Transplanting bacterial community inocula on hydroponic lettuce

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The different components of the root zone (plant, microbiome, and rooting medium) can be engineered to enhance crop production sustainably by applying plant growth-promoting rhizobacteria (PGPR). Multispecies microbial inocula may be more effective in improving plant performance than single species PGPRs because they can better establish. In soilless systems, the selection of the plant growing medium raw materials plays a crucial role in the effectiveness of bacterial community amendment. Therefore, we investigated the effect of transplanting bacterial community inocula on lettuce (Lactuca sativa L.) root-associated bacterial community functioning inside a vertical farm. We found that the raw materials had distinct resident bacterial community structures and amending a bacterial community to the plant growing media allowed more control over the final root-associated bacterial community structure than a single species PGPR inoculum.

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; Toju et al., 2018; Kumar and Dubey, 2020; Van Gerrewey, et al., 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 amendments 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 selection of the plant growing medium might be critical in engineering the rhizosphere in soilless crop cultivation systems (Van Gerrewey, et al., 2020; Van Gerrewey, 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 fiber, 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 and 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 activity of the compost microbiome. They observed differences in microbial community composition between the unplanted compost and the tomato rhizosphere, suggesting the plant host was a driver for rhizosphere microbiome recruitment. Moreover, Bacillus spp. was the most abundant taxon in both the unplanted compost and tomato rhizosphere, which possesses plant growth-promoting 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 plant growth-promoting rhizobacteria (PGPR). PGPRs can promote plant growth through various mechanisms like N-fixation, phosphate solubilization, siderophore production, phytohormone production, stress alleviation, and pathogen resistance (Lugtenberg and Kamilova, 2009; Bulgarelli et al., 2013). However, the positive effects of single species PGPR application do not always transfer from lab conditions to a realistic environment (Parnell et al., 2016; Compant et al., 2019). 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). In addition, the impact of inoculation may depend on the plant host genotype (Sasaki et al., 2010; Zhong et al., 2019). Also, the inoculum dosage can affect the plant growth response (Bai et al., 2002).

Instead, the amendment of microbial consortia may be more effective in improving plant performance than single species inocula (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 was shown to increase with increasing inoculum diversity (Hu et al., 2016). Moreover, higher Pseudomonas inoculum diversity reduced Ralstonia solanacearum disease incidence in the tomato rhizosphere due to resource competition and direct interference with the pathogen. Gu et al. (2020) showed that inoculation with soil bacterial communities increased the rhizosphere bacterial community diversity, which played an essential role in plant growth promotion. However, the effect of inoculation on the rhizosphere 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) on the performance of lettuce (Lactuca sativa L.) grown in ten different plant growing media (M1–10) composed of peat (black peat or white peat), other organics (coir pith or wood fiber), composted materials (composted bark or green waste compost), and inorganic materials (perlite or sand) inside a vertical farm (Van Gerrewey, et al., 2020). We showed that microbe-plant growing medium interactions were major determinants of lettuce performance. For example, BCI S3 possessed plant growth-promoting 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 material selection is critical when amending bacterial community inocula to promote plant growth.

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, et al., 2020). Metagenomic analyses on the 16S rRNA gene sequences were performed to determine the root-associated bacterial community structures, diversity, and compositions. Moreover, the raw materials and inocula were associated with the introduction of potential PGPRs. 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) Do plant growing media composition and bacterial community inoculation promote bacterial diversity in the root zone, benefiting plant performance? (3) Do plant growing media composition and bacterial community inoculation introduce OTUs linked to improved plant performance?

We identified changes in the lettuce root-associated bacterial community structure depended on the raw materials used. The raw material batches generated large differences in community structure across experimental replicates. The physiochemical and biological properties of the raw materials can vary, even at the batch level. For example, compost is known for its strong variability in quality from batch to batch due to the many different primary organic materials and composting conditions (Barrett et al., 2016; Gruda, 2019). Therefore, certification systems exist to assure a good quality product 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 can lead to slight variations in physicochemical and biological properties of the final mixtures. These variations affect the intricate physical, chemical, and biological relations in plant growing media, which in turn influence the microbial community structure (Grunert, et al., 2016; Grunert, et al., 2016; Montagne et al., 2017).

