Revista Brasileira de Ciência do Solo

Division – Soil Use and Management | Commission – Soil Fertility and Plant Nutrition

Rhizosphere microbiome engineering of *Triticum aestivum* L.

Shabana Wagi[(1)]*, Peer Schenk[(2)], Ambreen Ahmed[(1)] and Eladl Eltanahay[(3)]

[(1)] University of the Punjab, Institute of Botany, Quaid- e- Azam Campus, Lahore, Pakistan.
[(2)] University of Queensland, School of Agriculture and Food Sciences, Brisbane, Australia.
[(3)] Faculty of Science Mansoura University, Botany Department, Egypt.

ABSTRACT: Root-associated microbiomes (RAMs) are complex microbial communities, essential for plant growth and development. The RAMs interact with the roots, maintain the root architecture, protect plants from a plethora of pathogens and biotic and abiotic stress and intensify nutrient uptake, i.e., improve plant growth and yield. A wide variety of microbial populations is usually found in the rhizosphere. Plant exudates also play a significant role in the establishment of rhizospheric microbial communities. This study deals with the approach of microbiome engineering to enhance the development of crops such as wheat. We focus on the idea of soil engineering to foster beneficial microbial communities that can improve plant growth effectively and reduce competition by gradually decreasing the number of pathogenic communities. This technique enables plants to thrive under adequate edaphic conditions. In the current study, the rhizosphere of *Triticum aestivum* L. was analyzed over four generations. Variations in the microbial diversity between batches one to four (B1-B4) were analyzed with regard to their capacity to improve plant growth. Microbial species richness in the rhizosphere microbiome of wheat was recorded in all investigated plant batches (B0 to B4). The major phyla across the four plant batches were *Proteobacteria, Chloroflexi* and *Actinobacteria.* Jaccard Similarity Coefficient indicated similarity between the batches B4-treated and B4-control. Taxonomic distances between the bacterial communities of Batches B0, B1 and B4 were the highest. Significant improvements in the growth parameters of plants treated with a microbiome-containing soil solution of the previous generation (batch) were recorded. Subsequently, their microbiome was also engineered, which facilitated plant growth effectively.

Keywords: root associated microbiome, proteobacteria, *Triticum aestivum* L., microbiome engineering.

*** Corresponding author:**
E-mail: shabanawagi@gmail.com

Received: October 31, 2022
Approved: June 02, 2023

How to cite: Wagi S, Schenk P, Ahmed A, Eltanahay E. Rhizosphere microbiome engineering of *Triticum aestivum* L. Rev Bras Cienc Solo. 2023;47:e0220141
https://doi.org/10.36783/18069657rbcs20220141

Editors: José Miguel Reichert and Marco Aurélio Carbone Carneiro.

INTRODUCTION

Rhizosphere microbiome composition is diverse and usually host-specific. It acts as a micro-ecosystem with complex interactions between roots and microorganisms, facilitating nutrient uptake and plants' biotic and abiotic stress tolerance (Haldar et al., 2022; Orozco-Mosqueda et al., 2022). With the advent of modern technologies, it became possible to evaluate the plant microbiome by analyses of below-ground and above-ground microbial communities. This will help identify, select and engineer beneficial microbial communities for higher crop yields (Rivera-Pinto et al., 2018). These plant-associated microbiomes have coordinated mechanisms that can induce plant growth improvement and provide an alternative sustainable way to improve crop productivity in an eco-friendly manner (Muller et al., 2015; Kumar and Dubey, 2020; Moreira et al., 2023). These selected microbial communities associated with the plant phenotype trigger microbial selection, compete with other bacterial communities and tolerate the chemical imbalance of the soil caused by inadequate agricultural inputs, i.e., even under stress conditions, they can improve plant growth and development.

Metagenomics is a genetic tool for identifying biological entities residing in the rhizosphere. In nature, polymicrobial interactions are highly significant and essential for developing a balanced ecosystem. Studies of microbial population dynamics provide more accurate knowledge and detailed understanding about the actual phenomena involved in microbe-microbe and plant-microbe interactions. Between 1960 and 1980, it became clear that crop-dependent approaches provided only insufficient information about microbial communities in a particular habitat, creating the demand for alternative approaches. Recently, various crop-independent approaches have been developed to analyze microbial consortia. Metagenomics, for example, is the study of sets of genomes from mixed microbial populations. This term was first coined by Handelsman and coworkers (Schlaeppi and Bulgarelli, 2015).

Rhizosphere microbiome assembly plays a significant role in improving plant growth by efficiently reinforcing the biotic and abiotic stress tolerance of plants. Genetic makeup of plant species and edaphic factors trigger metabolic activities of the microbiome. Plant exudates directly feed rhizosphere microbial communities. These exudates are responsible for the type of developing microbial communities, in other words, the plant species, varieties and even cultivars are significantly important for the rhizospheric microbiome (Pérez-Jaramillo et al., 2017).

Metagenomes actually provide a clear insight into the physiology of any microbial community. Rhizosphere microbiome engineering can be utilized to reshape and maintain beneficial plant-microbe interactions to get more beneficial outcomes. This approach was developed to promote the growth of beneficial microbes, by evolving plants to develop beneficial host relationships naturally by triggering root exudates (Mohanram and Kumar, 2019). Plant-associated bacteria, especially rhizobacteria, play a vital role in plant health by using several direct and indirect mechanisms of action e.g., nutrient availability, hormone production, siderophore production, pathogen resistance and stress tolerance.

