Received: 6 September 2023 | Accepted: 21 January 2024

DOI: 10.1111/1751-7915.14422

MICROBIAL BIOTECHNOLOGY Applied Microbiology International

RESEARCH ARTICLE

Bacterially enhanced plant-growing media for controlled environment agriculture

Thijs Van Gerrewey[1,2,3,4] | **Oscar Navarrete**[3] | **Maarten Vandecruys**[3] | **Maaike Perneel**[5] | **Nico Boon**[2] | **Danny Geelen**[1]

[1]HortiCell, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Gent, Belgium

[2]Center for Microbial Ecology and Technology (CMET), Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Gent, Belgium

[3]Urban Crop Solutions BVBA, Waregem, Belgium

[4]Agaris Belgium NV, Gent, Belgium

[5]Cropfit, Faculty of Bioscience Engineering, Ghent University, Gent, Belgium

Correspondence
Danny Geelen, HortiCell, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Gent, 9000, Belgium.
Email: danny.geelen@ugent.be

Funding information
Agentschap Innoveren en Ondernemen, Grant/Award Number: HBC.2017.0209

Abstract

Microbe–plant interactions in the root zone not only shape crop performance in soil but also in hydroponic cultivation systems. The biological and physicochemical properties of the plant-growing medium determine the root-associated microbial community and influence bacterial inoculation effectiveness, which affects plant growth. This study investigated the combined impact of plant-growing media composition and bacterial community inoculation on the root-associated bacterial community of hydroponically grown lettuce (*Lactuca sativa* L.). Ten plant-growing media were composed of varying raw materials, including black peat, white peat, coir pith, wood fibre, composted bark, green waste compost, perlite and sand. In addition, five different bacterial community inocula (BCI S1–5) were collected from the roots of lettuce obtained at different farms. After inoculation and cultivation inside a vertical farm, lettuce root-associated bacterial community structures, diversity and compositions were determined by evaluating 16S rRNA gene sequences. The study revealed distinct bacterial community structures among experimental replicates, highlighting the influence of raw material variations on root-associated bacterial communities, even at the batch level. However, bacterial community inoculation allowed modulation of the root-associated bacterial communities independently from the plant-growing medium composition. Bacterial diversity was identified as a key determinant of plant growth performance with green waste compost introducing *Bacilli* and *Actinobacteria*, and bacterial community inoculum S3 introducing *Pseudomonas*, which positively correlated with plant growth. These findings challenge the prevailing notion of hydroponic cultivation systems as sterile environments and highlight the significance of proper plant-growing media raw material selection and bacterial community inoculation in shaping root-associated microbiomes that provide stability through microbial diversity. This study supports the concept of creating bacterially enhanced plant-growing media to promote plant growth in controlled environment agriculture.

INTRODUCTION

Engineering the root-associated microbiome has been proposed as a method to sustainably enhance crop production to challenge a future where we have to feed the growing global population under reduced chemical fertilizer and agrochemical use (Dessaux et al., 2016; Kumar & Dubey, 2020; Soberón-Chávez, 2023; Toju et al., 2018; Van Gerrewey, Boon, & Geelen, 2021). The different components of the root zone can be

exploited to improve plant performance. For example, plant breeding techniques can introduce traits of interest, soil improvers can be added to improve the soil's physicochemical and biological properties, microbial inocula can promote plant growth and plant–microbe interactions can be steered through manipulation of root exudation (Dessaux et al., 2016).

Following our previous work, we postulated that the selection of the plant-growing medium might be critical in engineering the root zone in soilless crop cultivation systems (Van Gerrewey, Ameloot, et al., 2020; Van Gerrewey, Vandecruys, et al., 2020). Plant-growing media may be simple stand-alone materials to highly complex mixtures of various inorganic or organic raw materials such as peat, coir pith, wood fibre, green waste compost, perlite and mineral wool (Carlile et al., 2015; Grunert, Hernandez-Sanabria, et al., 2016). The composition and the physicochemical characteristics of the plant-growing medium determine the microbial community associated with the plant roots (Grunert, Hernandez-Sanabria, et al., 2016; Grunert, Reheul, et al., 2016; Montagne et al., 2017; Van Gerrewey, Vandecruys, et al., 2020). Plants shape their root-associated microbiome by selecting microbes from the environment to adapt to the local nutrient conditions (Garcia & Kao-Kniffin, 2018). For example, Antoniou et al. (2017) showed that sterile peat amended with green waste compost improved tomato plant growth compared to sterile peat-compost mixes, indicating plant growth-promoting (PGP) activity of the compost microbiome. They observed differences in microbial community composition between the unplanted compost and the tomato root-associated microbiome, suggesting the plant host was a driver for root-associated microbiome recruitment. Moreover, *Bacillus* spp. was the most abundant taxon in both the unplanted compost and tomato root-associated microbiome, which possesses PGP properties (Antoniou et al., 2017; Kashyap et al., 2019; Tiwari et al., 2019).

Another approach to engineering the root-associated microbiome is the application of PGP bacteria. PGP bacteria can promote plant growth through various mechanisms like N-fixation, phosphate solubilization, siderophore production, phytohormone production, stress alleviation and pathogen resistance (Bulgarelli et al., 2013; Lugtenberg & Kamilova, 2009). However, the positive effects of single-species PGP bacteria application do not always transfer from lab conditions to a realistic environment (Compant et al., 2019; Parnell et al., 2016). Multiple extraneous variables may cause this dissimilarity in effectiveness. For example, the bacterial inoculum may be unable to compete with the resident community (Eisenhauer et al., 2013; Mawarda et al., 2022). In addition, the impact of inoculation may depend on the plant host genotype (Sasaki et al., 2010; Xu et al., 2020; Zhong et al., 2019). Also, the inoculum dosage can affect the plant growth response (Bai et al., 2002; Kumar et al., 2020).

Instead, inoculation with microbial consortia may be more effective in improving plant performance than single-species inocula (Batista & Singh, 2021; Wagg et al., 2019). They may be better equipped to fill in the niches that vary greatly depending on the plant host, the resident root-associated microbiome and the environment's physicochemical and biological characteristics. For example, the survival of introduced *Pseudomonas* strains improved with increasing inoculum diversity (Hu et al., 2016). Moreover, higher *Pseudomonas* inoculum diversity reduced *Ralstonia solanacearum* disease incidence in the tomato root-associated microbiome due to resource competition and direct interference with the pathogen. Gu et al. (2020) showed that inoculation with soil bacterial communities increased the root-associated bacterial community diversity, which played an essential role in plant growth promotion. However, the effect of inoculation on the root-associated bacterial community and plant growth depended on the bacterial community inoculum source.

In previous work, we investigated the effect of five bacterial community inocula (BCI S1–5), collected from the roots of lettuce (*Lactuca sativa* L.) obtained at different farms, on the performance of lettuce grown in 10 different plant-growing media (M1–10) composed of peat (black peat or white peat), other organics (coir pith or wood fibre), composted materials (composted bark or green waste compost) and inorganic materials (perlite or sand) inside a vertical farm (Van Gerrewey, Vandecruys, et al., 2020). We showed that microbe–plant-growing medium interactions were major determinants of lettuce performance. For example, BCI S3 possessed PGP properties, but only in half of the plant-growing media. Moreover, BCI S3 amended to the two mixtures containing black peat and green waste compost (M8 and M10) created a synergistic effect, outperforming the commercial control plant-growing medium. These results showed that proper raw material selection is critical when amending bacterial community inocula to promote plant growth.

The biological and physicochemical characteristics of the plant-growing medium determine the root-associated microbial community. In addition, the impact of bacterial community inoculation on plant growth depends on the properties of the selected plant-growing medium. It is, therefore, necessary to further investigate the effect of plant-growing medium composition and bacterial community inoculation on the root-associated microbiome in hydroponic cultivation. The root-associated bacterial communities were sampled during the previous work to investigate further the role of the plant-growing media materials, inocula and their interactions in microbiome functioning (Van Gerrewey, Vandecruys, et al., 2020). 16S rRNA gene sequences were evaluated to determine

the root-associated bacterial community structures, diversity and compositions. The raw materials and inocula were associated with the introduction of potential PGP bacteria. Using these analyses, we sought to answer the following questions: (1) Do plant-growing media composition and inoculation shift the root-associated bacterial community structure? (2) Does bacterial diversity in the root zone promote plant performance? (3) Do plant-growing media composition and bacterial community inoculation introduce candidate PGP bacteria, improving plant performance?

EXPERIMENTAL PROCEDURES

Plant growth experiment: Bacterial community inocula, plant-growing media, growth conditions and plant sample analysis

The design and experimental procedures of the plant growth experiment have been described before (Van Gerrewey, Vandecruys, et al., 2020). In brief, bacterial community inocula (BCI S1–5) were collected from the roots of lettuce (*Lactuca sativa* L.) at five different locations in Flanders, Belgium: three open-field organic farms and two soilless farms. Sampling and root-associated bacterial community extraction were performed following the method described by Barillot et al. (2013). The amount of live bacterial cells present in the root-associated bacterial community samples was estimated using flow cytometric analysis to standardize bacterial inoculation (Van Nevel et al., 2013).