In addition to the high variability in microbiome structure introduced by the raw materials even at the batch level, the expected exponential growth of the plant growing media market will result in the development of more novel materials and the use of more different blends (Blok et al., 2021). This high variability and uncertainty make manipulating the resident communities in plant growing media raw materials a daunting task. However, we showed that amending a bacterial community allowed more control over the structure across different plant growing media mixtures. Moreover, our previous research showed that bacterial community amendment could improve plant performance over different plant growing media mixtures (Van Gerrewey, et al., 2020). Therefore, the amendment of multifunctional bacterial consortia may be a better approach to steer root-associated community structure for plant growth promotion.

We asked whether plant growing media composition and bacterial community inoculation can introduce potential PGPRs. Our observations showed that raw material selection and inoculation shifted the root-associated bacterial community. The representative OTUs of the raw materials were mainly classified as a few common phyla: *Proteobacteria*, *Bacteroidota*, *Planctomycetota*, *Actinobacteriota*, *Verrucomicrobiota*, *Acidobacteriota*, *Firmicutes*, and *Chloroflexi*, indicating that lettuce selects OTUs from only a few core taxa to establish a distinct root-associated microbial community. Indeed, Cardinale *et al.* (2015) identified these taxa among the core taxa of lettuce. This observation highlights the narrow selection the plant host exerts on the root-associated bacterial community through niche adaptation (de la Fuente Cantó *et al.*, 2020).

Although the root-associated bacterial communities were mainly derived from select core taxa, a few taxa were associated with specific raw materials. For example, *Patescibacteria* was a distinct phylum that has been detected in peat bogs, fens, and moderately acidic water, suggesting that this is a core phylum present in the raw material that settles in the root zone (Juottonen *et al.*, 2017; Lemos *et al.*, 2019; Ivanova *et al.*, 2020).

Our previous research also observed that changing the substrate type did not affect microbial activity, following the absence of carbon in these generally sterile raw materials (Van Gerrewey, *et al.*, 2020; Regeling Handelspotgronden, 2021). The lack of effect indicates that the presence of organic carbon in the raw materials is required to have a resident community that can induce changes in the root-associated bacterial community.

Conclusion

Our results showed high variability in root-associated bacterial community structure introduced by the raw materials even at the batch level. However, the community structure can be controlled across different plant growing media mixtures by using bacterial community inocula. The results suggest that introducing more bacterial diversity in the root zone through raw material selection and bacterial community inoculation is a driver for plant performance. The good performing raw materials introduce multiple OTUs classified as Bacilli and Actinobacteria in the root zone, which are known to possess plant growth-promoting properties. The use of substrate and inocula likely resulted in a synergistic effect on plant performance by introducing more bacterial diversity and PGPRs. Therefore, we need to transition towards selecting raw materials that harbor diverse and multifunctional microbiomes and support bacterial community amendment, creating bacterially enhanced plant growing media that secure plant performance in a hydroponic environment.

References

Antoniou, A., Tsolakidou, M.-D., Stringlis, I.A., and Pantelides, I.S. (2017) Rhizosphere Microbiome Recruited from a Suppressive Compost Improves Plant Fitness and Increases Protection against Vascular Wilt Pathogens of Tomato. *Front Plant Sci* **8**: 1–16.

Bai, Y., Pan, B., Charles, T.C., and Smith, D.L. (2002) Co-inoculation dose and root zone temperature for plant growth promoting rhizobacteria on soybean [Glycine max (L.) Merr] grown in soil-less media. *Soil Biol Biochem* **34**: 1953–1957.

Bais, H.P., Fall, R., and 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 Physiol* **134**: 307–319.

Barillot, C.D.C., Sarde, C.O., Bert, V., Tarnaud, E., and Cochet, N. (2013) A standardized method for the sampling of rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. *Ann Microbiol* **63**: 471–476.

Barrett, G.E., Alexander, P.D., Robinson, J.S., and Bragg, N.C. (2016) Achieving environmentally sustainable growing media for soilless plant cultivation systems – A review. *Sci Hortic (Amsterdam)* **212**: 220–234.