To meet the current food demand and improve yield per hectare, chemical fertilizers are used in wheat growth (Kavamura et al., 2021). Applying microbial inoculants as biofertilizers is more appropriate than chemical fertilizers, which are toxic and undegradable and consequently affect the ecosystem negatively (Sun Xiaoxiao et al., 2019). The objective of this study is to investigate how the plant phenotype triggers microbial selection and to engineered the microbiome of the wheat rhizosphere to improve its yield in a sustainable manner. In this study, the rhizosphere microbiome of *Triticum aestivum* L. was engineered and the microbial communities were analyzed over four generations by repeated inoculations from the preceding microbial generation.

MATERIALS AND METHODS

Engineering of rhizosphere microbiome

Fresh garden soil was collected in Brisbane, Australia, in May, 2018. Debris and stones were removed and the soil was placed on a tray with 36 sections. Wheat seeds of var. FSD 2008 were surface-disinfected with 1 % sodium hypochlorite, followed by several washings with autoclaved distilled water to remove traces of NaOCl. Five surface-disinfected seeds per tray section in a growth chamber were sown. For the first batch (B0), a total of 160 seeds were sown. After three weeks of plant growth, five plants with optimum growth were selected and their roots were washed in autoclaved distilled water. This rhizospheric solution was used to treat the plants of the batch (B1), while control plants were treated with distilled water. A similar procedure was repeated soil for the second and third batch, up to the fourth generation (B4). For each generation, the rhizospheric water of the previous batch was used to water the next one (B0-B1-B2-B3-B4). Plants of all four batches were harvested and different growth parameters, such as shoot length, root length and number of leaves, were recorded for each generation and compared with control plants. A total of four batches (B0-B4) of microbiome treatments were analyzed in the current study.

Microbiome analysis by next-generation sequencing

Rhizospheric soil of treated and control plants was taken and genomic DNA was extracted using soil SV genomic DNA extraction purification kit, a commercial DNA-extraction/purification kit (Promega). Extracted DNA was then subjected to PCR amplification using 27F and 1492R primers. Amplified products were analyzed using a NanoDrop™ One/One Microvolume UV-Vis Spectrophotometer (Thermo Fischer) and agarose gel electrophoresis. The DNA samples were sent to Western Sydney University, Australia, for next-generation sequence analysis. A library was constructed using bacterial primers (16S) 341F-5'CCTACGGGNGGCWGCAG and 805R- 5' GACTACHVGGGTATCTAATCC and Illumina™ overhang adaptors forward 5'TCGTCGGCAGCGTCAGATGTTGTATAAGAGACAG [locus-specific sequence] and reverse overhang 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-sequence subjected to adaptor trimming, were then sequenced using Illumina MiSeq with read length of 2*301 bp.

Metagenomic data analysis

The sequences were first quality filtered and then de-replicated using the QIIME script split_libraries.py with the homopolymer filter deactivated and checked for chimeras against the Green Genes database (October 2013 release) using UCHIME ver. 3.0.617. Operational taxonomic units (OTUs) with 97 % similarity were selected and representative sequences were assigned which generated tables that show the abundance and taxonomic position of samples. We analyzed the species richness and evenness contained in the soil clone library using single diversity indices. In addition, in the co-occurrence analysis, α-and β-diversity of the samples was also assessed.

RESULTS

Rhizospheric microbiome engineering

Different growth parameters (shoot length, root length and number of leaves) of *Triticum aestivum* L. were evaluated and compared with the control plants of each batch. A reduction in shoot length was recorded in inoculated batch B1 plants, compared to the control plants, and was found to be further decreased in batch two (B2), compared to the control plants. In batch three (B3), shoot length increased over that of control plants. This increment was also observed in plants of batch four (B4). Negligible improvement

in root length over control plants was recorded in three batches (B1, B3 and B4). No effect on root length was observed in the second batch (B2), although a longer root length was recorded in batches three and four (B3 and B4). No significant improvement was observed in the number of leaves of the treated compared to control plants.The number of leaves of control plants was found to be higher than that of treated plants in batch two (B2) and almost equal to control plants in batches three and four (B3 and B4) (Figures 1 and 2).

Variations in OTU Richness

Rhizospheric microbial communities of *Triticum aestivum* L. were analyzed for α and β-diversity. Bacterial α-diversity was measured as OTU richness within batches (B0-B4), while β-diversity was measured among various batches. Bacterial community richness in the rhizosphere microbiome of wheat differed during different generations of plant growth from batch B0 to B4 (Figures 3, 4, 5 and 6).

Microbial community composition

A total of 39, 978 and 956 Pass Filter (PF) reads were obtained from all five batches. *Plancomycetes, Proteobacteria, Acidobacteria, Actinobacteria* and *Chloroflexi* were the most commonly recorded microbial phyla in the wheat rhizosphere. The most frequently observed bacterial classes associated with the wheat rhizosphere were *Alphaproteobacteria*, *Solibacter*, *Chloriacidobacteria*, *Phycisphaerae*, *Plancomycetia*, *Actinobacteria*, *Acidomicrobiia*, *Gemm-1*, *OD1*, *TM7-1*, *TM7*-3, *Fimbriimonadia* and *Ellin* 6529. Various genera associated with the wheat root microbiome included *Candidatus, Solibacter*, *Pedomicrobium*, *Sphingomonas*, *Gemmata, Streptomyces*, *Catellatospora*, *Kribbella*, *Agrobacterium*, *Devosia*, *Kaistobacter*, *Mycobacterium*, *Sphingopyxis*, *Rhodococcus*, *Bdellovibrio*, *Sphingobium*, *Bradyrhizobium*, *Corynebacterium*, *Pseudonocardia*, *Phenylobacterium*, *Candidatus, Xiphinematobacter*, *Modestobacter*, *Fimbriimonas*, *Ochrobactrum* and *Microlunatus* (Figure 3).