Using fractional factorial design (Van Gerrewey, Vandecruys, et al., 2020), 10 different plant-growing media were composed (M1–10) at Agaris Belgium NV (Gent, Belgium) by varying five raw material groups: (a) peat (PT; black peat BP or white peat WP), (b) other organics (OO; coir pith CP or wood fibre WF), (c) composted materials (CM; composted bark CB or green waste compost GC), (d) inorganic materials (IM; perlite P or sand S) and (e) Arabic gum (AG) dosed at 1 kg.m^{-3} or 5 kg.m^{-3} (Table S1). All plant-growing media have the following volumetric composition: 60% v/v PT, 20% v/v OO, 10% v/v CM and 10% v/v IM. The physicochemical properties of the plant-growing media were measured in triplicate before the start of the experiment (Verdonck & Gabriels, 1992). The chemical properties were as follows: pH and EC ($\mu S.cm^{-1}$) and NO_3^-, NH_4^+, P_2O_5, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Na^+, Cl^-, Fe^{2+} and Mn^{2+} concentrations (mg.L^{-1} substratum). The physical properties were as follows: dry matter content (DM; % FW), organic matter content (OM; % DW), ash content (% DW), bulk density (ρ_b; g.L^{-1}), shrinkage (% v/v), moisture content (% v/v), water capacity (WC; g.(100 g dry matter)$^{-1}$), air volume at water saturation point (V_a; % v/v), water volume at water saturation point (V_w; % v/v), total pore volume (TPV; % v/v) and water-filled porosity at water saturation point (WFP; % v/v).

Sterilized hydroponic mesh pots fitted with hydroponic paper (Ellepot, Esbjerg, Denmark) were filled with 200 mL of the plant-growing media. Batavia lettuce seeds (Enza Zaden, Enkhuizen, The Netherlands) were sown in 10 pots of each plant-growing medium. The pots were placed 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 $\mu mol.m^{-2}\cdot s^{-1}$. Six pots with uniform lettuce seedlings were selected per plant-growing medium and placed in trays 2 weeks after sowing.

At this point, the five bacterial community inocula (BCI S1–5) were applied to all plant-growing media. One millilitre of inoculum diluted in tryptic soy broth (TSB) was applied at the plant base and provided a dose of 3.2×10^9 colony forming units (CFU) per L plant-growing medium. As a positive control treatment (PGPR), a commercial product containing *Bacillus* sp. with PGP properties (VITACT® R, De Ceuster Meststoffen – DCM, Grobbendonk, Belgium) 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. After inoculation, the trays were placed inside a vertical farm (Urban Crop Solutions, Beveren–Leie, Belgium) for 3 weeks under the growing conditions mentioned above. During these 3 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 whole plant growth experiment described above was replicated three times. New batches of plant-growing media were composed for each experimental replicate, while the same bacterial community inoculum sources were used.

The plants were harvested 3 weeks after inoculation. Shoot fresh weight (FW), root fresh weight (RW), lettuce head area (LHA), percentage shoot dry weight (% DW), total phenolic content (TPC), chlorophyll a+b (Chl_{a+b}), carotenoids and nitrate (NO_3^-) concentration were measured and analysed as described before (Van Gerrewey, Vandecruys, et al., 2020).

Root-associated bacterial community analysis

Root-associated bacterial community sample collection

After processing the lettuce head, the plant roots and plant-growing medium of the sample were used to

extract the root-associated bacterial community following the procedure described by Barillot et al. (2013) and Van Gerrewey, Vandecruys, et al. (2020) with slight modifications. In brief, any loosely attached plant-growing medium was removed by manually shaking and pounding the plant root system. The plant root systems were pooled per treatment (six plants) and washed twice. First, the pooled plant root systems were added to 250 mL of a sterile 0.9% NaCl solution and placed on a rotary shaker at 125 × rpm for 90 min at 4°C to obtain the rhizosphere fraction. Second, the pooled plant root systems were transferred to 250 mL of sterile 0.9% NaCl +0.01% Tween 80 and placed on a rotary shaker at 125 × rpm for 90 min at 4°C to obtain the rhizoplane fraction. After the second washing, both fractions were centrifuged at 9425 × *g* for 10 min at room temperature to collect the bacteria in the pellet. Finally, the bacterial pellets were resuspended in TSB + 15% glycerol and stored at −80°C.

16S rRNA gene amplicon sequencing

16S rRNA gene amplicon sequencing was performed as described before (De Paepe et al., 2017; De Vrieze et al., 2016). In brief, equal volumes of the stored rhizosphere and rhizoplane fractions were pooled per treatment. Three replicates of the original inoculum sources were also included in the sequencing run. DNA extraction was performed using bead beating with a PowerLyzer (Qiagen, Venlo, the Netherlands) and phenol/chloroform extraction. After DNA extraction, the samples were purified with the Zymo Research OneStep PCR Inhibitor Removal Kit (Irvine, CA, USA). The 16S rRNA gene V3–V4 hypervariable regions were amplified by PCR using primers 341F (5′-CCT ACG GGN GGC WGC AG-3′) and 785Rmod (5′-GAC TAC HVG GGT ATC TAA KCC-3′). The reverse primer was adapted from Klindworth et al. (2013) to make it more universal. Quality control PCR was performed using *Taq* DNA polymerase with the Fermentas PCR Kit according to the manufacturers' specifications (Thermo Fisher Scientific, Waltham, MA, USA). The obtained PCR product was run along with the DNA extract on a 2% agarose gel for 30 minutes at 100 V. Ten microlitre of the original genomic DNA extract was sent out to LGC Genomics GmbH (Berlin, Germany) for library preparation and sequencing on an Illumina Miseq platform with v3 chemistry and the primers mentioned above. A mock community was included in triplicate in the sequencing run to assess the sequencing quality. Read assembly and clean-up were derived mainly from the MiSeq SOP described by the Schloss lab (Kozich et al., 2013; Schloss et al., 2011). In brief, mothur (v. 1.42.3) was used to assemble reads into contigs, perform alignment-based quality filtering (alignment to the mothur-reconstructed SILVA SEED alignment, v. 138 only containing the 341–785 fragments), remove chimeras (vsearch v. 2.13.0) and assign taxonomy using a naive Bayesian classifier (Wang et al., 2007) and SILVA NR v. 138 (Quast et al., 2013). These contigs were clustered into operational taxonomic units (OTU) at 97% sequence similarity. All sequences classified as Eukaryota, Archaea, Chloroplasts and Mitochondria were removed (lineages were checked and correctly classified, but no non-bacterial sequences were detected). If sequences could not be classified at all (even at (super)Kingdom level), they were removed. For each OTU, the representative sequence was picked as the most abundant sequence within that OTU cluster. The raw fastq files used to create the OTU table have been deposited in the National Center for Biotechnology Information (NCBI) database (accession number PRJNA814889).

Root-associated bacterial community statistical analysis

The statistical analyses were carried out as described before by Van Gerrewey, El-Nakhel, et al. (2021). In summary, all the statistical analyses were performed in R (v. 4.1.0) using the *phyloseq* package (v. 1.36.0) to import the data (McMurdie & Holmes, 2013; R Core Team, 2021). Singleton OTUs were considered noise and were removed from the dataset before analysis. After removing singleton OTUs, 10,129,030 16S rRNA gene sequences from 231 samples remained in the data set, with an average of 43,849 sequences per sample (Figure S1). The 16S rRNA gene sequences represented 45,907 OTUs, which consisted of 47 phyla, 146 classes, 382 orders, 688 families and 1528 genera. A summary of the sequencing metrics grouped per BCI treatment and raw material can be found in Tables S2 and S3.

Three alpha-diversity metrics were estimated, considering both unobserved rare taxa and the uncertainty resulting from using samples as proxies for the entire microbiome (Willis et al., 2017). The *breakaway* package (v. 4.7.3) was used to estimate richness (i.e. the total number of OTUs) (Willis & Bunge, 2015). The *DivNet* package (v. 0.3.7) was used to estimate Shannon's diversity and Simpson's diversity at the genus level (Willis & Martin, 2022). The Shannon and Simpson diversity indices allow us to quantify alpha diversity accounting for both the richness and evenness (i.e. the distribution of taxa relative abundances) of the samples. Shannon's diversity index is a measure of the uncertainty of predicting individual genera. Simpson's diversity index is the probability that two random observations taken from a sample represent the same genus, meaning that a high Simpson's diversity index coincides with low diversity and vice versa. Any values outside 1.5 times the IQR were imputed with the mean.

After quantifying the alpha-diversity indices, the *breakaway* package *betta_random()* function statistically compared the alpha-diversity indices between BCI treatments or plant-growing media using the variance of the estimates in a mixed-model approach (Willis et al., 2017). When fitting the model with BCI treatment or plant-growing medium composition as a fixed effect, replication was added as a random effect. Finally, Pearson's correlation analysis between the alpha-diversity and plant performance metrics (growth: FW, RW and LHA; quality: % DW, TPC, Chl_{a+b}, carotenoids and NO_3^--concentration) was carried out and visualized with the *correlation* (v. 0.6.1) and *corrplot* (v. 0.89) packages using the Benjamini–Hochberg procedure to adjust the *p*-values for multiple testing (Makowski et al., 2020; Wei & Simko, 2021).

Beta diversity was analysed by principal coordinates analysis (PCoA) based on the abundance-based Bray–Curtis dissimilarity matrix (*phyloseq* package) and visualized with the *ggplot2* package (v. 3.3.4) (Wickham, 2020). A PerMANOVA (permutations = 10,000; $\alpha = 0.05$) on the Bray–Curtis distances evaluated the homogeneity of variances and determined differences in community structure between the BCI treatments and the plant-growing media with Holm adjustment for multiple comparisons (*vegan* package v. 2.5–7) (Oksanen et al., 2020).