Blok, C., Eveleens, B., and van Winkel, A. (2021) Growing media for food and quality of life in the period 2020-2050. *Acta Hortic* **1305**: 341–356.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E.V.L., and Schulze-Lefert, P. (2013) Structure and Functions of the Bacterial Microbiota of Plants. *Annu Rev Plant Biol* **64**: 807–838.

De Cáceres, M. and Legendre, P. (2009) Associations between species and groups of sites: Indices and statistical inference. *Ecology* **90**: 3566–3574.

Cardinale, M., Grube, M., Erlacher, A., Quehenberger, J., and Berg, G. (2015) Bacterial networks and co-occurrence relationships in the lettuce root microbiota. *Environ Microbiol* **17**: 239–252.

Carlile, W.R., Cattivello, C., and Zaccheo, P. (2015) Organic Growing Media: Constituents and Properties. *Vadose Zo J* **14**: vzj2014.09.0125.

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 Microbiol Ecol* **92**: fiw197.

Compant, S., Samad, A., Faist, H., and Sessitsch, A. (2019) A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *J Adv Res* **19**: 29–37.

Csardi, G. and Nepusz, T. (2006) The igraph software package for complex network research. *InterJournal, complex Syst* **1695**: 1–9.

David, B. V., Chandrasehar, G., and Selvam, P.N. (2018) Pseudomonas fluorescens : A Plant-Growth-Promoting Rhizobacterium (PGPR) With Potential Role in Biocontrol of Pests of Crops. In *Crop Improvement Through Microbial Biotechnology*. Elsevier, pp. 221–243.

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. *Nat Commun* **7**: 10541.

Dessaux, Y., Grandclément, C., and Faure, D. (2016) Engineering the Rhizosphere. *Trends Plant Sci* **21**: 266–278.

Dufrêne, M. and Legendre, P. (1997) Species assemblages and indicator species: The need for a flexible asymmetrical approach. *Ecol Monogr* **67**: 345–366.

Eisenhauer, N., Schulz, W., Scheu, S., and Jousset, A. (2013) Niche dimensionality links biodiversity and invasibility of microbial communities. *Funct Ecol* **27**: 282–288.

Faust, K., Bauchinger, F., Laroche, B., Buyl, S. de, Lahti, L., Washburne, A.D., et al. (2018) seqtime: Time Series Analysis of Sequencing Data.

Gabriels, D., Hartmann, R., Verplancke, H., Cornelis, W., and Verschoore, P. (1998) Werkwijzen voor grondanalyses. *Univ Gent, Gent, Belgium*.

Garcia, J. and Kao-Kniffin, J. (2018) Microbial group dynamics in plant rhizospheres and their implications on nutrient cycling. *Front Microbiol* **9**: 1516.

Van Gerrewey, T., Ameloot, N., Navarrete, O., Vandecruys, M., Perneel, M., Boon, N., and Geelen, D. (2020) Microbial activity in peat-reduced plant growing media: Identifying influential growing medium constituents and physicochemical properties using fractional factorial design of experiments. *J Clean Prod* **256**: 120323.

Van Gerrewey, T., Boon, N., and 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., and Geelen, D. (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.

GRIMES, D.J., WOESE, C.R., MacDONELL, M.T., and COLWELL, R.R. (1997) Systematic Study of the Genus Vogesella gen. nov. and Its Type Species, Vogesella indigofera comb. nov. *Int J Syst Bacteriol* **47**: 19–27.

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. *Sci Rep* **6**: 18837.

Grunert, O., Reheul, D., Van Labeke, M.-C., Perneel, M., Hernandez-Sanabria, E., Vlaeminck, S.E., and Boon, N. (2016) Growing media constituents determine the microbial nitrogen conversions in organic growing media for horticulture. *Microb Biotechnol* **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 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**:.

Ivanova, A.A., Beletsky, A. V., Rakitin, A.L., Kadnikov, V. V., Philippov, D.A., Mardanov, A. V., et al. (2020) Closely Located but Totally Distinct: Highly Contrasting Prokaryotic Diversity Patterns in Raised Bogs and Eutrophic Fens. *Microorganisms* **8**: 484.

