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Phosphorus Availability Modulates Rhizospheric Bacterial Communities in Elite and Landrace Wheat Genotypes

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Abstract

Phosphorus is a limiting nutrient directly impacting yield and plant associated microbiome. We studied the effect of phosphorus stress in wheat genotype (C306 landrace vs. PBW 725 cultivar) on rhizospheric bacterial communities at the *tillering* stage. Rhizosphere bacterial community established by tillering stage with a conserved core dominated by Firmicutes and Proteobacteria. Phosphorous stress and genotype caused minimal restructuring of abundance weighted rhizospheric community evidenced by Bray-Curtis based PCoA and comparable Shannon and Simpson indices. P-starvation increased bacterial richness without disrupting community evenness particularly in landrace indicating selective recruitment of rare rhizospheric taxa. KEGG Orthology separated samples based on genes related to metabolism, redox processed and early functional differentiation in soil microbial communities. The data generated in this study has been deposited in the NCBI under Bio-project code PRJNA1139961. Phosphorus starvation primarily expands the rare biosphere and alters functional potential without disrupting the core microbiome, highlighting early rhizosphere stability with implications for microbiome-informed nutrient management and breeding strategies.

Introduction

Wheat is an important staple crop for ensuring food security for the growing world population (www.ifpri.org/topic/food-security, www.oecd.org). It is an excellent source of carbohydrates (75%-80%) and proteins (9%-18%) and a significant source of vitamins and minerals. It provides an average of 500 kcal of energy per capita per day. Most of the post-Green revolution breeding programs of wheat have prioritized yield and disease resistance with little thought given to root exudation traits and microbe-responsiveness of a variety (Dixon et al 2022). These traits have gained importance lately because of recognition of role of microbes in enhancing plant growth and yield. Also, sustainable farming operations using beneficial microbes can meet the many challenges faced by agriculture today. These microbes provide many benefits to the plant which include nutrient acquisition, plant growth promotion, improving tolerance to biotic-abiotic stresses and bio-control traits (Garcha and Samredhi 2024, Pirttilä et al 2021, Santos et al 2019, Verma et al 2019, Mazid and Khan 2015, Bhardwaj et al 2014). Isolation of these microbes from plants grown under stress and from the associated microflora of wild varieties are the contemporary approaches in screening for beneficial microbes (Seybold et al 2020, Kohl et al 2019, Legrand et al 2017).

Several reports have established that the rhizospheric and endophytic bacterial community of crops including wheat is influenced by many factors which include the genotype, soil physico-chemical and biological traits, crop-rotation scheme, agronomic practices and fertilization, developmental stage of the plant, etc (Kavamura et al 2018, Mahoney et al 2017, Gdanetz et al 2017, Robinson et al 2016, Mauchline et al 2015). Most of the studies have related genotype with rhizospheric microbiome of wheat at maturity stage. Landraces have been reported to have abundance of Verrumicrobia and Bacteroidetes and domesticated cultivars have revealed abundance of Proteobacteria and Actinobacteria (Gholizadeh et al 2022). Tcakz et al 2020 reported abundance of Bacteroidetes in hybrid wheat whereas Kinnunan-

Grubb et al 2020 reported abundance of Firmicutes and Verrumicrobia in modern cultivars of wheat. Clearly, there is an interplay of many factors. The studies cited above were carried out in different geographical areas where soil characteristics vary greatly. Evidently, soil physical and chemical characteristics also determine the composition of the indigenous microflora.

Much work on rhizospheric community structure has been reported at maturity stage but scant reports have studied how soon the plant genotype manifests its impact on rhizospheric soil microbiome. In this study, we have focused on early developmental stage, tillering stage. Our first independent variable was genotype. We used a landrace C306 and elite genotype PBW 725. The elite genotype is popularly cultivated in North-Western states of India which contribute more than 75% to national grain production kitty. We used P level as the second independent variable. Between N/P/K we choose P because P starvation changes the tillering emergence pattern and reduces the rate of tiller emergence for each tiller (Rodriguez et al 1999). We hypothesized that wheat genotype is a key determinant of rhizospheric bacterial community assembly and that phosphorus availability modifies these genotype-specific recruitment patterns, leading to significant shifts in bacterial diversity, composition, and community structure by the tillering stage under field conditions. N level and K level were excluded from this study for uncomplicated interpretation of results and to prevent association without causation.