Differential abundance testing was used to identify abundant bacteria in the BCI treatments compared to the control treatment and the inoculum sources and to detect differences in bacterial abundances between the plant-growing media. In addition, differentially abundant bacteria in the BCI treatments were associated with high or low plant performance. The differential abundance tests were performed with the *DESeq2* package (v. 1.32.0) using a Wald test with a local fit ($\alpha = 0.01$) (Love et al., 2014).

Indicator taxon analysis using multi-level pattern analysis (permutations = 10,000) with the indicator value index 'IndVal.g' of Dufrêne and Legendre (1997) was utilized to identify indicator OTUs for each BCI treatment and plant-growing medium (*indicspecies* package v. 1.7.9) (De Cáceres & Legendre, 2009). *p*-values were adjusted for multiple testing with the Benjamini–Hochberg adjustment.

Before constructing bacterial co-occurrence networks for each BCI treatment and plant-growing medium, the OTU table was filtered by keeping only OTUs present in more than 40–60% of the samples using the *seqtime* package (v. 0.1.1) to reduce sparsity (Faust et al., 2018; Zamkovaya et al., 2021). The *SpiecEasi* package (v. 1.1.0) was used to normalize the filtered data and compute the sparse inverse covariance matrix using the Meinshausen–Buhlmann neighbourhood selection method (Kurtz et al., 2015). The stability approach to regularization selection (StARS) was used to estimate the optimal penalty parameter λ, with a minimal λ ratio set to $5e^{-5}$, the number of λ penalties set to 100 and the number of subsamples for StARS set to 20. The resulting adjacency matrix was then converted into an igraph object, removing unconnected nodes (i.e. network OTUs). The *igraph* package (v. 1.2.6) was used to visualize the microbial networks and detect network clusters (using the fast greedy modularity optimization algorithm) and hubs (using Kleinberg's hub centrality scores) (Csardi & Nepusz, 2006).

Experimental replicate R1 was omitted from the dataset when comparing alpha diversity, co-occurrence networks and the root-associated bacterial community composition between the BCI treatments because a *Sciara* larvae infestation strongly affected germination and early seedling growth during this replicate. This infestation made it difficult to observe the effectiveness of BCI treatment S3 in R1 specifically, which showed improved plant performance (FW and LHA) in experimental replicates R2 and R3 (Table S4).

RESULTS

Do plant-growing media composition and inoculation shift the root-associated bacterial community structure?

Raw materials determined the root-associated bacterial community structures

Previously, it was shown that plant-growing media composition strongly influences the performance of hydroponically grown lettuce (Van Gerrewey, Vandecruys, et al., 2020). To investigate whether bacteria associated with the root zone contribute to differential crop performance, we analysed the bacterial community structure of the root zone samples. Principal coordinates analysis (PCoA) of the Bray–Curtis dissimilarity matrix indicated that the root-associated bacterial community structures varied strongly between three experimental replicates (R1–3) (Figure 1). R1 clustered apart from R2 and R3 along the first PCoA axis, while R3 clustered apart from R1 and R2 along the second PCoA axis. A PerMANOVA on the Bray–Curtis distances with pairwise comparisons revealed significant differences in root-associated bacterial community structure between the three experimental replicates ($R^2 = 0.19$, $p < 0.0001$; Table S5). In addition, multiple indicators OTUs for each experimental replicate (R1: 674 OTUs; R2: 1366 OTUs; R3: 2155 OTUs) and many differentially abundant OTUs (Figure S2) were identified.

While lettuce variety and growing conditions were identical across experimental replicates, fresh batches of plant-growing media were prepared for each replicate. These batches displayed differences in chemical (e.g. EC; Table S6) and physical properties

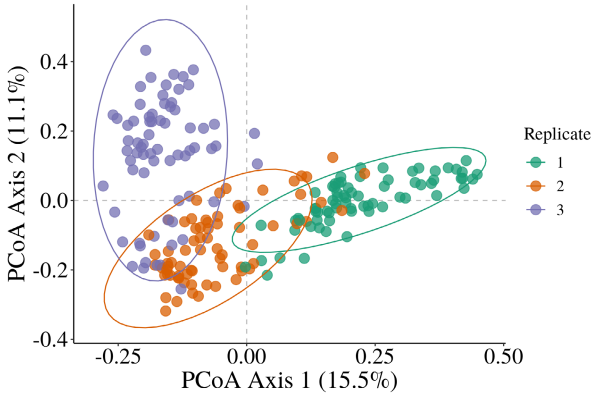

FIGURE 1 Bray–Curtis dissimilarity principal coordinates analysis (PCoA) of the lettuce root-associated bacterial community samples. Colours indicate the experimental replicate (71 samples per replicate). Confidence ellipses are drawn using a multivariate t-distribution.

(Table S7). These variations were attributed to qualitative differences in the raw materials obtained from the supplier and may be the cause for differences in microbial communities observed across replicates (Figure 1).

With this hypothesis in mind, we set out a correlative analysis of the raw materials used to prepare mixtures and the root-associated bacterial community structure recorded (Table S8). The following significant correlations were identified: (1) black peat mixtures showed distinct root-associated bacterial community structures from mixtures containing white peat; (2) the bacterial community structures of coir pith mixtures were distinct from wood fibre mixtures; and (3) mixtures with composted bark differed in bacterial community structure from green waste compost mixtures. In contrast, mixtures containing perlite did not differ in root-associated bacterial community structure from mixtures with sand. Collectively, the results show that raw materials in plant-growing media are a determining factor for the bacterial community structure in the root zone.

Inoculation with a bacterial community can shape the root-associated bacterial community structure

Since the primary goal is to modulate the root-associated bacterial community in hydroponics, we next assessed the effect of inoculation with different bacterial community inocula (BCI). The addition of BCI led to the creation of distinct root-associated bacterial community structures, although with varying success across the experimental replicates (Figure 2 and Table 1). Inoculation with a single-species PGPR inoculum (*Bacillus* sp.) had a limited effect, altering the

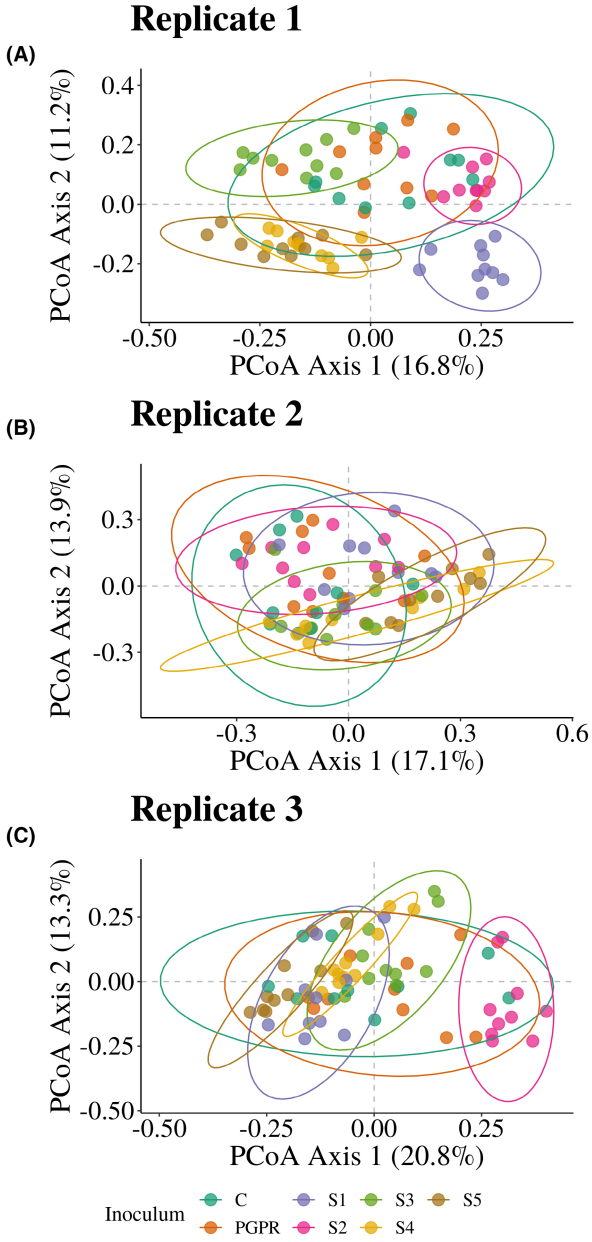

FIGURE 2 Bray–Curtis dissimilarity principal coordinates analysis (PCoA) of the lettuce root-associated bacterial community samples per experimental replicate: (A) replicate 1, (B) replicate 2 and (C) replicate 3. Colours indicate the inoculum treatments (10 samples per inoculum). S1–5 indicate the bacterial community inocula treatments. C indicates the negative control treatment without inoculum, and PGPR indicates the positive control treatment with a *Bacillus* sp. inoculum. Confidence ellipses are drawn using a multivariate t-distribution.

root-associated bacterial community structure in experimental replicate R3 (Table 1).

The variances in community structure were generally smaller after BCI treatment compared with the non-inoculated control (Table 1). This reduction in variance was not noticed after applying a single species PGPR inoculum, suggesting that complex bacterial mixtures are required to determine the root-associated bacterial

TABLE 1 PerMANOVA with Holm adjustment for multiple comparisons on Bray–Curtis distances to determine differences in lettuce root-associated bacterial community composition (β-diversity) and homogeneity of variances between inoculum treatments per experimental replicate (R1–3) or across all experimental replicates (All R).