Jørgensen, N.O.G., Brandt, K.K., Nybroe, O., and Hansen, M. (2010) Vogesella mureinivorans sp. nov., a peptidoglycan-degrading bacterium from lake water. *Int J Syst Evol Microbiol* **60**: 2467–2472.

Juottonen, H., Eiler, A., Biasi, C., Tuittila, E.-S., Yrjälä, K., and Fritze, H. (2017) Distinct Anaerobic Bacterial Consumers of Cellobiose-Derived Carbon in Boreal Fens with Different CO 2 /CH 4 Production Ratios. *Appl Environ Microbiol* **83**: 1–15.

Kashyap, B.K., Solanki, M.K., Pandey, A.K., Prabha, S., Kumar, P., and Kumari, B. (2019) Bacillus as Plant Growth Promoting Rhizobacteria (PGPR): A Promising Green Agriculture Technology. In *Plant Health Under Biotic Stress*. Ansari, R.A. and Mahmood, I. (eds). Singapore: Springer Singapore, pp. 219–236.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., and Glöckner, F.O. (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* **41**: e1–e1.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and 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. *Appl Environ Microbiol* **79**: 5112–5120.

Kumar, A. and Dubey, A. (2020) Rhizosphere microbiome: Engineering bacterial competitiveness for enhancing crop production. *J Adv Res* **24**: 337–352.

Kurtz, Z.D., Müller, C.L., Miraldi, E.R., Littman, D.R., Blaser, M.J., and Bonneau, R.A. (2015) Sparse and Compositionally Robust Inference of Microbial Ecological Networks. *PLoS Comput Biol* **11**:.

de la Fuente Cantó, C., Simonin, M., King, E., Moulin, L., Bennett, M.J., Castrillo, G., and Laplaze, L. (2020) An extended root phenotype: the rhizosphere, its formation and impacts on plant fitness. *Plant J* **103**: 951–964.

Lemos, L.N., Medeiros, J.D., Dini-Andreote, F., Fernandes, G.R., Varani, A.M., Oliveira, G., and Pylro, V.S. (2019) Genomic signatures and co-occurrence patterns of the ultra-small Saccharimonadia (phylum CPR/Patescibacteria) suggest a symbiotic lifestyle. *Mol Ecol* **28**: 4259–4271.

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. *Int J Mol Sci* **19**: 622.

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

Lugtenberg, B. and Kamilova, F. (2009) Plant-Growth-Promoting Rhizobacteria. *Annu Rev Microbiol* **63**: 541–556.

Makowski, D., Ben-Shachar, M., Patil, I., and Lüdecke, D. (2020) Methods and Algorithms for Correlation Analysis in R. *J Open Source Softw* **5**: 2306.

McMurdie, P.J. and 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., and Lebeau, T. (2017) Bacterial and fungal communities vary with the type of organic substrate: implications for biocontrol of soilless crops. *Environ Chem Lett* **15**: 537–545.

Nadeem, S.M., Imran, M., Naveed, M., Khan, M.Y., Ahmad, M., Zahir, Z.A., and Crowley, D.E. (2017) Synergistic use of biochar, compost and plant growth-promoting rhizobacteria for enhancing cucumber growth under water deficit conditions. *J Sci Food Agric* **97**: 5139–5145.

Van Nevel, S., Koetzsch, S., Weilenmann, H.-U., Boon, N., and Hammes, F. (2013) Routine bacterial analysis with automated flow cytometry. *J Microbiol Methods* **94**: 73–76.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2020) vegan: Community Ecology Package.

Oteino, N., Lally, R.D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K.J., and Dowling, D.N. (2015) Plant growth promotion induced by phosphate solubilizing endophytic Pseudomonas isolates. *Front Microbiol* **6**: 1–9.

De Paepe, K., Kerckhof, F.M., Verspreet, J., Courtin, C.M., and Van de Wiele, T. (2017) Inter-individual differences determine the outcome of wheat bran colonization by the human gut microbiome. *Environ Microbiol* **19**: 3251–3267.

Parnell, J.J., Berka, R., Young, H.A., Sturino, J.M., Kang, Y., Barnhart, D.M., and DiLeo, M. V. (2016) From the Lab to the Farm: An Industrial Perspective of Plant Beneficial Microorganisms. *Front Plant Sci* **7**: 1–12.