Variations in α-Diversity

In batch zero (B0), *Planctomycetes* followed by *Proteobacteria*, *Actinobacteria*, *Gemmatimonades*, *Acidobacteria*, *TM7* and *Chloroflexi* were the most abundantly observed taxonomic phyla. The relative density of the bacterial phylum *Proteobacteria,* followed by *Plancomycetes*, *Acidobacteria*, *Chloroflexi* and *Actinobacteria,* were the most abundant phyla in B1, and in B2, *Proteobacteria* followed by *Planctomycetes*. The *Protobacteria* followed by *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, *Plancomycetes*, *Verrucomicrobia*, TM7, OD1 and WS6 were commonly recorded in B3 populations, but in the final community rhizosphere, *Actinobacteria* followed by *Chloroflexi*, *Proteobacteria*, *Acidobacteria*, *Gemmetamonadetes* and *TM7* were the most abundant taxonomic phyla (Figure 3).

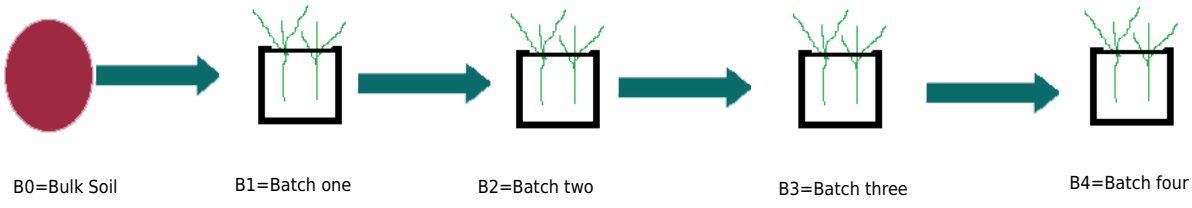

Figure 1. Experimental design to test the effect of microbial communities on growth of *Triticum aestivum L.*

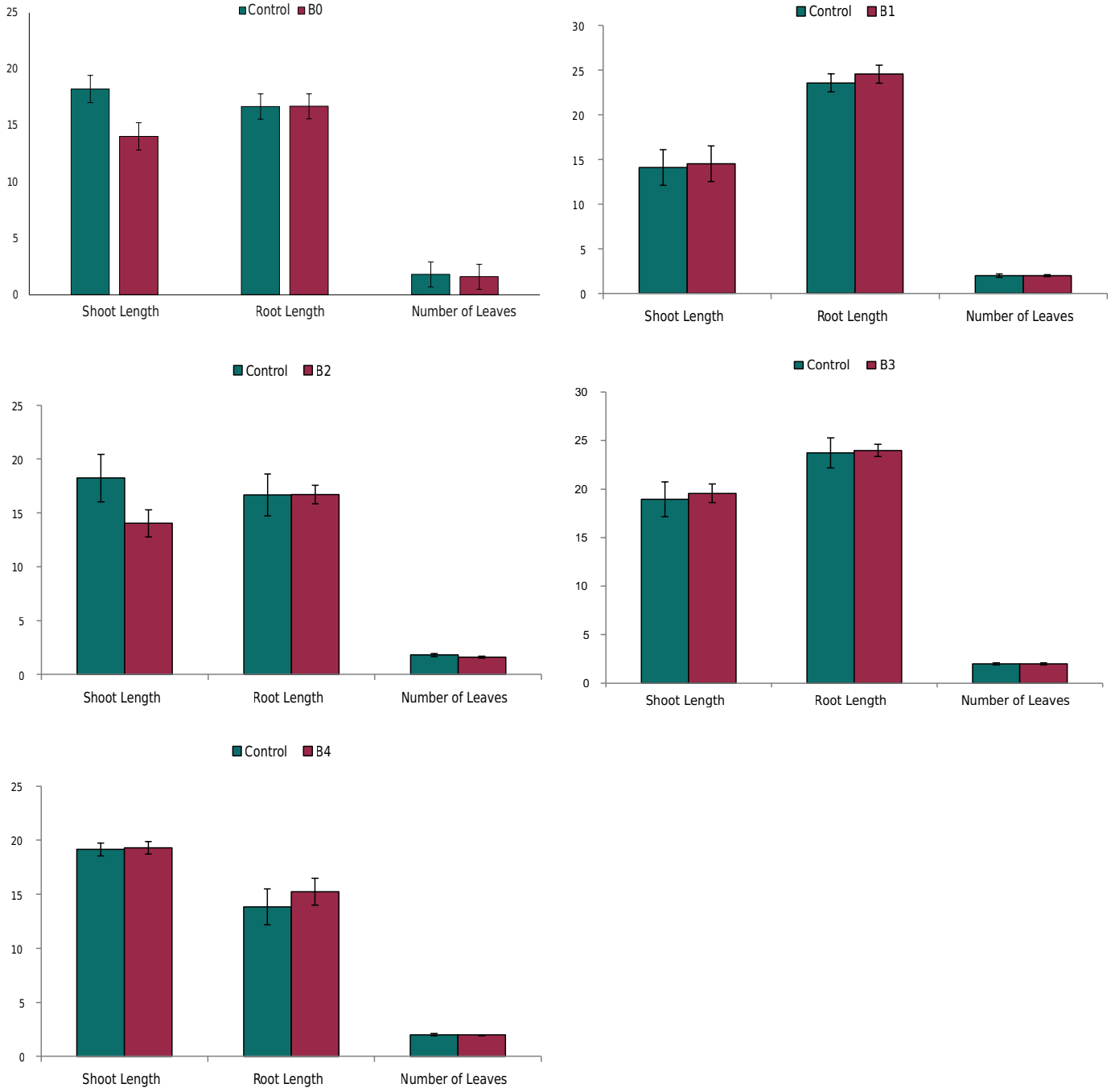

Figure 2. Effect of microbial communities on growth of *Triticum aestivum* L.