	β-Diversity				Homogeneity of variances			
Inoculum	**All R**	**R1**	**R2**	**R3**	**All R**	**R1**	**R2**	**R3**
C	a	a	abc	a	a	a	a	a
PGPR	b	a	a	b	ab	ab	ab	a
S1	c	b	d	c	bc	c	ab	b
S2	d	c	b	d	cd	cd	b	abc
S3	e	d	c	e	ab	bd	ab	ac
S4	f	e	c	f	d	cd	ab	bc
S5	g	f	e	g	b	cd	ab	b
Global	***	***	***	***	***	***	n.s.	*

Note: S1–5 indicates the bacterial community inocula treatments. C indicates the negative control treatment without inoculum, and PGPR indicates the positive control treatment with a *Bacillus* sp. inoculum. Asterisks indicate global level of significance: not significant (n.s.), $p<0.05$ (*) and $p<0.001$ (***). Different letters within each column indicate significant differences ($\alpha=0.05$).

community structure across different plant-growing media.

Does bacterial diversity in the root zone promote plant performance?

Black peat, green waste compost and wood fibre increased bacterial diversity in the root zone

Microbial community diversity, as measured by three different metrics (richness, Shannon's and Simpson's diversity index), was significantly affected by the raw materials in the plant-growing media (Figure 3). The richness in BP and GC mixtures was more than 500 OTUs higher compared to WP ($p<0.01$) and CB ($p<0.001$) respectively. In mixtures containing BP and GC, Shannon's diversity index was significantly higher, and Simpson's diversity index was significantly lower ($p<0.001$), indicating that BP and GC increased the root-associated bacterial community diversity (Figure 3). Higher diversity was recorded in WF mixtures than in CP, while changing the type of IM or dose of AG did not affect the root-associated bacterial community diversity (Figure S3). The plant-growing media mixtures containing at least two materials of BP, GC or WF (M7, M8, M9 and M10) showed higher diversity than the other mixtures (Figure S4).

A co-occurrence network analysis was conducted to assess whether raw material selection resulted in more complex root-associated bacterial community networks (Table S9). BP and GC generated more complex networks containing more OTUs and a significantly higher amount of connections per OTU ($p<0.001$) than WP and CB respectively. The networks of the CP and WF mixtures were of similar size, although WF showed more connections per OTU ($p<0.001$). Finally, the networks of the materials in the IM and AG groups were similar in complexity.

Bacterial community inoculation increased bacterial diversity in the root zone

The bacterial diversity metrics were also compared between the BCI treatments. In general, inoculation did not affect the richness compared to the non-inoculated control (Figure S5), yet Shannon's and Simpson's diversity indices indicated an increase in diversity when inoculating with BCI S1, S3 and S5 while inoculation with BCI S2 decreased diversity (Figure S5).

When comparing the network complexity between the BCI treatments based on the OTU degree (i.e. the number of connections of an OTU), three groups were distinguished: (1) negative control C and BCI S3 with the highest OTU degree; (2) BCI S1 and S5 with middle OTU degree; and (3) single-species PGPR inoculum, BCI S2 and S4 with the lowest OTU degree (Table S10). These results revealed that bacterial community inocula enhanced the diversity in the root zone, depending on the inoculum source.

Raw materials and bacterial community inocula as sources of bacterial diversity correlated with improved plant performance

Previously, we have shown that plant growth performance is affected by the plant-growing media composition and BCI (Van Gerrewey, Vandecruys, et al., 2020). In this follow-up study, Pearson's correlation analysis showed that plant growth performance (FW, LHA and RW) positively correlated with bacterial diversity (Figure S6). However, the plant performance metrics did not correlate with bacterial richness. According to Simpson's diversity, high bacterial diversity in the root zone was also correlated with high % DW and Chl_{a+b} content (Figure S6). These correlations indicate that good-performing plants were associated with higher root-associated bacterial community diversity.

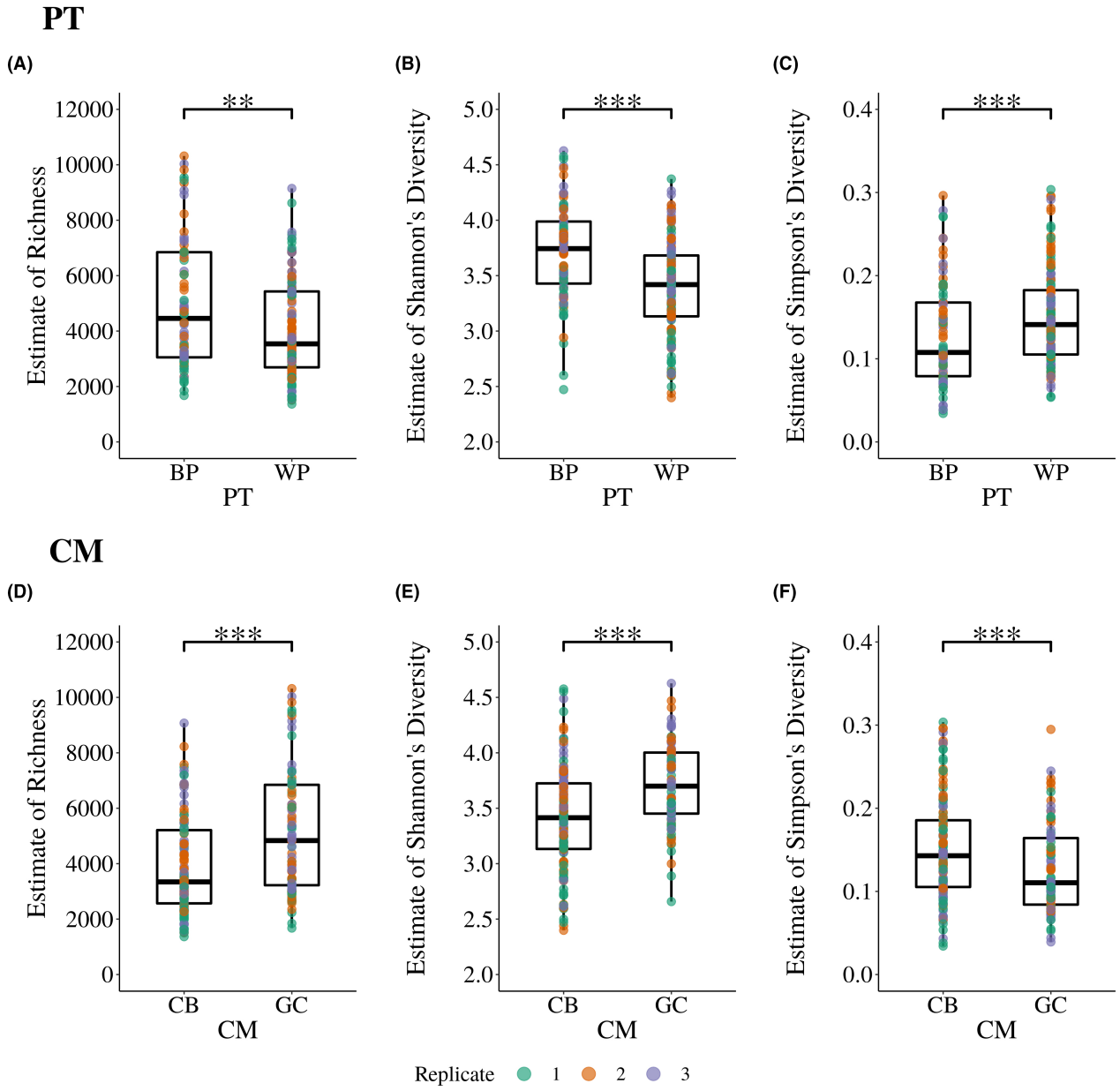

FIGURE 3 Boxplots of the estimated (a; d) richness, (b; e) Shannon's diversity and (c; f) Simpson's alpha diversity indices of the lettuce root-associated bacterial communities grouped per type of peat (PT; black peat BP and white peat WP) and type of composted material (CM; composted bark CB and green waste compost GC) over the three experimental replicates. The alpha-diversity indices were estimated by taking unknown taxa into account. Shannon's diversity and Simpson's diversity indices were determined at the genus level. Statistical comparison of the alpha-diversity indices using the estimates' variance in a mixed-model approach. When fitting the model, replication was added as a random effect. Asterisks indicate the level of significance: $p < 0.01$ (**) and $p < 0.001$ (***). $n = 84$ (BP; GC) and $n = 126$ (WP; CB).

Do plant-growing media composition and bacterial community inoculation introduce candidate PGP bacteria, improving plant performance?

Root-associated bacterial community composition varied with plant-growing medium composition and inoculation

Distinct lettuce root-associated bacterial community compositions were identified depending on the type of raw materials used in the growing media mixtures. Comparing peat materials, the root zones of lettuce grown in BP mixtures contained 2758 indicator OTUs and 955 indicator OTUs in WP mixtures (Table S11). At the same time, 1646 OTUs were differentially abundant between BP and WP mixtures (Table S12). Most of the OTUs found in the BP, and WP-grown lettuce root zones belong to the phyla *Proteobacteria*, *Bacteroidota*, *Planctomycetota*, *Actinobacteriota*, *Acidobacteriota* and *Verrucomicrobiota*. Bacteria from the classes *Parcubacteria* and *Saccharimonadia*,

members of the phylum *Patescibacteria*, were unique for BP samples.

