-M8) to 22.67 mg/100 g FW (S5-M7). Multiple treatments (C-M5, PGPR-M3, S1-M6, S2-M2, S2-M4, S2-M6, S2-M8, S2-M9, S2-M10, S5-M8, and S5-M10) showed significantly ($P < 0.05$) lower Chl_{a+b} levels than the commercial plant growing medium (21.48 mg/100 g FW). The effect of BCI treatment on Chl_{a+b} in each plant growing medium is shown in Figure S29. Overall, no clear trends in Chl_{a+b} levels, caused by BCI treatment or plant growing medium composition, were observed. However, DOE analysis revealed that BCI S2 treatment significantly ($P < 0.05$) decreased (−12%) Chl_{a+b} levels compared to C (Figure S30). Bacterial community inoculum S2 showed a significant ($P < 0.05$) interaction with the type of composted materials, where no difference in Chl_{a+b} levels was observed between BCI S2 and C in the composted bark plant growing media. However, BCI S2 treatment strongly decreased Chl_{a+b} levels in the green waste compost plant growing media compared to C (Figure S31). Contrary, BCI S4 treatment did not affect Chl_{a+b} levels in the green waste compost plant growing media compared to C. Instead, BCI S4 treatment increased Chl_{a+b} levels compared to C in the composted bark plant growing media (Figure S32). Indeed, response optimization towards maximal Chl_{a+b} showed that the combination of green waste compost with no BCI S2 application and BCI S4 application in combination with composted bark was optimal (Table S10).

When comparing all treatments, we observed that BCI treatment ($P < 0.001$), plant growing medium composition ($P < 0.001$), and their interaction ($P < 0.001$) significantly affected lettuce carotenoid content. Carotenoid levels were located between 3.12 mg/100 g FW (S2-M4) and 4.16 mg/100 g FW (S5-M7). Carotenoid content of lettuce grown in the commercial plant growing medium (4.11 mg/100 g FW) was significantly ($P < 0.05$) higher than of lettuce from S1-M8, S2-M2, S2-M4, S2-M6, S2-M8, S2-M10, S3-M8, and S3-M9. When examining the effect of BCI treatment grouped per plant growing medium (Figure S33), no clear shifts in carotenoid levels can be distinguished. Also, DOE analysis did not show any significant effects of the plant growing medium raw material groups on carotenoid content (Figure S34). However, it was revealed that BCI S2 significantly ($P < 0.05$) decreased carotenoid content compared to C (−6%). Additionally, BCI S2 treatment was the largest contributor to lettuce carotenoid content, compared to the plant growing medium raw material groups (Figure S35). A significant ($P < 0.05$)

interaction effect between composted materials and inorganic materials was observed under BCI S3 treatment (Figure S36a). When using perlite as inorganic material, carotenoid content was not affected by the type of compost. However, carotenoid content decreased when using sand in combination with green waste compost compared to composted bark. Bacterial community inoculum S3 directly interacted ($P < 0.05$) with the type of inorganic material (Figure S36b). The carotenoid content of the plant growing media containing sand was not affected by BCI S3 treatment, while BCI S3 treatment decreased carotenoid content in the plant growing media containing perlite. When treating with BCI S4, carotenoid content of the composted bark plant growing media was higher than the green waste compost plant growing media. This difference in carotenoid content was not present under C treatment (Figure S37). The type of other organics did not affect carotenoid content under a low dose of Arabic gum nor under C treatment. However, a high dose of Arabic gum or inoculation with BCI S5 increased carotenoid content in the plant growing media containing wood fiber (Figure S38a,b). No difference in carotenoid content was observed between C and BCI S5 treatment in the composted bark plant growing media. But, BCI S5 increased carotenoid levels when using green waste compost (Figure S38c). Maximizing carotenoid content depended on the BCI treatment (Table S11). Bacterial community inocula S4 and S5 were required to reach maximal carotenoid levels, while maximal carotenoid levels cannot be reached when treated with BCI S2 or S3.