Variation in β-Diversity

Jaccard Similarity Coefficient indicated that batch B4-treated and B4-control were similar and most closely related to each other (Figure 4). On the other hand, B3 and B2-control were up to 50 % similar to each other, while B1 was rather distinct and farther away from all other batches. Batches B0 and B3 were also similar to each other. Significant differences were also observed between wheat communities based on weighted unifraction distances by PCoA analysis (ANOSIM R = 0.4; P = 0.0001). The distribution of pairwise weighted unifraction distances was also explored. It was found that the profile of the wheat communities was significantly different among the four batches (Figure 4).

Figure 3. Evaluation of microbial community composition.

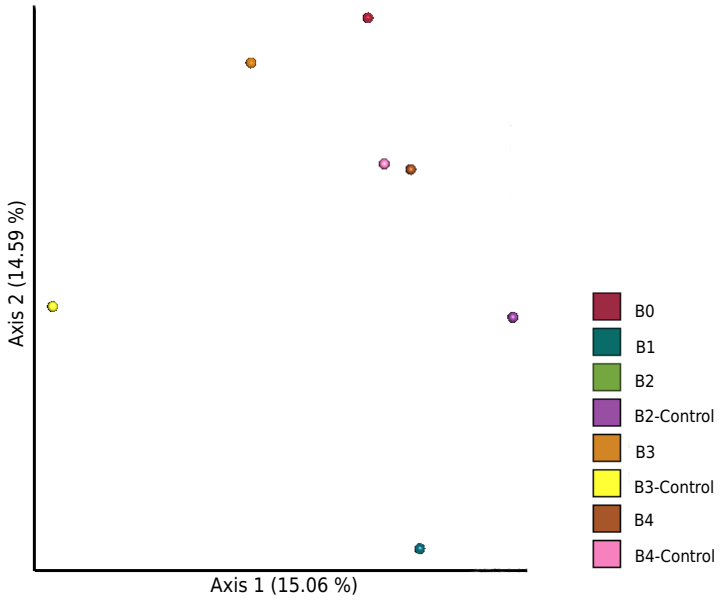

Figure 4. Jaccard Similarity Coefficient indicating the composition of microbiome.

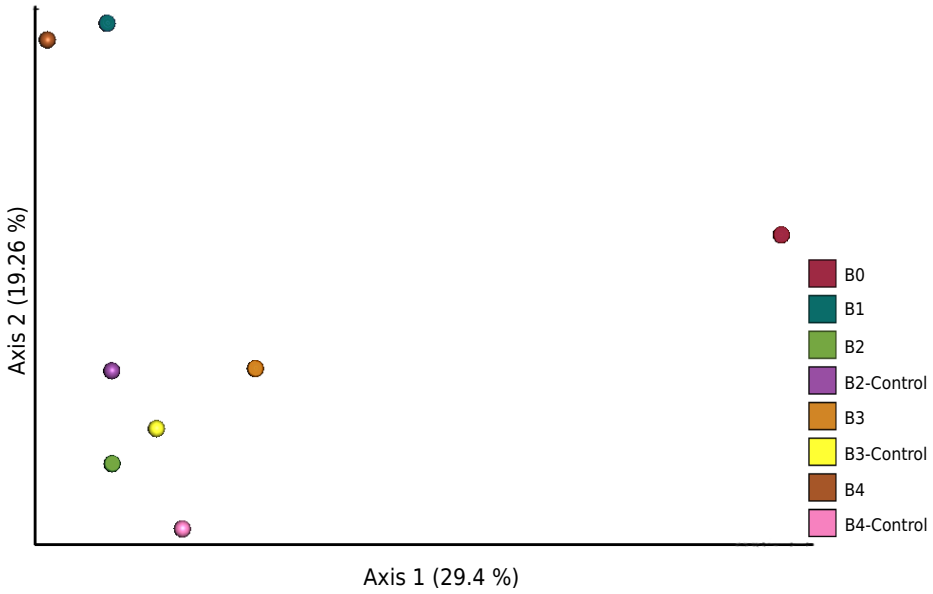

Figure 5. Unifraction (Qualitative) analysis of wheat rhizosphere microbiome.

Taxonomic distances between bacterial communities of the batches B0, B1 and B4 were found to be highest, and those between B2, B2-control, B3, B3-control and B4-control the lowest. These results with taxonomic composition data suggested less variation in the batches B3, B3-control and B4-control (Figures 5 and 6). The phyla Plancomycetes and Proteobacteria were more abundant in the rhizosphere of B2-control than of B2-treated plants. The classes Acidobacteria, Solibacteria, Actinobacteria, Alphaproteobacteria, Actinomycetes, Chloroacidobacteria, Gemmatimonadetes had a higher relative percentage in batch B2 than the control plants (B2-control). The relative percentage of Plancomycetes, Acidobacteria (Solibacteria), Actinobacteria was significantly higher in B3-treated than in B3-control plants. At the same time, relative percentage of the phyla Proteobacteria

(α-proteobacteria), Caulobacteria (Brevundimonas), Acidobacteria, Proteobacteria (Rhodospirillales), Proteobacteria and Plancomycetes was higher in B3 plants compared to control plants of this batch while the relative abundance of Chloroflexi was found to be similar in both B3-treated and control plants. The classes Planctomycetes, Chloroflexi, Actinomycetes and orders such as Rhodobacterales and Solibacterales had a higher relative frequency in B4 than B4-control plants. Moreover, the microbial classes Chloroflexisellin, Planctomycetes Gemmate, Chloroflexis S085, Proteobacteria, Deltaproteobacteria and order Myxococcales were more abundant in the plant rhizosphere of all batches (B0-B4) and are therefore frequently found bacterial communities in the rhizosphere (Figures 5 and 6).