Patten, C.L. and Glick, B.R. (2002) Role of Pseudomonas putida Indoleacetic Acid in Development of the Host Plant Root System. *Appl Environ Microbiol* **68**: 3795–3801.

Pauwelyn, E., Vanhouteghem, K., Cottyn, B., De Vos, P., Maes, M., Bleyaert, P., and Höfte, M. (2011) Epidemiology of Pseudomonas cichorii, the Cause of Lettuce Midrib Rot. *J Phytopathol* **159**: 298–305.

Phour, M. and Sindhu, S.S. (2020) Amelioration of salinity stress and growth stimulation of mustard (Brassica juncea L.) by salt-tolerant Pseudomonas species. *Appl Soil Ecol* **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 Res* **41**: D590–D596.

R Core Team. (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rameshkumar, N., Lang, E., and Tanaka, N. (2016) Description of Vogesella oryzae sp. nov., isolated from the rhizosphere of saline tolerant pokkali rice. *Syst Appl Microbiol* **39**: 20–24.

Regeling Handelspotgronden (2021) RHP the European knowledge centre for growing media since 1963.

Sarris, P.F., Trantas, E.A., Mpalantinaki, E., Ververidis, F., and 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 Sci Plant Nutr* **56**: 636–644.

Sathya, A., Vijayabharathi, R., and 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., and Westcott, S.L. (2011) Reducing the Effects of PCR Amplification and Sequencing Artifacts on 16S rRNA-Based Studies. *PLoS One* **6**: e27310.

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

Thilagam, R. and Hemalatha, N. (2019) Plant growth promotion and chilli anthracnose disease suppression ability of rhizosphere soil actinobacteria. *J Appl Microbiol* **126**: 1835–1849.

Tiwari, S., Prasad, V., and Lata, C. (2019) Bacillus: Plant Growth Promoting Bacteria for Sustainable Agriculture and Environment. In *New and Future Developments in Microbial Biotechnology and Bioengineering*. 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. *Nat Plants* **4**: 247–257.

Vacheron, J., Moënne-Loccoz, Y., Dubost, A., Gonçalves-Martins, M., Muller, D., and Prigent-Combaret, C. (2016) Fluorescent Pseudomonas Strains with only Few Plant-Beneficial Properties Are Favored in the Maize Rhizosphere. *Front Plant Sci* **7**: 1–13.

Verdonck, D. ir. O. and Gabriels, D. ir. R. (1992) INTERNATIONAL SOCIETY FOR HORTICULTURAL SCIENCE. *Acta Hortic* 169–180.

De Vrieze, J., Coma, M., Debeuckelaere, M., Van der Meeren, P., and Rabaey, K. (2016) High salinity in molasses wastewaters shifts anaerobic digestion to carboxylate production. *Water Res* **98**: 293–301.

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

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

Wei, T. and Simko, V. (2021) R package "corrplot": Visualization of a Correlation Matrix.

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

Weidner, S., Koller, R., Latz, E., Kowalchuk, G., Bonkowski, M., Scheu, S., and Jousset, A. (2015) Bacterial diversity amplifies nutrient-based plant–soil feedbacks. *Funct Ecol* **29**: 1341–1349.

Wickham, H. (2020) ggplot2: elegant Graphics for Data Analysis.

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

Willis, A., Bunge, J., and Whitman, T. (2017) Improved detection of changes in species richness in high diversity microbial communities. *J R Stat Soc Ser C Appl Stat* **66**: 963–977.

Willis, A.D. and Martin, B.D. (2020) Estimating diversity in networked ecological communities. *Biostatistics*.

Xin, X.-F., Kvitko, B., and He, S.Y. (2018) Pseudomonas syringae: what it takes to be a pathogen. *Nat Rev Microbiol* **16**: 316–328.

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

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

Zhong, Y., Yang, Y., Liu, P., Xu, R., Rensing, C., Fu, X., and Liao, H. (2019) Genotype and rhizobium inoculation modulate the assembly of soybean rhizobacterial communities. *Plant Cell Environ* **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. *Microb Biotechnol* **14**: 488–502.