Materials and Methods

Experimental design and soil sampling

The objective of this study was to study the combined influence of phosphate starvation and genotype on the rhizospheric community structure in tillering stage of wheat. Wheat varieties- landrace (locally adapted wild variety) C306 and a cultivar PBW 725 were procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University Ludhiana. Seeds were surface sterilized by treating with 70% ethanol for 2–3 minutes followed by 0.1% $HgCl_2$ for 1 minute. The sterile seeds were washed with autoclaved water for at least 3–5 minutes. Seeds were placed on 0.5% Nutrient Agar plates and incubated at 25–28°C for 24–48 h. Sterility was confirmed by absence of microbial growth on the agar plates. Sterile seeds were sown in 1.5m X 1.5m plots. Approximately, 22–23 g of seeds (@40kg/acre) were sown into manually dug 5 cm deep tranches in the winter season of 2024–2025. Plots of each variety (C 306 and PBW 725) and two treatments, P-amended and P-starved, were replicated in randomized block design. These plots were in a research field where rice-wheat monoculture cropping system is extensively practiced. Plots were managed manually for agronomic operations as weeding and examined visually for appearance of any disease. It was managed as per standard protocol for cultivation of wheat mirroring the agronomic practices practised by farmers https://www.pau.edu/content/ccil/pf/pp_rabi.pdf. Two treatments were maintained- P amended in which 48.6 g urea per plot (@50kg N/acre) and 30.2 g diammonium phosphate DAP (@25kg P_20_5 /acre) were applied. In P-starved plots, DAP was not applied but 48.6 g urea per plot (@50kg N/acre) was applied. Urea contains 46% N and DAP contains 18% N and contains 46% P_20_5. Soil moisture level was

maintained at 40% and checked subjectively. We did not record incidence of any disease. Three replicates were maintained.

The seedlings were harvested carefully at tillering stage by digging around each plant to a depth of at least 6–8 inches. Plants were placed in plastic sample bags and transported to the laboratory. Roots were shaken to remove the bulk soil. Roots were placed in sterile containers having 50 ml of sterile 0.85% (w/v) NaCl. These containers were placed in orbital shaker for 20 minutes. The contents were centrifuged at 5000 rpm for 15 minutes. The pellet having rhizospheric soil was stored at -20°C. Rhizospheric soil samples from five plants per plot were collected.

DNA extraction and high throughput sequencing

Approximately, 5g soil was used for DNA extraction using Xploregen kit following the manufacturer`s instructions. Extracted DNA from the samples were subjected to Nano Drop and GEL Check before being taken for PCR amplification. QCPurity of DNA was determined by recording readings at 260/280 for a value between 1.8-2.0. For PCR Amplification of 16s region, primer 16s F-5'GAGTTTGATGMTGGCTCAG3' 16s R- 5' TTACCGCGGCMGCSGGCAC3' were used. The amplicons from each sample were purified with Ampure beads to remove unused primers and an additional 8 cycles of PCR was performed using Illumina barcoded adapters to prepare the sequencing libraries. Libraries were purified using Ampure beads and quantitated using Qubit dsDNA High Sensitivity assay kit. Sequencing was performed using Illumina Miseq with 2x300PE v3-v4 sequencing kit.