3.8. Principal Component Analysis

The first two components of the PCA analysis explained 65% of the variance in the lettuce dataset (PC 1: 37.8; PC 2: 27.2%) (Figure 4). Quality of representation (cos^2) values of the plant performance parameters showed that FW (96%), LHA (93%), RW (88%), and DW (71%) are well represented in PC 1 and PC 2, while the representation of NO_3 (55%), TPC (39%), Chl_{a+b} (34%), and carotenoids (44%) is low (Figure 4b). Correlation analysis of the yield and quality parameters (Table 3) demonstrated that FW, LHA, RW, and NO_3-content were significantly positively correlated to PC 1, while TPC, Chl_{a+b}, and carotenoids were significantly negatively correlated to PC 1. Correlation analysis on PC 2 revealed that LHA, RW, DW, TPC, Chl_{a+b}, and carotenoids correlated positively and NO_3-content was negatively correlated. Hereby, we observed grouping of the yield parameters (FW, LHA, and RW) along the positive PC 1 and PC 2 axis, while the quality parameters DW, TPC, Chl_{a+b}, and carotenoids were clustered towards the negative PC 1 axis and the positive PC 2 axis. NO_3-content was separated from the other yield and quality parameters along the positive PC 1 and negative PC 2 axis (Figure 4a). Shoot fresh weight (18.5%), LHA (17.8%), RW (16.8%), and DW (13.6%) were the dominant variables, contributing the most to PC 1:2 (Figure 4d). The PC 1:2 contribution values of NO_3 (10.7%), TPC (7.5%), Chl_{a+b} (6.5%), and carotenoids (8.5%) remained below the expected average contribution.

Table 3. Dimension Description of the Lettuce Yield and Quality Variables to PC 1 and PC 2 at the 95% Confidence Level.

	PC 1 (37.8%)		**PC 2 (27.2%)**	
	Correlation	***P* Value**	**Correlation**	***P* Value**
FW	0.961	1.71×10^{-37}	/	n.s.
LHA	0.848	2.62×10^{-19}	0.457	1.13×10^{-4}
RW	0.731	3.15×10^{-12}	0.585	2.53×10^{-7}
DW	/	n.s.	0.806	3.41×10^{-16}
TPC	−0.390	1.21×10^{-3}	0.491	2.90×10^{-5}
NO_3	0.615	3.96×10^{-8}	−0.420	4.46×10^{-4}
Chl_{a+b}	−0.347	4.28×10^{-3}	0.466	8.15×10^{-5}
Carotenoids	−0.373	2.07×10^{-3}	0.550	1.68×10^{-6}

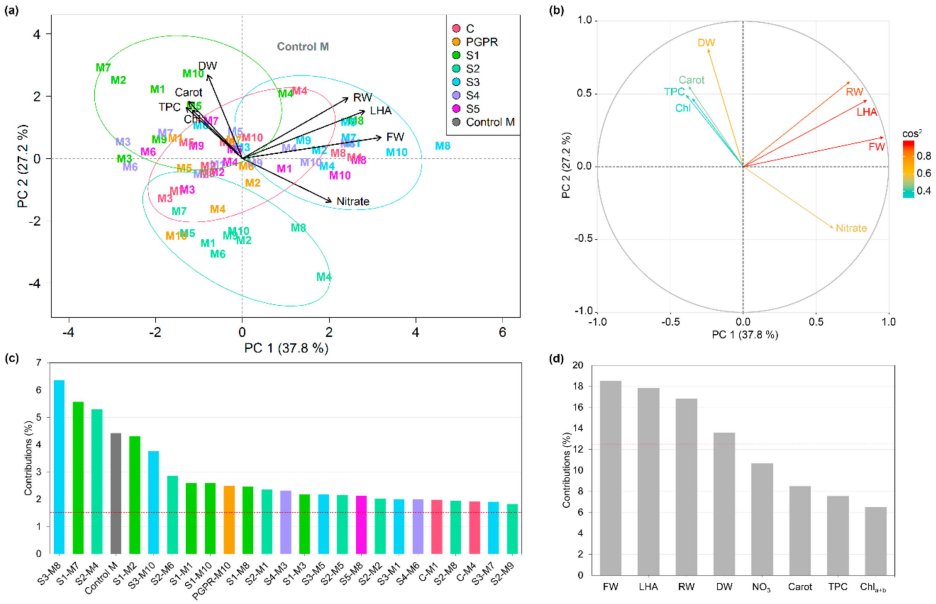

Figure 4. Principal component analysis (PCA) of the lettuce yield and quality variables under different BCI-plant growing medium treatments. (**a**) PCA biplot of individual samples to PC 1 and PC 2. Symbols indicate the type of plant growing medium (M1–10 and control M, the commercial plant growing medium) and colors indicate BCI treatment (S1–5, negative control C, and positive control PGPR). Ellipses denote 95% confidence interval of C, S1, S2, and S3. The plant performance parameters are shoot fresh weight (FW), lettuce head area (LHA), root fresh weight (RW), shoot dry weight (DW), total phenolic content (TPC), Nitrate content, chlorophyll a+b (Chl), and carotenoids (Carot); (**b**) Quality of representation (cos^2) correlation circle of variables to PC 1 and PC 2. The color gradient indicates the quality of representation of the variables; (**c**) Contribution plot of the top 25 samples to PC 1 and PC 2. Colors are the same as in a. The dashed line indicates the expected average contribution if the contribution of the samples were uniform; (**d**) Contribution plot of variables to PC 1 and PC 2. The dashed line indicates the expected average contribution if the contribution of the variables were uniform.