Root zones of lettuce grown in CP mixtures contained 639 indicator OTUs and 592 in WF mixtures (Table S13). A total of 725 OTUs were differentially abundant between CP and WF mixtures (Table S14). The majority of the CP and WF OTUs belong to the phyla *Bacteroidota*, *Planctomycetota*, *Proteobacteria* and *Verrucomicrobiota*. *Actinobacteriota*, *Bdellovibrionota* and *Myxococcota* were distinct phyla for WF samples and *Patescibacteria* for CP samples.

In the root zones of GC-grown lettuce, we identified 1977 indicator OTUs and 259 in CB samples (Table S15), while 722 OTUs were differentially abundant between the two composted materials (Table S16). Most of the GC and CB OTUs belong to the phyla *Firmicutes*, *Proteobacteria*, *Bacteroidota*, *Actinobacteriota* and *Planctomycetota*. *Acidobacteriota* and *Chloroflexi* were distinct phyla of GC, and *Verrucomicrobiota* and *Patescibacteria* were distinct phyla for the CB samples. In addition, the classes *Bacilli*, *Clostridia* and *Limnochordia* were distinct representatives of GC in the phylum *Firmicutes*. Interestingly, changing between perlite and sand only had a small impact on the variation in the abundance of OTUs recorded (Table S17).

Inoculation with the 5 BCIs generated up to 28 indicator OTUs for each BCI, most belonging to the phylum *Proteobacteria* (Table S18). Differentially abundant OTUs between BCIs and non-inoculated control are shown in Table S19. For each BCI (S1 to S5), unique OTUs in the inocula sources were recurrent in the root zone samples (Table S20). This suggests that unique OTUs were introduced via the S1 to S5 inocula in the root zone.

The OTU of the single-species PGPR treatment that showed the largest $\log_2$fold change (11.93) was classified as *Bacillus* (OTU6) and was also the most abundant in the PGPR inoculum source (99.21% relative abundance). The PGPR-treated samples showed a high relative abundance (11.06%), showing that a single-species inoculum is sufficient to gain persistence in the root zone (Table S20).

Specific bacterial taxa correlated with lettuce performance

The occurrence of specific OTUs in the different root zone samples suggested that some of these were associated with plant growth and quality. To determine possible links between OTUs and plant parameters, we estimated the OTU $\log_2$-fold changes per unit increase in the plant metric, irrespective of the raw materials used or inocula applied.

A large number of OTUs were significantly more abundant in the root zones of good-performing plants (119 OTUs correlated with high FW, 232 with high LHA and 110 with high RW). Many OTUs were also significantly more abundant in low-performing plants (159 OTUs correlated with low FW, 339 with low LHA and 184 with low RW) (Table S21). For example, *Bacilli* and *Limnochordia* were distinct classes containing OTUs that were more abundant in the root zones of good-performing plants (high FW, LHA and RW), while *Bacteroidia* and *Verrucomicrobiae* were distinct classes of low-performing plants (low FW, LHA and RW).

Likewise, many OTUs were significantly more abundant in the root zones of high-quality plants (131 OTUs to % DW, 257 to NO_3^--content, 8 to TPC, 321 to Chl_{a+b} and 207 to carotenoids) or low-quality plants (137 OTUs to % DW, 164 to NO_3^--content, 10 to TPC, 492 to Chl_{a+b} and 164 to carotenoids) (Table S22). OTUs belonging to the *Acidobacteriota* and *Planctomycetota* showed a strong correlation with high plant quality (high % DW, TPC, Chl_{a+b}, and carotenoids and low NO_3^-), and OTUs of the *Firmicutes* correlated with low plant quality (low % DW, TPC, Chl_{a+b} and carotenoids, and high NO_3^-).

Green waste compost and BCI S3 inoculation introduce candidate PGP bacteria in the root zone

Following up on the previous study whereby it was shown that green waste compost and inoculum S3 improved plant performance and S2 reduced it (Van Gerrewey, Vandecruys, et al., 2020), the differential abundance of OTUs in the root zone samples was analysed and correlated with plant performance.

Representative OTUs found in the root zone of plants grown in GC mixtures that positively correlated with plant performance belonged to the phyla *Firmicutes*, *Actinobacteriota*, *Proteobacteria* and *Chloroflexi*, with *Bacilli* and *Actinobacteria* as the largest classes (Table S23). OTU41 (classified as *Bacillales*) showed the greatest $\log_2$-fold change per unit increase in FW, LHA and RW, with $\log_2$-fold change values of 0.11 per g FW, 0.02 per cm^2 LHA and 1.70 per g RW. OTUs that positively correlated with plant performance were also detected in the root zone of plants grown in BP mixtures and mainly belonged to the phyla *Acidobacteriota*, *Actinobacteriota* and *Chloroflexi* (Table S24).

Four BCI S3 representative OTUs showed a positive $\log_2$-fold change per unit increase in FW, LHA or RW: OTU73 (classified as *Vogesella*; $\log_2$-fold change values of 0.12 per g FW, 0.02 per cm^2 LHA and 1.18 per g RW), OTU156 (classified as *Pseudomonas*; $\log_2$-fold change value of 9.46 per g RW), OTU198 (classified as *Pseudomonas*; $\log_2$-fold change values of 0.18 per g FW and 2.61 per g RW) and OTU1269 (classified as *Pseudomonas*; $\log_2$-fold change values of 0.12 per g FW and 1.57 per g RW). The three OTUs classified as *Pseudomonas* (OTU156, OTU198 and OTU1269) were likely introduced through inoculation since they

were detected in the BCI S3 inoculum source but not in the negative control (Table S20). OTU73 was more abundant in the BCI S3 inoculum source compared to the negative control (0.02% and 0.002%, respectively), indicating that inoculation contributed to its increase in relative abundance in the root zone (0.32%) (Table S20). In addition, another BCI S3 representative OTU classified as *Vogesella* (OTU210) was linked to increased NO_3^--content (log_2-fold change value of 0.004 per mg/kg FW). This OTU was likely introduced through inoculation since it was not detected in the negative control (Table S20).

BCI S2 resulted in low growth performance (Van Gerrewey, Vandecruys, et al., 2020). Eleven BCI S2 differentially abundant OTUs showed a negative log_2-fold change per unit increase in FW, LHA or RW (Table S25). Four OTUs were classified as *Pseudomonas* (OTU1, OTU22, OTU40 and OTU1106) and three OTUs as *Acinetobacter* (OTU63, OTU69 and OTU140). OTU1 (classified as *Pseudomonas*) dominated the root zone of BCI S2-treated lettuce (30.70% relative abundance), being almost double in relative abundance compared to the negative control (18.40%) (Table S20). The large presence of OTU1 (classified as *Pseudomonas*) in the BCI S2 inoculum source (19.58%) likely introduced OTU1 in excess, allowing it to take over the lettuce root zone.

DISCUSSION

Plant-growing medium composition and bacterial community inocula steer the root-associated bacterial community

The growth and quality of lettuce in hydroponic cultivation are strongly influenced by plant-growing medium composition and the application of bacterial inocula (Van Gerrewey, Vandecruys, et al., 2020). In this follow-up study, we determined how variations in medium composition and inoculation with bacterial communities affect the complexity of the root-associated microbiome. The lettuce root-associated bacterial community structure depended on the type and the batch of raw materials used. The physicochemical and biological properties of the raw materials turned out to vary at the batch level, which altered the lettuce root-associated microbiome. The properties of growing medium components can vary widely according to the conditions under which it is produced (Barrett et al., 2016; Gruda, 2019). Therefore, certification systems are put in place to ensure quality to the end user (Regeling Handelspotgronden, 2021). Nonetheless, changing raw material bulk storage conditions, raw material handling and high turnover at the plant-growing media supplier may have led to the variations we recorded. These variations affect the intricate physical, chemical and biological relations in plant-growing media, which in turn influence the microbial community structure (Grunert, Hernandez-Sanabria, et al., 2016; Grunert, Reheul, et al., 2016; Montagne et al., 2017).

Given the expected increase in the use of plant-growing media and the need to substitute peat with novel materials (Blok et al., 2021), it will be important to monitor how future plant-growing media affect the root-associated microbiome. Only a few OTUs that were significantly more abundant in BP compared to WP mixtures showed a positive correlation with plant growth performance (FW and LHA). This coincides with our previous observation that plant yield did not differ between BP and WP mixtures (Van Gerrewey, Vandecruys, et al., 2020). Contrary, the application of GC introduced many OTUs classified as *Bacilli* and *Actinobacteria* into the root zone, which positively correlated with improved plant growth performance (FW, LHA and RW). Members of the *Bacilli* are among the most frequently reported PGP bacteria that promote plant growth by enhancing nutrient solubilization, N-fixation, phytohormone production and antimicrobial activity in a variety of crops (Kashyap et al., 2019; Tiwari et al., 2019). *Bacilli* are highly abundant in unplanted compost and the root-associated microbiome of tomatoes grown in plant-growing media including compost (Antoniou et al., 2017), and were shown to possess PGP traits improving chickpea growth under greenhouse and field conditions (Sreevidya & Gopalakrishnan, 2017). PGP members of the *Actinobacteria* show anti-fungal activity, and improve nutrient acquisition, phytohormone production and stress tolerance (Sathya et al., 2017; Thilagam & Hemalatha, 2019). Therefore, GC is a valuable component of plant-growing media either by providing *Bacillus* and *Actinobacterium* PGP bacteria or by promoting the establishment of these strains in the lettuce root-associated microbiome.