Data processing

NCBI database was used for 16s V3-V4 region for bioinformatics analysis. The data received from the sequencer was de-multiplexed into fastq raw data. It was checked using Fastqc (Version 0.11.9) and Multiqc (Version 1.10.1) tools. The QC passed samples are qualified for further analysis and for 16s metagenomic analysis. The Phred score of sequences used for downstream processing was ≥ 30. Sequences with less than 350 nt were removed. Filtered sequences after merging of OUT clustering were subjected to chimeric detection with > 97% similarity. The workflow of the pipeline was- the abundance feature tables and the top ten organisms in each sample were constructed using Microsoft excel. The other analyses like Heatmap, core microbiome, Dendrogram, Alpha diversity, Beta diversity, PCOA plot, Rarefaction curve was built using online resource of www.microbiomeanalyst.ca. For Metabolic pathway visualization another online tool name Metageneassist was used (http://www.metagenassist.ca/).The data input was filtered and normalized. For alpha diversity analysis, over 0.1 million reads counts were considered. The average contig length (V3-V4) was 469 bp. Alpha diversity was measured by calculating the Chao1, Shannon and Simpson indexes with the statistical method of T-test / ANOVA. The beta diversity was constructed at the taxonomic level of Genus with Bray-Curtis index distance method used was based on Permutational MANOVA (PERMANOVA) statistical method. The dendrogram was constructed based on the distance measure of Bray-Curtis Index with Ward clustering algorithm. Unit

variance scaling WAS applied to sequence abundance. Both taxon rows and samples columns were clustered using correlation distance and average linkage. Top 50 organisms (genus level) were taken for the heatmap construction.

Functional analysis

PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), computational method that predicts the functional potential of microbial communities based on 16S rRNA gene sequencing data was used in this study. It utilized reference phylogeny and ancestral state reconstruction to infer the presence and abundance of gene families in a metagenome; gg13, specific version of the GreenGenes database. Results are presented graphically.

Results

The experiment was carried out during the wheat growing season of year 2024–2025. The soil of the research fields was sampled for physico-chemical characterization. The soil was sandy loam in texture. It had normal pH and electrical conductivity. However, it was low in organic carbon content and content of available N, P and K. The site of the experiment was inland subtropical, semiarid to subhumid (North-Western India). The mean temperature during the early growth stages (tillering) was 25.1°C (October), 19.5°C (November). The mean RH was 63–65 g/m^3. Rainfall of 54 mm was recorded in October. Sunshine hours were 7.6 (October) and 5.2 (November) which is less than the optimum sunshine hours of wheat ≥ 8.0. Wind speed of 2.1 m/s was recorded (Online Resource 1 and Online Resource 2).

A total of 24 phyla, 48 classes, 99 orders, 191 families, and 370 genera were identified in the rhizospheric soil of these varieties sampled at tillering stage. We did not observe any statistically significant difference in relative abundance of phyla relating to genotype and P level. The predominant phyla in P amended soils was Firmicutes (C306- 56.15% and 16679 OTUs; PBW 725- 53.47% and 17173 OTUs); Proteobacteria (C306- 35.38% and 10511 OTUs; PBW 725- 37.99% and 12202 OTUs); Bacteroidetes (C306- 6.74% and 2002 OTUs; PBW 725 – 6.59% and 2116 OTUs) and Actinobacteria (C306- 1.4%, 418 OTUs and PBW 725 – 1.6%, 525 OTUs). The predominant phyla in P starved soils was Firmicutes (C306- 54.35% and 15043 OTUs; PBW 725- 55.06% and 20670 OTUs); Proteobacteria (C306- 36.04% and 9976 OTUs and PBW 725- 37.51% and 14084 OTUs); Bacteroidetes (7.65% and 2118 OTUs and 5.62% and 2109 OTUs) and Actinobacteria C306- 1.57% and 435 OTUs; PBW 725 – 1.57% and 589 OTUs) (Online Resource 3 and Online Resource 4 and Fig. 1a).