Principal component analysis showed grouping of the BCI-plant growing medium samples depending on BCI treatment (Figure 4a). Plant growing media treated with BCI S3 were separated from the C treatment towards FW, LHA, RW, and NO_3. Similarly, separation of BCI treatment S1 was observed towards DW, TPC, Chl_{a+b}, and carotenoids. Bacterial community inoculum S2 treated plant growing media clustered in the opposite direction of the plant performance parameters. We did not observe any clear grouping of BCI-plant growing medium samples based on plant growing medium type. The dominant treatments were S3-M8, S1-M7, S2-M4, the commercial plant growing medium, S1-M2, and S3-M10, each contributing more than 3% to PC 1:2 (Figure 4c).

4. Discussion

Reported evidence shows that plant growing media properties can enhance the beneficial impact of specific microbes on plant performance and stress resistance [41,42,46]. However, the role of plant growing medium composition and its interaction with rhizosphere bacterial communities in successful PGPR amendment and plant performance in soilless cultivation systems is not well understood. The presented study shows that microbe-plant growing medium interactions are important during the young plant stage for plant growth-promoting responses.

4.1. Plant Growing Medium Constituents Have Differing Effects on Lettuce Performance

The five plant growing media raw material groups peat, other organics, composted materials, inorganic materials, and Arabic gum had varied effects on the tested plant performance parameters. First, changing black peat to white peat significantly increased RW (Figure S9). Mathers et al. [70] reviewed that proper plant growing medium aeration is a vital physical characteristic influencing root growth. We observed that the air volume of the white peat growing media varied from 19.33% *v/v* (M5) to 26.33% *v/v* (M2), and for the black peat growing media from 13% *v/v* (M3) to 17% *v/v* (M7) (Table S3). Brückner [71] also reported higher air volume in white peat (24% *v/v*) compared to black peat (17% *v/v*). Thus, the positive impact of white peat on air volume improved the rooting of lettuce. Although white peat improved root weight compared to black peat, this advantage did not result in increased FW. This may be caused by the fact that after transplantation, for both white and black peat plant growing media, the roots grew out of the plant growing medium into the nutrient solution, having direct access to abundant nutrients. Since PFALs require high energy input, the production of non-salable plant parts must be minimized to reduce energy consumption [72]. The black peat growing media reduced RW of lettuce without affecting shoot FW, compared to the white peat growing media. So, the use of black peat blended with alternative materials as a plant growing medium can help minimize PFAL energy consumption through reduced lettuce root mass production. Alternatively, the use of white peat combined with alternative materials may be more advantageous for crops where the root system is the prime salable plant part.

Second, the use of perlite increased FW compared to sand, with LHA and RW showing similar trends (Figure 2, Figures S5 and S9). Similar to what we observed in the peat raw material group, the increase in plant growth likely resulted from a higher air volume and water capacity of plant growing media amended with perlite, compared to sand. Perlite is commonly amended to plant growing media to increase the air-filled pore space and water-holding capacity [73]. Contrary, sand has a small water buffer and pore volume [74]. Brückner [71] observed air volumes in sand-peat growing media ranging from 14–18% and 24–27% in perlite-peat growing media. In a previous study, we reported a higher air volume in perlite mixtures (20.5% *v/v*) compared to sand mixtures (17.8% *v/v*). Moreover, the water-holding capacity of perlite mixtures (615 g.(100 g dry matter)$^{-1}$) was double of that from sand mixtures (269 g.(100 g dry matter)$^{-1}$) [62]. The current physical analysis also showed a higher air volume and water-holding capacity of the plant growing media amended with perlite (20.8% *v/v* and 604 g.(100 g dry matter)$^{-1}$ respectively) compared to sand amendment (17.4% *v/v* and 287 g.(100 g dry matter)$^{-1}$ respectively) (Table S3).