In addition to plant-growing medium components, the application of root-associated bacterial community inocula promoted bacterial diversity in the root zone. A high root zone microbial diversity was positively correlated with enhanced plant performance. Even bacterial communities isolated from different sources (forest, soybean and tomato soil) have been shown to increase the root-associated bacterial community diversity and promote the growth and yield of tomatoes (Gu et al., 2020). For instance, the increase in crop growth has been associated with positive effects on nutrient assimilation (Gu et al., 2020) and N availability (Weidner et al., 2015). The improved yields observed in intercropping systems have also been linked to a higher root-associated bacterial community diversity (Li et al., 2018). Root-associated bacterial community diversity reduced pathogen invasion by increasing resource use and niche overlap and reducing competition between generalist and specialist

resource users (Wei et al., 2015). The raw materials and inocula that increased bacterial diversity introduced or stimulated the establishment of microbes in the root zone with functional traits that promote lettuce growth. It allows the host plant to select from a larger pool of bacteria with beneficial functional traits that the host requires to adapt to the environment and support its growth (Delgado-Baquerizo et al., 2016; Yan et al., 2017).

The transplantation of a root-associated bacterial community successfully introduced PGP bacteria into the root zone of lettuce. Indeed, the good-performing BCI S3 inoculum (Van Gerrewey, Vandecruys, et al., 2020) introduced three OTUs classified as *Pseudomonas* (OTU156, OTU198 and OTU1269) associated with high plant performance. The genus *Pseudomonas* contains multiple members with various PGP traits like phosphate solubilization, phytohormone production, biocontrol and abiotic stress tolerance (Cipriano et al., 2016; David et al., 2018; Oteino et al., 2015; Patten & Glick, 2002; Phour & Sindhu, 2020; Vacheron et al., 2016; Zhuang et al., 2021). In addition, BCI S3 inoculation contributed to the increased abundance of *Vogesella* in the root zone, also linked to high plant performance. *Vogesella* is closely related to *Pseudomonas*, and *Vogesella* strains have been isolated from the root of salt-tolerant Pokkali rice, although they have no known PGP traits (Grimes et al., 1997; Rameshkumar et al., 2016).

Also, a negative impact of microbial transplantation is possible as BCI S2 turned out to inhibit lettuce growth (Van Gerrewey, Vandecruys, et al., 2020). BCI S2 contained several OTUs classified as *Pseudomonas* (OTU1, OTU22, OTU40 and OTU1106). *Pseudomonas* species with plant pathogenic properties are, for example, *Pseudomonas syringae*, *P. viridiflava* and *P. cichorii* (Pauwelyn et al., 2011; Sarris et al., 2012; Xin et al., 2018). *Pseudomonas syringae* is a well-studied plant pathogen infecting almost all economically important crops (Xin et al., 2018). *P. syringae* is considered a colonizer of the phyllosphere, although it has been found to colonize the root of Arabidopsis, where it causes severe root rot (Bais et al., 2004). Therefore, the transplantation of root-associated bacterial community isolates is not without risk, and in the case of S2, *Pseudomonads* with plant growth-inhibiting properties have likely been introduced into the lettuce root zone.

Lessons and future perspectives for creating bacterially enhanced plant-growing media for controlled environment agriculture

Plant-growing media are stable when their physical, chemical and biological properties are in a steady state (Verhagen, 2009). Biological stability generally concerns the microbial breakdown of organic matter, which causes a change in physical structure, chemical properties and nitrogen mobility (Verhagen, 2009). This definition gives a negative connotation to the presence of microbes as they can destabilize the plant-growing medium. However, our findings and other studies reveal that a diverse microbiome provides stability against a varying (a)biotic environment (Bulgarelli et al., 2013; de la Fuente Cantó et al., 2020; Fierer, 2017; Grunert, Hernandez-Sanabria, et al., 2016; Wagg et al., 2019). The current definition of biological stability does not include favourable microbial properties. Therefore, the definition of biological stability better includes the presence of a microbially diverse and competitive community, providing functional diversity and resilience to (a)biotic environmental changes. This updated definition highlights the positive aspects of the microbiome in providing a stable plant-growing medium that promotes plant health.

Plant-growing media are a staple good in controlled environment agriculture (CEA). Selecting the right growing media mixture is as crucial for optimal plant growth as light, temperature, humidity, water and nutrients. Hydroponic cultivation with plant-growing media is an expanding system expected to increase exponentially (Blok et al., 2021). Therefore, growing media must be sustainably produced, disposed of, recycled or reused. Next to optimal physicochemical properties, they can provide a multifunctional microbial community that fosters resilience to (a)biotic stress. Our advice to the growing media industry is to efficiently develop and optimize growing media mixtures that are fit for multiple purposes (e.g. microbial multifunctionality and organic fertilizer availability).

The prevention of disease and pests in CEA is pivotal. Strict hygiene measures like protective clothing and disinfection limit the entry of diseases and pests, while pesticides and biocontrol are remedying measures (van Delden et al., 2021). To prevent contamination and spread of pathogens, nutrient solutions are sterilized using disinfection systems like hydrogen peroxide, filters and UV radiation to prevent the spread of pathogens (Son et al., 2016). This also applies to plant-growing media, where mineral wool is the most popular constituent for the hydroponic cultivation of most vegetables (e.g. tomato, cucumber and bell pepper) because of its sterile production process making it initially free of pathogens (Carlile et al., 2015; Gruda, 2012). As a result, according to popular belief, hydroponic cultivation is considered a sterile production system. However, it is technically extremely difficult to prevent the occurrence of pests and diseases in CEA. They can be introduced through accidental contamination via employees, consumables, ventilation systems, doorways, inadequate phytosanitation and poor maintenance (Roberts et al., 2020). In this context, the aspiration of CEA to be sterile creates a pathogen-vulnerable environment (Roberts et al., 2020; van Delden et al., 2021). Indeed,

mineral wool is quickly colonized by microorganisms, but it lacks the microbial diversity to provide a resilient root environment that protects the plant against pathogen invasion (Grunert, Hernandez-Sanabria, et al., 2016). Microbiome diversity is critical for microbiome multifunctionality, and complex PGP bacteria consortia provide more efficient and consistent plant growth promotion (Batista & Singh, 2021; de la Fuente Cantó et al., 2020; Wagg et al., 2019). The use of bacterially enhanced plant-growing media can help in this respect. Our results showed that proper selection of the growing medium raw materials and the application of bacterial consortia improve bacterial diversity in the root zone, introducing bacteria that positively correlated with plant growth. In conclusion, growers should be aware that there is always a risk of contamination and should be prepared for the eventuality of disease proliferation in their system. Therefore, phytosanitary measures have to go hand in hand with exploiting the root-associated microbiome to minimize disease development and promote plant performance.

The maximization of root-associated microbiome functioning offers an excellent opportunity to secure plant performance and health in CEA. This can be achieved by amending a diverse bacterial inoculum. The current gaps in our knowledge are how to properly isolate, cultivate, formulate and store bacterial consortia (Batista & Singh, 2021). To allow formulation, the plant-growing medium can act as a carrier material for the bacterial consortia. Therefore, rather than solely focusing on the physicochemical properties when composing new plant-growing media, we need to transition towards selecting raw materials that harbour diverse microbiomes and have supportive properties to inoculation with bacterial consortia. Ideally, the bacterial consortia would be added during plant-growing medium manufacturing, avoiding the extra step of adding the inoculum at the start of cultivation. However, plant-growing media amended with bacterial consortia may not maintain their functionality after long-term storage or during long cultivation cycles. It may very well be that multiple applications are required for crops with a long cultivation cycle to sustain functional levels of the inoculum. Several hurdles still need to be overcome before bacterial community inocula become customary.

CONCLUSIONS

This study showed that the lettuce root-associated bacterial community structure depended on the type of plant-growing media raw materials used, even at the batch level. However, we can shape the community structure independently from the plant-growing media composition by amending bacterial community inocula. The introduction of bacterial diversity in the root zone through raw material selection and bacterial community inoculation was a driver for plant performance. The good-performing raw material GC introduced candidate PGP bacteria identified as *Bacilli* and *Actinobacteria* in the root zone. The transplantation of a root-associated bacterial community (BCI S3) also introduced potential PGP bacteria (*Pseudomonas*) in the root zone. This work supports the idea of creating bacterially enhanced plant-growing media by selecting raw materials and amending bacterial communities that introduce diverse and multifunctional microbiomes that support plant growth and health in CEA.

AUTHOR CONTRIBUTIONS

Thijs Van Gerrewey: Data curation (lead); formal analysis (lead); investigation (lead); visualization (lead); writing – original draft (lead). **Oscar Navarrete:** Supervision (supporting). **Maarten Vandecruys:** Supervision (supporting). **Maaike Perneel:** Conceptualization (supporting); project administration (supporting). **Nico Boon:** Conceptualization (equal); funding acquisition (equal); writing – review and editing (equal). **Danny Geelen:** Conceptualization (equal); funding acquisition (equal); supervision (lead); writing – review and editing (equal).

ACKNOWLEDGEMENTS

The authors acknowledge dr. Jeroen De Zaeytijd and dr. Nele Ameloot for supervision of the research and Tim Lacoere for 16s rRNA gene amplicon-sequencing support. Thank you to prof. dr. Kris Verheyen, prof. dr. Leen De Gelder, prof. dr. Aurélien Carlier, dr. Oliver Grunert and prof. dr. Leo Marcelis for their valuable feedback. This research was funded by the project grant VLAIO Baekeland mandate HBC.2017.0209.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses or interpretation of data, in the writing of the manuscript or in the decision to publish the results.