A total of 191 families were detected in the soil samples. Their richness between the 4 samples was not notably different. The descending order of their abundance was Lactobacillaceae (> 25%), Staphylococcusceae, Enterobacteriaceae (> 15%), Moraxellaceae (> 10%), Bacillaceae and Paenibacillaceae (> 5%). Less than 5% abundance of Pseudomonadaceae, Flavobacteriaceae, Sphingobacteriaceae, Prevotellaceae and Comamonadaceae was revealed (Fig. 1b). At genera level, 370 genera were shared by samples of P amended and P starved soils of both varieties. For consideration,

we grouped them according to abundance- with dominance of ≥ 10- *Lactobacillus* and *Staphylococcus*; between 5–10% abundance- *Acinetobacter, Escherichia, Paenibacillus*, *Pseudomonas, Salmonella*; with presence of < 5% - *Bacillus, Sphingobacterium, Candidatus, Chryseobacterium, Prevotell*a and *Psychrobacter*. Core microbiome analysis based on relative abundance and sample prevalence of bacterial OTUs grouped by genera is presented in Fig. 1c. The core microbiome was visualized with the sample prevalence of 20% and abundance of at least 0.01%. Descending order of relative abundance was shown by *Lactobacillus, Staphylococcus, Escherichia, Acinetobacter, Salmonella, Paenibacillus, Bacillus* and *Pseudomonas*. Though abundant these genera showed low prevalence (%). Heatmap of top 50 genera is presented in Fig. 1d.

Venn diagram for comparative representation of all the samples is provided in Fig. 2a. 122 genera (33%) were found prevalent in soil samples of *both* genotype (C306 and PBW725) and *both* conditions (P-amended and P-starved). It included bacteria of agricultural importance- *Azospirillum, Mesorhizobium, Pantoea, Bacillus and Pseudomonas.* Principal Coordinates Analysis (PCoA) in Fig. 2b, revealed distinct effects of both wheat genotype and phosphorus availability on rhizospheric bacterial community composition. The first coordinate axis (PCoA1) accounted for 89.2% of the total variation, indicating that the major source of variation among samples was associated with differences in genotype and/or phosphorus treatment, whereas PCoA2 explained 11.6% of the variation. The two phosphorus treatments of the elite genotype PBW725 (Samples 3 and 4) were separated primarily along PCoA1, indicating a substantial shift in bacterial community structure under phosphorus deficiency. In contrast, the landrace genotype C306 (Samples 1 and 2) showed greater separation along PCoA2 than along PCoA1, suggesting that phosphorus starvation induced a different pattern of microbial restructuring in the landrace background. Furthermore, P-starved PBW725 (Sample 4) was clearly separated from all other samples, indicating the development of a distinct rhizospheric bacterial assemblage under phosphorus limitation. The intermediate positioning of P-amended PBW725 (Sample 3) suggests partial overlap with both C306 treatments, reflecting a combination of genotype- and nutrient-driven influences on microbial community assembly.

Microbiome diversity was quantified by alpha and beta diversity indexes. Landrace and cultivars in *both* P-amended and P-starved conditions exhibited an alpha diversity analysis revealed a high Simpson diversity index (~ 0.9), indicating low dominance and an even community structure. The Shannon index (2.58–2.68) further suggested moderate to high species richness with balanced abundance distribution, reflecting a stable and functionally diverse bacterial assemblage across samples. Chao1 index of 87.5 ± 1.0 (sample 1-P amended C306), 107.5 ± 6.4 (sample 2-P starved C306), 98.7 ± 2 (sample 3 P amended PBW 725) and 98.3 ± 0.7 (sample 4 P starved PBW 725) was recorded. The apparent discrepancy among alpha diversity indices reflects the distinct ecological attributes captured by each metric. Chao1 is a richness estimator that emphasizes rare taxa (singletons and doubletons), whereas Shannon and Simpson indices incorporate both richness and evenness and are more strongly influenced by dominant community members. In the present study, phosphorus starvation, particularly in the landrace C306, resulted in a marked increase in Chao1 estimates, indicating the recruitment or enrichment of additional low-abundance taxa. However, the relative abundance distribution of dominant taxa remained largely

unchanged across treatments, leading to comparable Shannon and Simpson values. This suggests that phosphorus stress expanded the rare biosphere without disrupting overall community evenness or dominance structure.