Taxonomic characterization

Rhizospheric community was represented by 39,978,956 reads. Pyrotag reads obtained from wheat showed 99 % similarity to the BLAST hit against the NCBI 16S database. Results indicated that the analyzed fraction dominated by bacteria was closely related to previously characterized and reported species. Variations in phylum, class, order, family and genus level of the taxonomic compositions of microbiomes in the wheat rhizosphere were assessed. The rhizospheric microbiome of *T. aestivum* was characterized by the abundance of the phyla *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *TM7, Armetimonadetes*, *Gemmatimonadetes*, *OD1* and *Verrucomycetes*. The classes *Deltaproteobacteri*, *Alphaproteobacteria*, *Solibacteres*, *Chloroacidobacteria*, *Phycisphaerae*, *Plancomycetia*, *Actinomicrobia*, *Acidomicrobiia, Gemm-1*, *Ellin 6529*, *Gitt-GS-136*, *Anaerolineae*, *Chloroflexi*, *SHA-20*, *Thermomicrobi*a, *TK-17*, *TK10, TM7-1*, *TM7-3, ZB-2*, *Fimbriimonadia* and *0319-6E2* were the most commonly found bacterial classes associated with the rhizosphere and were also abundant in the wheat microbiome. The relative abundance of taxa varied between the various batches of wheat (B0-B4). Interestingly, some poorly represented groups such as *Verrucomicrobia* were also observed (Figures 7 and 8).

DISCUSSION

This study deals with microbiome engineering of the wheat rhizosphere, to establish sustainable agricultural practices. The root microbiome provides multiple health benefits to the plants. The idea behind this study was to engineer the soil successively with continuous applications of multiple microbe communities of the same soil to make it suitable for developing beneficial bacteria that can improve and maximize plant growth organically. A plant growth experiment was conducted with wheat, and the root microbiome and growth potential were assessed after successive applications of bacterial inoculants from the soil under the best plants of each harvest. Batch B0 was planted in the untreated raw soil collected from Peer's garden in Australia. An increase in shoot and root length was recorded in the B1 wheat population, while no increase was recorded in the number of leaves. However, *Proteobacteria, Planctomycetes, Acidobacteria, Chloroflexi* and *Actinobacteria* were common residents of the root microbiome in B1. A stable microbial community structure was also reported by Yousaf and Elshahed (2009) in a soil dominated by the nine major bacterial phyla *Chloroflexi, Actinobacteria*, *Acidobacteria*, *Firmicutes*, *Plancomycetes*, *Gammetimonidates* and *Verrucomicrobia*. These authors stated that more diverse communities contribute more to the ecosystem functionality than the less diverse communities. No effect on

shoot length was recorded in B2, while root length was almost equal in the treated and control plants. Interestingly, an increase in number of leaves was recorded in B2 plants (Figures 1 and 2).

In the root microbiome of this B2, *Actinobacteria* and *Proteobacteria* were the most abundant classes. Other classes such as *Planctomycetes, Acidobacteria, Chlroflexi* and unidentified *Gemmatimonadetes* and TM7 were also recorded in this batch. In B3, shoot and root length were also improved, while no effect on number of leaves was recorded (Figure 3). This batch showed richness in microbial diversity. *Proteobacteria*, *Actinobacteria* and *Chloroflexi* were the most abundant microbial communities and similarly, *Planctomycetes* and unidentified Verrucomicrobia, TM7, OD1 and WS6 were also recorded in the root microbiome of B3 plants (Figure 3). McPherson et al. (2018) also reported high diversity of *Proteobacteria* and *Actinobacteria* followed by *Chloroflexi* on the root microbiome of grass. In the fourth batch (B4), shoot and root length increases were recorded, while the number of leaves remained the same. *Actinobacteria, Chloroflexi* and *Proteobacteria* were the most abundant inhabitants of wheat roots in B4. *Planctomycetes* and *Acidobacteria* were also observed, and only *TM7* was recorded in plants of this batch. An earlier study found that physical and geochemical parameters influence bacterial communities (Rascovan et al., 2016). With regard to the wheat root microbiome across all batches, the most abundant phylum was *Proteobacteria* and the least abundant OD1. Significant differences in microbiomes of the different batches (B0-B4) were observed. Beta diversity of the wheat rhizosphere microbiome indicated that in B3 and B4, there was less variation in microbial communities, i.e., they are more or less consistent, while variations were greater in B0, B1 and B2. One possible reason might be that the microbial populations became consistent (reached a stable climax stage) and were less affected by the successive generations (Figures 4, 5, 6 and 7).

Proteobacteria is a major phylum of Gram-negative bacteria that can fix nitrogen. *Chloroflexi* is green non-sulphur bacteria that are actually aerobic thermophiles.