ORCID

Thijs Van Gerrewey https://orcid.org/0000-0001-8913-4803
Maarten Vandecruys https://orcid.org/0000-0001-6548-0664
Nico Boon https://orcid.org/0000-0002-7734-3103
Danny Geelen https://orcid.org/0000-0001-8105-3937

REFERENCES

Antoniou, A., Tsolakidou, M.-D., Stringlis, I.A. & Pantelides, I.S. (2017) Rhizosphere microbiome recruited from a suppressive compost improves plant fitness and increases protection against vascular wilt pathogens of tomato. *Frontiers in Plant Science*, 8, 1–16.

Bai, Y., Pan, B., Charles, T.C. & Smith, D.L. (2002) Co-inoculation dose and root zone temperature for plant growth promoting

rhizobacteria on soybean [*Glycine max* (L.) Merr] grown in soilless media. *Soil Biology and Biochemistry*, 34, 1953–1957.
Bais, H.P., Fall, R. & Vivanco, J.M. (2004) Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant Physiology*, 134, 307–319.
Barillot, C.D.C., Sarde, C.-O., Bert, V., Tarnaud, E. & Cochet, N. (2013) A standardized method for the sampling of rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. *Annales de Microbiologie*, 63, 471–476.
Barrett, G.E., Alexander, P.D., Robinson, J.S. & Bragg, N.C. (2016) Achieving environmentally sustainable growing media for soilless plant cultivation systems – a review. *Scientia Horticulturae*, 212, 220–234.
Batista, B.D. & Singh, B.K. (2021) Realities and hopes in the application of microbial tools in agriculture. *Microbial Biotechnology*, 14, 1258–1268.
Blok, C., Eveleens, B. & van Winkel, A. (2021) Growing media for food and quality of life in the period 2020–2050. *Acta Horticulturae*, 1305, 341–356.
Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E.V.L. & Schulze-Lefert, P. (2013) Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*, 64, 807–838.
Carlile, W.R., Cattivello, C. & Zaccheo, P. (2015) Organic growing media: constituents and properties. *Vadose Zone Journal*, 14, 1–13.
Cipriano, M.A.P., Lupatini, M., Lopes-Santos, L., da Silva, M.J., Roesch, L.F.W., Destéfano, S.A.L. et al. (2016) Lettuce and rhizosphere microbiome responses to growth promoting *Pseudomonas* species under field conditions. *FEMS Microbiology Ecology*, 92, fiw197.
Compant, S., Samad, A., Faist, H. & Sessitsch, A. (2019) A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research*, 19, 29–37.
Csardi, G. & Nepusz, T. (2006) The igraph software package for complex network research. *InterJournal, Complex Systems*, 1695, 1–9.
David, B.V., Chandrasehar, G. & Selvam, P.N. (2018) *Pseudomonas fluorescens*: a plant-growth-promoting rhizobacterium (PGPR) with potential role in biocontrol of pests of crops. In: Prasad, R., Gill, S.S. & Tuteja, N. (Eds.), *New and future developments in microbial biotechnology and bioengineering: crop improvement through microbial biotechnology*. Amsterdam, the Netherlands: Elsevier, pp. 221–243.
De Cáceres, M. & Legendre, P. (2009) Associations between species and groups of sites: indices and statistical inference. *Ecology*, 90, 3566–3574.
de la Fuente Cantó, C., Simonin, M., King, E., Moulin, L., Bennett, M.J., Castrillo, G. et al. (2020) An extended root phenotype: the rhizosphere, its formation and impacts on plant fitness. *Plant Journal*, 103, 951–964.
De Paepe, K., Kerckhof, F.M., Verspreet, J., Courtin, C.M. & Van de Wiele, T. (2017) Inter-individual differences determine the outcome of wheat bran colonization by the human gut microbiome. *Environmental Microbiology*, 19, 3251–3267.
De Vrieze, J., Coma, M., Debeuckelaere, M., Van der Meeren, P. & Rabaey, K. (2016) High salinity in molasses wastewaters shifts anaerobic digestion to carboxylate production. *Water Research*, 98, 293–301.
Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D. et al. (2016) Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications*, 7, 10541.
Dessaux, Y., Grandclément, C. & Faure, D. (2016) Engineering the rhizosphere. *Trends in Plant Science*, 21, 266–278.
Dufrêne, M. & Legendre, P. (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, 67, 345–366.
Eisenhauer, N., Schulz, W., Scheu, S. & Jousset, A. (2013) Niche dimensionality links biodiversity and invasibility of microbial communities. *Functional Ecology*, 27, 282–288.
Faust, K., Bauchinger, F., Laroche, B., de Buyl, S., Lahti, L., Washburne, A.D. et al. (2018) Signatures of ecological processes in microbial community time series. *Microbiome*, 6, 120.
Fierer, N. (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews. Microbiology*, 15, 579–590.
Garcia, J. & Kao-Kniffin, J. (2018) Microbial group dynamics in plant rhizospheres and their implications on nutrient cycling. *Frontiers in Microbiology*, 9, 1516.
Grimes, D.J., Woese, C.R., MacDonell, M.T. & Colwell, R.R. (1997) Systematic study of the genus *Vogesella* gen. nov. and its type species, *Vogesella indigofera* comb. nov. *International Journal of Systematic Bacteriology*, 47, 19–27.
Gruda, N. (2012) Current and future perspective of growing media in Europe. *Acta Horticulturae*, 960, 37–43.
Gruda, N. (2019) Increasing sustainability of growing media constituents and stand-alone substrates in soilless culture systems. *Agronomy*, 9, 298.
Grunert, O., Hernandez-Sanabria, E., Vilchez-Vargas, R., Jauregui, R., Pieper, D.H., Perneel, M. et al. (2016) Mineral and organic growing media have distinct community structure, stability and functionality in soilless culture systems. *Scientific Reports*, 6, 18837.
Grunert, O., Reheul, D., Van Labeke, M.-C., Perneel, M., Hernandez-Sanabria, E., Vlaeminck, S.E. et al. (2016) Growing media constituents determine the microbial nitrogen conversions in organic growing media for horticulture. *Microbial Biotechnology*, 9, 389–399.
Gu, Y., Dong, K., Geisen, S., Yang, W., Yan, Y., Gu, D. et al. (2020) The effect of microbial inoculant origin on the rhizosphere bacterial community composition and plant growth-promotion. *Plant and Soil*, 452, 105–117.
Hu, J., Wei, Z., Friman, V.-P., Gu, S., Wang, X., Eisenhauer, N. et al. (2016) Probiotic diversity enhances rhizosphere microbiome function and plant disease suppression. *mBio*, 7, e01790-16.
Kashyap, B.K., Solanki, M.K., Pandey, A.K., Prabha, S., Kumar, P. & Kumari, B. (2019) *Bacillus* as plant growth promoting rhizobacteria (PGPR): a promising green agriculture technology. In: Ansari, R.A. & Mahmood, I. (Eds.) *Plant health under biotic stress*. Singapore: Springer Singapore, pp. 219–236.
Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M. et al. (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, 41, e1.
Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. & Schloss, P.D. (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Applied and Environmental Microbiology*, 79, 5112–5120.
Kumar, A. & Dubey, A. (2020) Rhizosphere microbiome: engineering bacterial competitiveness for enhancing crop production. *Journal of Advanced Research*, 24, 337–352.
Kumar, V., Kumar, P. & Khan, A. (2020) Optimization of PGPR and silicon fertilization using response surface methodology for enhanced growth, yield and biochemical parameters of French bean (*Phaseolus vulgaris* L.) under saline stress. *Biocatalysis and Agricultural Biotechnology*, 23, 101463.
Kurtz, Z.D., Müller, C.L., Miraldi, E.R., Littman, D.R., Blaser, M.J. & Bonneau, R.A. (2015) Sparse and compositionally robust inference of microbial ecological networks. *PLoS Computational Biology*, 11, e1004226.
Li, Q., Chen, J., Wu, L., Luo, X., Li, N., Arafat, Y. et al. (2018) Belowground interactions impact the soil bacterial community, soil fertility, and crop yield in maize/peanut intercropping systems. *International Journal of Molecular Sciences*, 19, 622.

Love, M.I., Huber, W. & Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15, 550.

Lugtenberg, B. & Kamilova, F. (2009) Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology*, 63, 541–556.

Makowski, D., Ben-Shachar, M., Patil, I. & Lüdecke, D. (2020) Methods and algorithms for correlation analysis in R. *Journal of Open Source Software*, 5, 2306.

Mawarda, P.C., Mallon, C.A., Le Roux, X., van Elsas, J.D. & Salles, J.F. (2022) Interactions between bacterial inoculants and native soil bacterial community: the case of spore-forming *Bacillus* spp. *FEMS Microbiology Ecology*, 98, 1–11.

McMurdie, P.J. & Holmes, S. (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, 8, e61217.

Montagne, V., Capiaux, H., Barret, M., Cannavo, P., Charpentier, S., Grosbellet, C. et al. (2017) Bacterial and fungal communities vary with the type of organic substrate: implications for biocontrol of soilless crops. *Environmental Chemistry Letters*, 15, 537–545.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B. et al. (2020) vegan: Community ecology package. [WWW document]. URL cran.r-project.org/web/packages/vegan/index.html

Oteino, N., Lally, R.D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K.J. et al. (2015) Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Frontiers in Microbiology*, 6, 745.