The predicted functional profiles of microbial communities were inferred using KEGG Orthology (KO) terms derived from 16S rRNA gene sequencing data across four soil samples (Online Resource 5). A total of 328 unique KO terms were identified, with varying abundance levels across samples, indicating a rich functional diversity. Principal Component Analysis (PCA) was performed on the KO abundance matrix to explore sample-level differences in predicted functional profiles. As shown in Fig. 3a, samples cluster distinctly in PCA space, with the first two principal components explaining a significant portion of the variation. This spatial separation reflects differences in microbial functional potential, possibly associated with variation in environmental conditions land-use history, etc.

To further investigate specific functional traits contributing to sample differentiation, the top 10 most variable KO terms across samples were identified and visualized. In Fig. 3b, we have shown that these KOs show large inter-sample variability, particularly in genes related to energy metabolism, transport systems, and redox reactions. For instance, KO terms such as K00001 (alcohol dehydrogenase) and K02003 (ABC transporter permease) demonstrated substantial variation, suggesting differential microbial adaptation strategies.

Discussion

Wheat (*Triticum aestivum*) is one of the four primary crops. As per estimates, the production of wheat must increase at the rate of 2.4% per year for ensuring food security for the growing world population (Tian et al 2021). Wheat breeding programs select varieties for high yield and disease resistance traits and these varieties are dependent on chemical fertilizers to sustain the high yield which has an adverse impact on environment and on the plant associated microbiome. Plants rely on their associated microbiome for nutrient acquisition and for tolerance to biotic-abiotic factors. Genotype and nutrient stress influence the plant associated microbiome.

Most of the work reported on rhizospheric communities influenced by wheat variety and N/P level have been conducted at reproductive and maturity of wheat. Few reports have studied the differences at vegetative growth stage (45 days after sowing). Our choice of early tillering stage for drawing samples was based on studies which have reported establishment of rhizospheric and endophytic microbiome within 30 days after sowing in rice. (Garcha and Chopra 2023). Both the varieties chosen for this study were heaxaploid bread wheat varieties. Landrace C306, a pan-India and pre-green revolution variety. It was developed through selection and hybridization among the then prevalent varieties in 1965. It is tall, erect and resistant to yellow rust. PBW 725, is an elite genotype and has been commercially cultivated in Indo-Gangetic plains. It is a double dwarf, rust resistant and high yielding variety. Other variables in this study like soil type, cropping pattern and agronomic procedures were kept constant except for P application to prevent any bias.

Our results indicate that the host type and P level had little impact of assembly of rhizospheric community composition at early developmental stage- tillering stage. The taxa was dominated by Firmicutes and Proteobacteria. Actinobacteria and Bacteroidetes were the minor taxa. Resilience of Firmicutes and Proteobacteria emphasize their adaptive capabilities as also stated by Philippot and workers (2013) and Ramirez and workers (2018). The dominance of *Lactobacillus* and *Staphylococcus* in was reported by Zhao et al. (2019), who identified these genera as key players in soil microbial communities. They revealed that *Acinetobacter, Escherichia, Paenibacillus, Pseudomonas*, and *Salmonella* were present in moderate amounts. Similar observations were made by Zhang et al. (2022). They reported that these genera had impacted plant growth and stress tolerance. *Bacillus, Sphingobacterium*, and others were less prevalent but contributed to overall diversity and niche functions in soil ecosystem (Sun et al. 2021; Yadav et al 2018). A number of workers (Bulgarelli et al.2012; Zhang et al. 2016d ller et al. 2020) have shown that while plant genotype have minimal influence on rhizosphere microbiomes, environmental factors such as nutrient availability often have a more pronounced effect.