Third, the application of green waste compost significantly increased lettuce growth (FW, LHA, RW) compared to composted bark (Figure 2,Figures S5 and S9). Spiers and Fietje [75] reported that green waste compost EC (3.43 dS·m^{-1}) was higher than bark compost EC (0.10 dS·m^{-1}). The high amount of available K^+ in green waste compost was mainly responsible for the high EC, with amounts reported up to 916 ppm for green waste compost compared to 19 ppm for composted bark. Previously, we also observed that plant growing media amended with green waste compost have higher EC (149 µS·cm^{-1}) than plant growing media containing bark compost (60 µS·cm^{-1}), with K^+ levels of 228 mg·L^{-1} and 70 mg·L^{-1} respectively [62]. In the current study, we also observed higher EC values for green waste compost growing media, varying from 130 µS·cm^{-1} (M4) to 275 µS·cm^{-1} (M8), compared to composted bark growing media, varying from 51 µS·cm^{-1} (M5) to 207 µS·cm^{-1} (M3) (Table S3). These differences in EC values between green waste compost and bark compost growing media were related to the K^+-content, respectively varying from 255.8 mg·L^{-1} (M8) to 335.5 mg·L^{-1} (M9), and from 84.7 mg·L^{-1} (M5) to 122.6 mg·L^{-1} (M6). The increased availability of salts, and especially K^+, in the plant growing media amended with green waste compost proved to be advantageous for lettuce growth.

Fourth, using wood fiber over coir pith, in the other organics raw material group, decreased all plant growth parameters tested (FW, LHA, and RW) (Figure 2, Figures S5 and S9). This reduction in growth may be caused by N-immobilization, which is a known problem in wood fiber growing

media [76]. To avoid N-immobilization it is necessary to apply fertilizer from the start of plant cultivation [77]. Contrary to the commercial plant growing medium, we did not apply starter fertilizer to the experimental plant growing media. Only after transplantation to the PFAL (2 weeks after sowing), plants were irrigated regularly with nutrient solution.

These examples highlight the strong variety in which different plant growing medium constituents affect the physicochemical properties of the plant growing medium and thus plant performance. Proper selection of plant growing medium raw materials is required to achieve the desired enhancement of specific plant performance parameters.

4.2. Microbe-Plant Growing Medium Interactions and the Bacterial Source Determine Plant Performance

Plant growth-promoting rhizobacteria technology is becoming increasingly popular. However, there is still much doubt about the effectiveness of microbial amendment [31]. Design of experiments analysis revealed that bacterial amendment was the main driver affecting plant performance. However, the effectiveness of bacterial amendment and the plant performance parameters affected depended on microbe-plant growing medium interactions and the bacterial source.

Statistical analysis showed a significant interaction between the BCI and plant growing medium-class variables for all the tested plant performance parameters. Both BCI S1 and S3 positively affected plant performance. But, the observed effects did not occur in each plant growing medium (Figures 1 and 3), suggesting the potential influence of plant growing medium composition on the effectiveness of BCI treatment. Vandecasteele et al. [78] also reported that successful microbial inoculation depended on the type of plant growing medium. Biocontrol fungi showed better colonization in defibrated pure miscanthus, reed straw and flax shives compared to peat since peat did not provide the necessary compounds for fungal growth. Also, DOE analysis revealed several interaction effects between BCI treatment and plant growing medium constituents. For example, lettuce TPC was not affected by the type of compost under control treatment. However, inoculating the plant growing media with BCI S1 raised the TPC of lettuce grown in composted bark growing media while the TPC levels observed in the green waste compost growing media were unchanged (Figure S21). The higher organic matter content of the bark compost growing media, compared to the green waste compost growing media, may have provided a specific source of nutrients for the bacterial community present in S1 [62]. Overall, plant growing media without the BCI amendment did not perform as well as the commercial peat-coir based growing medium. We did not add starter fertilizer to the experimental plant growing media, while NPK levels of the commercialized plant growing medium were much higher, which may have caused retardation in growth (Table S3). However, we did observe that the BCI S3 amendment improved plant growth and even outperformed the commercial plant growing medium when amending BCI S3 to M8 and M10. This proves that specific microbe-plant growing medium combinations can create a synergistic effect that can outperform commercialized plant growing media.