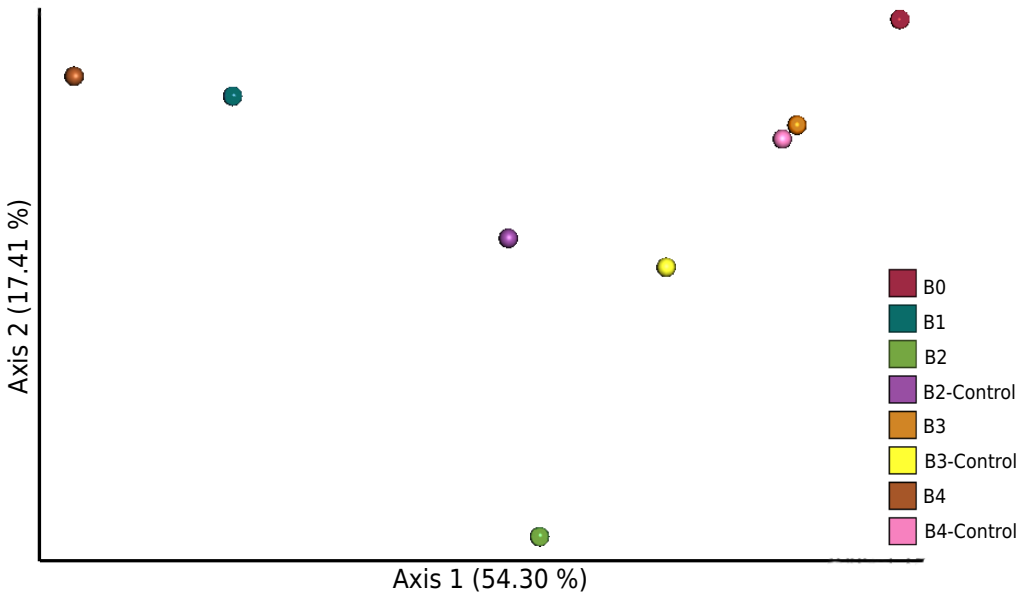

Figure 6. Unifraction (Quantitative) analysis of wheat rhizosphere microbiome.

Actinobacteria are gram-positive bacteria, extremely important for agriculture and forests, as their contribution is essential for forest development. They contribute to soil development. *Acidobacteria* is also a rather important bacterial phylum. The expansion of knowledge about bacteria and high throughput sequencing made it easy to apply the microbiome concept to agriculture practices with good results (Srhan et al., 2018). Tao et al. (2017) reported that *Acidobacteria* and *Verrucobacteria* improved corn yield significantly. The microbial flora of batch zero (B0) was moderately expressed, while those of batches B1, B2, B3 and B4 were highly expressed and more diverse (Figure 3). Unifraction weighted (quantitative) analysis showed that more bacterial communities were detected in B0, B1 and B4 than in B2. Moreover, the population density in B3 and B4-control was intermediate.

Bacteria density in batches B3 and B4 was very similar but relatively lower than in B0 (Figure 4). Unifraction qualitative analysis showed that microbial communities in batches B0 and B1 were closely related to each other, with highest similarity percentages, while the community in B4 was distinct, and all other batch communities were quite closely related to each other (Figures 5, 7 and 8). Plants along with the associated microbes are known as holobiont, which constitutes a complex system, owing to a greater representation of *Pseudomonas, Copiotrophs, Oligotrophs and Actinobacteria* (Rascovan et al., 2016). *Proteobacteria* are predominant in silty soil (Youssef and Elshahed, 2009). According to Chandra et al. (2021), *Chloroflexi* and *Gemmatimonadetes* are the dominant members of the sugarcane rhizosphere and improve crop yield under drought stress.