Parnell, J.J., Berka, R., Young, H.A., Sturino, J.M., Kang, Y., Barnhart, D.M. et al. (2016) From the lab to the farm: an industrial perspective of plant beneficial microorganisms. *Frontiers in Plant Science*, 7, 1110.

Patten, C.L. & Glick, B.R. (2002) Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Applied and Environmental Microbiology*, 68, 3795–3801.

Pauwelyn, E., Vanhouteghem, K., Cottyn, B., De Vos, P., Maes, M., Bleyaert, P. et al. (2011) Epidemiology of *Pseudomonas cichorii*, the cause of lettuce midrib rot. *Journal of Phytopathology*, 159, 298–305.

Phour, M. & Sindhu, S.S. (2020) Amelioration of salinity stress and growth stimulation of mustard (*Brassica juncea* L.) by salt-tolerant *Pseudomonas* species. *Applied Soil Ecology*, 149, 103518.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41, D590–D596.

R Core Team. (2021) *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. [WWW document]. URL www.r-project.org

Rameshkumar, N., Lang, E. & Tanaka, N. (2016) Description of *Vogesella oryzae* sp. nov., isolated from the rhizosphere of saline tolerant pokkali rice. *Systematic and Applied Microbiology*, 39, 20–24.

Regeling Handelspotgronden. (2021) RHP the European knowledge centre for growing media since 1963. [WWW document]. URL www.rhp.nl

Roberts, J.M., Bruce, T.J.A., Monaghan, J.M., Pope, T.W., Leather, S.R. & Beacham, A.M. (2020) Vertical farming systems bring new considerations for pest and disease management. *Annals of Applied Biology*, 176, 226–232.

Sarris, P.F., Trantas, E.A., Mpalantinaki, E., Ververidis, F. & Goumas, D.E. (2012) *Pseudomonas viridiflava*, a multi host plant pathogen with significant genetic variation at the molecular level. *PLoS One*, 7, e36090.

Sasaki, K., Ikeda, S., Eda, S., Mitsui, H., Hanzawa, E., Kisara, C. et al. (2010) Impact of plant genotype and nitrogen level on rice growth response to inoculation with *Azospirillum* sp. strain B510 under paddy field conditions. *Soil Science & Plant Nutrition*, 56, 636–644.

Sathya, A., Vijayabharathi, R. & Gopalakrishnan, S. (2017) Plant growth-promoting actinobacteria: a new strategy for enhancing sustainable production and protection of grain legumes. *3 Biotech*, 7, 102.

Schloss, P.D., Gevers, D. & Westcott, S.L. (2011) Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PLoS One*, 6, e27310.

Soberón-Chávez, G. (2023) Some insights on traditional and novel approaches in microbial biotechnology that contribute to the United Nations sustainable development goals. *Microbial Biotechnology*, 16, 2015–2018.

Son, J.E., Kim, H.J. & Ahn, T.I. (2016) Hydroponic systems. In: Kozai, T., Niu, G. & Takagaki, M. (Eds.), *Plant factory*. London, UK: Academic Press, pp. 213–221.

Sreevidya, M. & Gopalakrishnan, S. (2017) Direct and indirect plant growth-promoting abilities of *Bacillus* species on chickpea, isolated from compost and rhizosphere soils. *Organic Agriculture*, 7, 31–40.

Thilagam, R. & Hemalatha, N. (2019) Plant growth promotion and chilli anthracnose disease suppression ability of rhizosphere soil actinobacteria. *Journal of Applied Microbiology*, 126, 1835–1849.

Tiwari, S., Prasad, V. & Lata, C. (2019) *Bacillus*: plant growth promoting bacteria for sustainable agriculture and environment. In: Singh, J.S. & Singh, D.P. (Eds.), *New and future developments in microbial biotechnology and bioengineering: microbial biotechnology in agro–environmental sustainability*. Amsterdam, the Netherlands: Elsevier, pp. 43–55.

Toju, H., Peay, K.G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K. et al. (2018) Core microbiomes for sustainable agroecosystems. *Nature Plants*, 4, 247–257.

Vacheron, J., Moënne-Loccoz, Y., Dubost, A., Gonçalves-Martins, M., Muller, D. & Prigent-Combaret, C. (2016) Fluorescent *Pseudomonas* strains with only few plant-beneficial properties are favored in the maize rhizosphere. *Frontiers in Plant Science*, 7, 1212.

van Delden, S.H., SharathKumar, M., Butturini, M., Graamans, L.J.A., Heuvelink, E., Kacira, M. et al. (2021) Current status and future challenges in implementing and upscaling vertical farming systems. *Nature Food*, 2, 944–956.

Van Gerrewey, T., Ameloot, N., Navarrete, O., Vandecruys, M., Perneel, M., Boon, N. et al. (2020) Microbial activity in peat-reduced plant growing media: identifying influential growing medium constituents and physicochemical properties using fractional factorial design of experiments. *Journal of Cleaner Production*, 256, 120323.

Van Gerrewey, T., Boon, N. & Geelen, D. (2021) Vertical farming: the only way is up? *Agronomy*, 12, 2.

Van Gerrewey, T., El-Nakhel, C., De Pascale, S., De Paepe, J., Clauwaert, P., Kerckhof, F.-M. et al. (2021) Root-associated bacterial community shifts in hydroponic lettuce cultured with urine-derived fertilizer. *Microorganisms*, 9, 1326.

Van Gerrewey, T., Vandecruys, M., Ameloot, N., Perneel, M., Van Labeke, M.-C., Boon, N. et al. (2020) Microbe-plant growing media interactions modulate the effectiveness of bacterial amendments on lettuce performance inside a plant factory with artificial lighting. *Agronomy*, 10, 1456.

Van Nevel, S., Koetzsch, S., Weilenmann, H.-U., Boon, N. & Hammes, F. (2013) Routine bacterial analysis with automated flow cytometry. *Journal of Microbiological Methods*, 94, 73–76.

Verdonck, O. & Gabriels, R. (1992) International society for horticultural science. *Acta Horticulturae*, 302, 169–180.

Verhagen, J.B.G.M. (2009) Stability of growing media from a physical, chemical, and biological perspective. *Acta Horticulturae*, 819, 135–142.

Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E.E. & van der Heijden, M.G.A. (2019) Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nature Communications*, 10, 4841.

Wang, Q., Garrity, G.M., Tiedje, J.M. & Cole, J.R. (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, 73, 5261–5267.

Wei, T. & Simko, V. (2021) R package "corrplot": visualization of a correlation matrix. [WWW document]. URL github.com/taiyun/corrplot

Wei, Z., Yang, T., Friman, V.P., Xu, Y., Shen, Q. & Jousset, A. (2015) Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. *Nature Communications*, 6, 8413.

Weidner, S., Koller, R., Latz, E., Kowalchuk, G., Bonkowski, M., Scheu, S. et al. (2015) Bacterial diversity amplifies nutrient-based plant–soil feedbacks. *Functional Ecology*, 29, 1341–1349.

Wickham, H. (2020) ggplot2: Elegant graphics for data analysis. [WWW document]. URL cran.r-project.org/web/packages/ggplot2/index.html.

Willis, A. & Bunge, J. (2015) Estimating diversity via frequency ratios. *Biometrics*, 71, 1042–1049.

Willis, A., Bunge, J. & Whitman, T. (2017) Improved detection of changes in species richness in high diversity microbial communities. *Applied Statistics*, 66, 963–977.

Willis, A.D. & Martin, B.D. (2022) Estimating diversity in networked ecological communities. *Biostatistics*, 23, 207–222.

Xin, X.-F., Kvitko, B. & He, S.Y. (2018) *Pseudomonas syringae*: what it takes to be a pathogen. *Nature Reviews. Microbiology*, 16, 316–328.

Xu, H., Yang, Y., Tian, Y., Xu, R., Zhong, Y. & Liao, H. (2020) Rhizobium inoculation drives the shifting of rhizosphere fungal community in a host genotype dependent manner. *Frontiers in Microbiology*, 10, 3135.

Yan, Y., Kuramae, E.E., de Hollander, M., Klinkhamer, P.G.L. & van Veen, J.A. (2017) Functional traits dominate the diversity-related selection of bacterial communities in the rhizosphere. *The ISME Journal*, 11, 56–66.

Zamkovaya, T., Foster, J.S., de Crécy-Lagard, V. & Conesa, A. (2021) A network approach to elucidate and prioritize microbial dark matter in microbial communities. *ISME Journal*, 15, 228–244.

Zhong, Y., Yang, Y., Liu, P., Xu, R., Rensing, C., Fu, X. et al. (2019) Genotype and rhizobium inoculation modulate the assembly of soybean rhizobacterial communities. *Plant, Cell & Environment*, 42, 2028–2044.

Zhuang, L., Li, Y., Wang, Z., Yu, Y., Zhang, N., Yang, C. et al. (2021) Synthetic community with six *pseudomonas* strains screened from garlic rhizosphere microbiome promotes plant growth. *Microbial Biotechnology*, 14, 488–502.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Van Gerrewey, T., Navarrete, O., Vandecruys, M., Perneel, M., Boon, N. & Geelen, D. (2024) Bacterially enhanced plant-growing media for controlled environment agriculture. *Microbial Biotechnology*, 17, e14422. Available from: https://doi.org/10.1111/1751-7915.14422