Our results suggest that specific microbe-plant growing medium interactions determine plant performance. Moreover, bacterial amendment resulted in different effects on plant performance depending on the bacterial source. The BCIs were collected at separate locations. Bacterial community inoculum S1, S2, and S5 were collected at three different open field organic farms, while S3 and S4 were collected at different greenhouse soilless farms. Differences in cultivation method, fertilizer management, soil type, and crop species among others may have affected the composition of the collected root-associated bacterial communities. For instance, organic systems show greater microbial community diversity and higher microbial activity than conventional systems [79]. Roesti [80] concluded that the bacterial community structure varied between high and low fertilization strategies. Pii et al. [81] detected different microbial communities in two bulk soils. The recruitment of microbes from the soil to the rhizosphere is host-specific [82]. Rhizobia—legume interactions are well-studied, and their symbiosis is so specific that certain rhizobial species only interact with a selection of legumes [83].

All these parameters shape the bacterial community of the collected BCI samples, resulting in different effects on plant performance when amended to lettuce. For example, PCA analysis showed a grouping of the S3-plant growing medium combinations towards increased plant growth (FW, LHA, and RW) and NO_3-content (Figure 4a). Design of experiments analysis confirmed this, showing a significant increase in lettuce FW, LHA, RW, and NO_3-content under BCI S3 treatment (Figure 2, Figures S5, S9 and S25). Plant growth-promoting rhizobacteria are known to improve plant growth by enhancing nitrate uptake [84]. Because BCI S3 treatment increased lettuce NO_3-content, we suspect that BCI S3 includes certain PGPRs that improve plant growth through better nutrient acquisition.

Contrary to BCI S3, we observed a separation of BCI treatment S1 towards DW, TPC, Chl_{a+b}, and carotenoids, and away from NO_3 in the PCA analysis (Figure 4a). Indeed, DOE analysis showed a significant increase in DW and TPC, and a significant decrease in NO_3-content (Figures S14, S18 and S25). Plants are known to produce more phenolics under N-deficient conditions [85]. Also, there is evidence that PGPR treatment can induce systemic resistance against plant pathogens, and an elevated content of phenolics is suggested to play a role [86,87]. Bacterial community inoculum S1 may contain PGPRs that induce systemic resistance as suggested by the elevation in TPC.

Both BCI S1 and S3 positively affected plant performance. Meanwhile, BCI S2 treatment resulted in negative plant performance (LHA, RW, DW, Chl_{a+b}, and carotenoids) (Figure 4a), which may indicate that BCI S2 contains plant pathogenic bacteria. Surprisingly, the PGPR biostimulant (*Bacillus* sp.), which we applied as a positive control, also reduced plant performance. Design of experiments analysis even indicated a negative effect on FW and DW (Figure 2 and Figure S14). Research suggests that the amendment of several PGPRs could be more effective than individual species due to different mechanisms being used [38,88]. Moreover, PGPR application efficacy can depend on local environmental conditions and crop specificity [26]. Our results show that a complex bacterial community is a driver for successful bacterial amendment.

5. Conclusions

In summary, the reported results display the potential of bacterial enhancement of plant growing media to modulate plant performance in horticultural systems. Plant growing medium composition determines plant performance, and successful bacterial amendment can result in improved plant performance. We revealed that bacterial amendment was a key driver affecting plant performance. Not only does the effectiveness of bacterial amendment on plant performance depend on the bacterial source, but it also depends on the interaction with the plant growing medium. Further research will focus on determining how the rhizosphere bacterial community structure is associated with the observed microbe-plant growing medium interactions, and identifying the modes of action of the PGPRs affecting plant performance.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/10/1456/s1, Figure S1: Pareto chart of the standardized effect (absolute) of the significant terms on shoot fresh weight under BCI S3 treatment, Figure S2: Interaction effects between substratum raw material groups on shoot fresh weight under BCI S3 treatment, Figure S3: Interaction effects between substratum raw material groups on shoot fresh weight under BCI S5 treatment, Figure S4: Boxplot of lettuce head area grouped per substratum, Figure S5: Main effects of substratum constituents on lettuce head area under different bacterial treatments, Figure S6: Pareto chart of the standardized effect (absolute) of the significant terms on lettuce head area under BCI S3 treatment, Figure S7: Pareto chart of the standardized effect (absolute) of the significant terms on lettuce head area under BCI S2 treatment, Figure S8: Interaction effect between other organics and composted materials on lettuce head area under BCI S1 treatment, Figure S9: Main effects of substratum constituents on root fresh weight under different bacterial treatments, Figure S10: Pareto chart of the standardized effect (absolute) of the significant terms on root fresh weight under BCI S2 treatment, Figure S11: Pareto chart of the standardized effect (absolute) of the significant terms on root fresh weight under BCI S3 treatment, Figure S12: Interaction effect between other organics and composted materials on root fresh weight under BCI S1 treatment, Figure S13: Interaction effects between substratum raw material groups on root fresh weight under BCI S4 treatment, Figure S14: Main effects of substratum constituents on shoot dry weight under different bacterial treatments, Figure S15: Pareto chart of the standardized effect (absolute) of the significant terms on shoot dry weight under BCI S1 treatment, Figure S16: Pareto chart of the standardized effect (absolute) of the significant terms on shoot dry weight under BCI S2 treatment, Figure S17: Boxplot of total phenolic content grouped per substratum, Figure S18: Main effects of