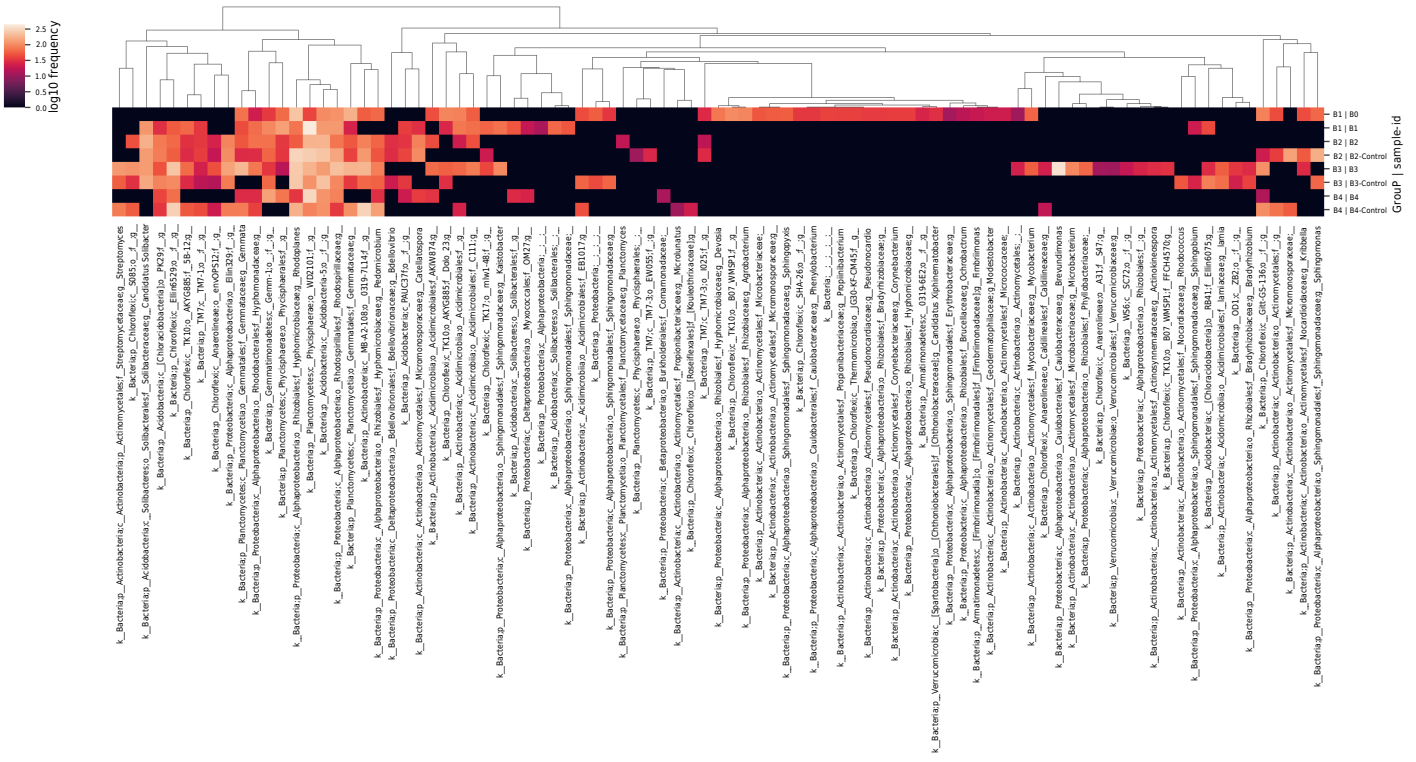

Figure 7. Relative frequency of wheat rhizosphere microbiome.

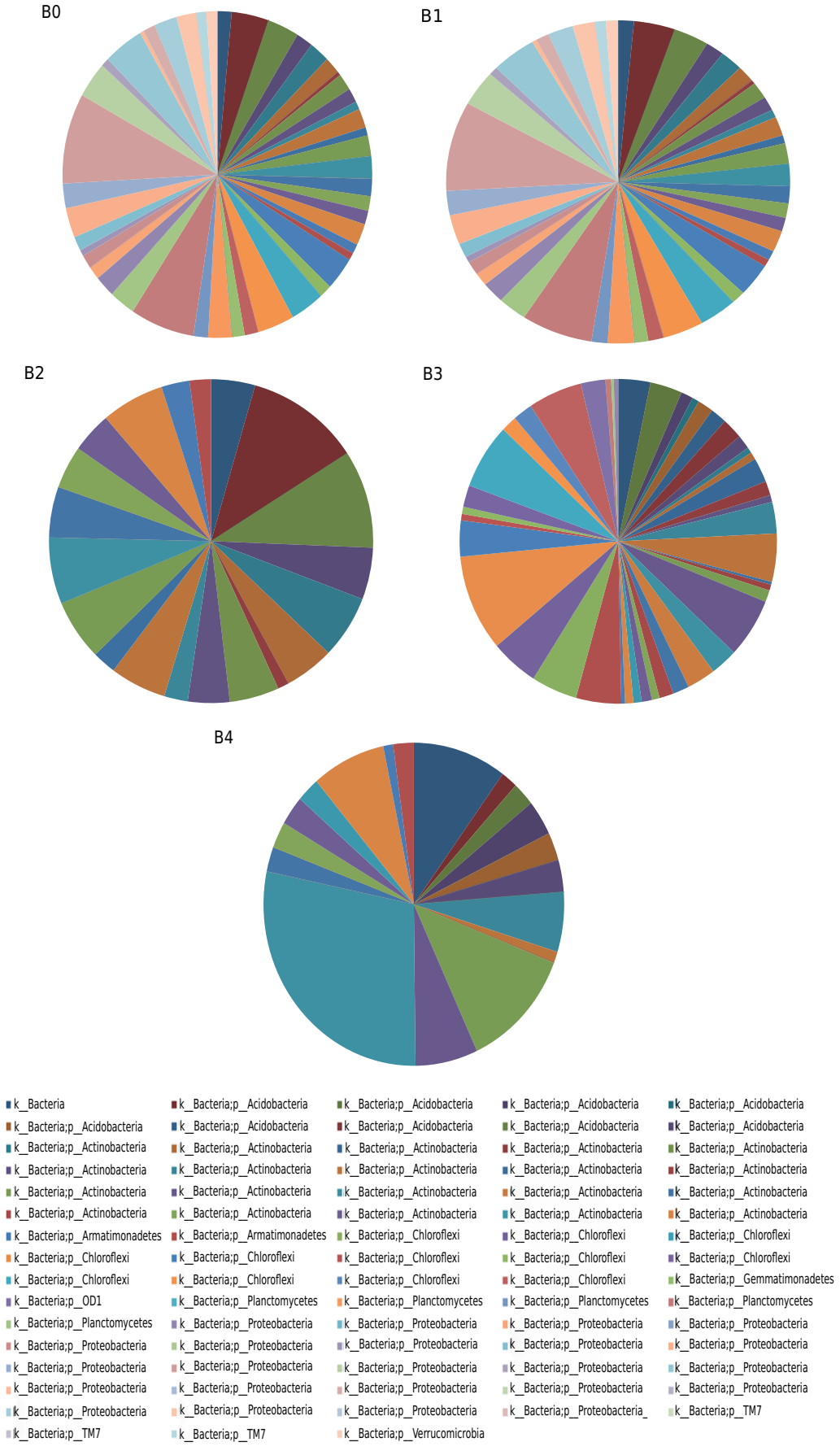

Figure 8. Effect on microbial communities associated with *Triticum aestivum* L. in (a) Batch B0, (b) Batch B1, (c) Batch B2, (d) Batch B3 and (e) Batch B4.

CONCLUSION

Root microbiome engineering provides an alternative natural way to improve wheat growth and yield by selective engineering and microbial organization in the wheat rhizosphere by a successive planting technique. The best-represented phyla in the plant rhizosphere of B1-B4 were *Proteobacteria, Chloroflexi* and *Actinobacteria*, Gram-negative and Gram-positive bacteria. This study suggests that these three phyla play a significant role in wheat growth and development. In addition, the authors believe that an amendment of the rhizhosphere with these phyla may be an effective strategy to replace the extensive use of chemical fertilizers.