substratum constituents on total phenolic content under different bacterial treatments, Figure S19: Pareto chart of the standardized effect (absolute) of the significant terms on total phenolic content under BCI S1 treatment, Figure S20: Pareto chart of the standardized effect (absolute) of the significant terms on total phenolic content (TPC) under BCI S4 treatment, Figure S21: Interaction effects between substratum raw material groups on total phenolic content (TPC; mg GAE/100 g FW) under BCI S1 treatment, Figure S22: Interaction effects between substratum raw material groups on total phenolic content under BCI S5 treatment, Figure S23: Interaction effect between other organics and BCI on total phenolic content under BCI S4 treatment, Figure S24: Boxplot of nitrate content grouped per substratum, Figure S25: Main effects of substratum constituents on nitrate content under different bacterial treatments, Figure S26: Pareto chart of the standardized effect (absolute) of the significant terms on NO_3-content under BCI S1 treatment, Figure S27: Pareto chart of the standardized effect (absolute) of the significant terms on NO_3-content under BCI S3 treatment, Figure S28: Interaction effect between other organics and Arabic gum on NO_3-content under BCI S5 treatment, Figure S29: Boxplot of chlorophyll a+b grouped per substratum, Figure S30: Main effects of substratum constituents on chlorophyll a+b content under different bacterial treatments, Figure S31: Interaction effect between composted materials and BCI on chlorophyll a+b content under BCI S2 treatment, Figure S32: Interaction effect between composted materials and BCI on chlorophyll a+b content under BCI S4 treatment, Figure S33: Boxplot of carotenoid content grouped per substratum, Figure S34: Main effects of substratum constituents on carotenoid content (mg/100 g FW) under different bacterial treatments, Figure S35: Pareto chart of the standardized effect (absolute) of the significant terms on carotenoid content under BCI S2 treatment, Figure S36: Interaction effects between substratum raw material groups on carotenoid content under BCI S3 treatment, Figure S37: Interaction effect between composted materials and BCI on carotenoid content under BCI S4 treatment, Figure S38: Interaction effects between substratum raw material groups on carotenoid content under BCI S5 treatment, Table S1: Control factors and level settings for substratum optimization, Table S2: The 2^{5-2}_{III} fractional factorial design, Table S3: Physicochemical properties of the experimental substrata and the commercial substratum, Table S4: Shoot fresh weight response optimization under each BCI treatment, Table S5: Lettuce head area response optimization under each BCI treatment, Table S6: Root fresh weight response optimization under each BCI treatment, Table S7: Shoot dry weight response optimization under each BCI treatment, Table S8: Total phenolic content response optimization under each BCI treatment, Table S9: NO_3-content response optimization under each BCI treatment, Table S10: Chlorophyll a+b content response optimization under each BCI treatment, Table S11: Carotenoid content response optimization under each BCI treatment.

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Abbreviations

Controlled-environment agriculture	CEA
Plant factory with artificial lighting	PFAL
Plant growth-promoting rhizobacteria	PGPR
Tryptic soy broth	TSB
Bacterial community inoculum	BCI S1–5
Experimental plant growing media	M1–10
Peat	PT
Black peat	BP
White peat	WP
Other organics	OO
Coir pith	CP
Wood fiber	WF
Composted materials	CM
Composted bark	CB
Green waste compost	GC
Inorganic materials	IM

Perlite	P
Sand	S
Arabic gum	AG
Shoot fresh weight	FW
Lettuce head area	LHA
Root fresh weight	RW
Shoot dry weight	DW
Total phenolic content	TPC
Gallic acid equivalents	GAE
Chlorophyll a+b	Chl_{a+b}
Design of experiments	DOE
Principal component analysis	PCA

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