AUTHOR CONTRIBUTIONS

Conceptualization: Peer Schenk (lead).

Data curation: Shabana Wagi (lead).

Formal analysis: Eladl Eltanahay (supporting) and Shabana Wagi (lead).

Methodology: Shabana Wagi (lead).

Project administration: Shabana Wagi (lead).

Resources: Peer Schenk (lead).

Software: Eladl Eltanahay (lead).

Supervision: Ambreen Ahmed (supporting) and Peer Schenk (lead).

Visualization: Ambreen Ahmed (equal) and Eladl Eltanahay (equal).

Writing – original draft: Shabana Wagi (lead).

Writing – review & editing: Ambreen Ahmed (equal) and Peer Schenk (equal).

REFERENCES

Chandra A, Gaur V, Tripathi P. Microbiome analysis of rhizospheres of plant and winter-initiated ratoon crops of sugarcane grown in sub-tropical India: utility to improve ratoon crop productivity. 3 Biotech. 2021;11:34. https://doi.org/10.1007/s13205-020-02603-9

Haldar S, Mondal S, Kumari A, Ghosh A, Chattopadhyay D, Ghosh A. Rhizosphere microbiome engineering. In: Joshi S, Pandey A, Sirohi R, Park SH, editors. Current developments in biotechnology and bioengineering. Amsterdan, Holanda: Elsevier; 2022. p. 377-96. https://doi.org/10.1016/B978-0-323-88504-1.00014-5

Kavamura VN, Mendes R, Bargaz A, Mauchline TH. Defining the wheat microbiome: Towards microbiome-facilitated crop production. Comput Struct Biotechnol J. 2021;19:1200-13. https://doi.org/10.1016/j.csbj.2021.01.045

Kumar A, Dubey A. Rhizosphere microbiome: Engineering bacterial competitiveness for enhancing crop production. J Adv Res. 2020;24:337-52. https://doi.org/10.1016/j.jare.2020.04.014

McPherson MR, Wang P, Marsh EL, Mitchell RB, Schachtman DP. Isolation and analysis of microbial communities in soil, rhizosphere, and roots in perennial grass experiments. JoVE J. 2018;137:e57932. https://doi.org/10.3791/57932

Mohanram S, Kumar P. Rhizosphere microbiome: Revisiting the synergy of plant-microbe interactions. Ann Microbiol. 2019;69:307-20. https://doi.org/10.1007/s13213-019-01448-9

Moreira ZPM, Chen MY, Ortuno DLY, Haney CH. Engineering plant microbiomes by integrating eco-evolutionary principles into current strategies. Curr Opin Plant Biol. 2023;71:102316. https://doi.org/10.1016/j.pbi.2022.102316

Mueller UG, Sachs JL. Engineering microbiomes to improve plant and animal health. Trends Microbiol. 2015;23:606-17. https://doi.org/10.1016/j.tim.2015.07.009

Orozco-Mosqueda MC, Fadiji AE, Babalola OO, Glick BR, Santoyo G. Rhizobiome engineering: Unveiling complex rhizosphere interactions to enhance plant growth and health. Microbiol Res. 2022;263:127137. https://doi.org/10.1016/j.micres.2022.127137

Pérez-Jaramillo JE, Carrión VJ, Bosse M, Ferrão LFV, Hollander M, Garcia AAF, Ramírez CA, Mendes R, Raaijmakers JM. Linking rhizosphere microbiome composition of wild and domesticated Phaseolus vulgaris to genotypic and root phenotypic traits. ISME J. 2017;11:2244-57. https://doi.org/10.1038/ismej.2017.85

Rascovan N, Carbonetto B, Perrig D, Díaz M, Canciani W, Abalo M, Alloati J, González-Anta G, Vazquez MP. Integrated analysis of root microbiomes of soybean and wheat from agricultural fields. Sci Rep. 2016;6:28084. https://doi.org/10.1038/srep28084

Rivera-Pinto J, Egozcue JJ, Pawlowsky-Glahn V, Paredes R, Noguera-Julian M, Calle ML. Balances: A new perspective for microbiome analysis. MSystems. 2018;3:e00053-18 https://doi.org/10.1128/mSystems.00053-18

Sarhan MS, Patz S, Hamza MA, Youssef HH, Mourad EF, Fayez M, Murphy B, Ruppel S, Hegazi NA. G3 PhyloChip analysis confirms the promise of plant-based culture media for unlocking the composition and diversity of the maize root microbiome and for recovering unculturable candidate divisions/phyla. Microbes Environ. 2018;33:317-25. https://doi.org/10.1264/jsme2.ME18023

Schlaeppi K, Bulgarelli D. The plant microbiome at work. Mol Plant Microbe In. 2015;28:212-7. https://doi.org/10.1094/MPMI-10-14-0334-FI

Sun X, Wang M, Guo L, Shu C, Zhang J, Geng L. Guanidine thiocyanate solution facilitates sample collection for plant rhizosphere microbiome analysis. Peer J. 2019;7:e6440. https://doi.org/10.7717/peerj.6440

Tao J, Liu X, Liang Y, Niu J, Xiao Y, Gu Y, Ma L, Meng D, Zhang Y, Huang W, Peng D, Yin, H. Maize growth responses to soil microbes and soil properties after fertilization with different green manures. Appl Microbiol Biotechnol. 2017;101:1289-99. https://doi.org/10.1007/s00253-016-7938-1

Youssef NH, Elshahed MS. Diversity rankings among bacterial lineages in soil. ISME J. 2009;3:305-13. https://doi.org/10.1038/ismej.2008.106