The phylogenetic distance between hosts impacts microbiome composition. This variation is not pronounced at tillering stage. Many workers have elucidated bacterial community structure in wheat at reproductive stage and have reported that domesticated cultivars have greater richness of Actinobacteria and Proteobacteria (Gholizadeh et al 2022) and landraces have a greater abundance of Bacteroidetes and Verrumicrobia (Gholizadeh et al 2022, Perez- Jaramillo et al 2017, Cardinale et al 2015, Bulgarelli et al 2015). Bread wheat at maturity is revealed to be dominated by Proteobacteria (40%), Actinobacteria-Acidobacteria-Bacteroidetes (10–15%) (Tcakz et al 2020, Prudence et al 2021). Kinnunan-Grubb et al 2020 reported greater presence of Firmicutes and Verrumicoriba in modern cultivars. Tckaz et al 2020 reported dissimilar rhizospheric communitites in wild ancestors and domesticated varieties. Reportedly, modern varieties had high profusion of Proteobacteria and decreased richness of Bacteriodetes in *Phaseolus vulgaris*.

Diversity of soil bacteria in the rhizospheric zone of wheat at the tillering stage has been reported by Kuźniar and co-workers (2023, 2020). They reported that core rhizomicrobiome of wheat contained generalist microorganisms from Proteobacteria, Actinobacteria, Hypomicrobiaceae, Bradyrhizobiaceae, and *Sphingomonas* sp. - The relative abundance of the core rhizomicrobiome ranged from 4.253% to 6.425% of the total sample, with *Sphingomonas* sp. and Burkholderiales as the dominant taxa. In our study, at the class level 654 OTUs were identified across all samples. Also, presence of unclassified Pelagibacteraceae and unclassified Oscillatoriales was revealed. We found that the bacterial community composition was majorly similar in terms of alpha and beta diversity indexes as well as distribution at phyla and family level. It indicated that the soils were not dominated by a single species. Venn diagram 122 genera (33%) overlap of microbial community in all samples. Reduced presence of Rhizobacteriaceae and Streptomyceae has been reported in modern varieties compared to the wild ancestors. This is true for our results as well. Differences in alpha diversity indices such as Chao1 (richness) versus Shannon and Simpson (richness + evenness) have been widely reported in rhizosphere microbiome studies, where nutrient availability or genotype primarily influence the presence/absence of rare taxa rather than the abundance distribution of dominant taxa. Similar patterns are discussed in

rhizosphere microbial ecology literature where host and environmental factors mediate recruitment of taxa without disrupting community evenness (Bakker et al. 2012; Bakker et al. 2015). Despite pronounced genotype-specific differences in richness as reflected by Chao1 estimates, particularly under phosphorus starvation in C306, Shannon and Simpson indices remained comparable across treatments, indicating stable evenness and dominance patterns.

The observed separation of samples in the Bray–Curtis PCoA indicates that both wheat genotype and phosphorus availability contribute to the structuring of rhizospheric bacterial communities. The strong contribution of PCoA1 (89.2%) suggests that the dominant driver of community variation was the interaction between host genotype and phosphorus status rather than stochastic microbial fluctuations. The marked separation between P-amended and P-starved PBW725 indicates that phosphorus deficiency substantially altered the rhizosphere microbiome associated with the elite genotype. Such shifts are consistent with previous reports showing that phosphorus deprivation modifies root exudation patterns, thereby selectively enriching bacterial taxa involved in phosphorus mobilization, organic acid production, and nutrient scavenging. Under low-phosphorus conditions, plants often recruit microbial communities capable of enhancing phosphorus acquisition through mineral solubilization and phosphatase activity. Interestingly, the landrace genotype C306 exhibited a different response pattern. Although phosphorus starvation altered community composition, the magnitude and direction of separation differed from that observed in PBW725. This suggests genotype-dependent microbial recruitment, whereby genetically distinct wheat backgrounds shape unique rhizosphere environments through differences in root architecture, exudate chemistry, and nutrient acquisition strategies.

The distinct positioning of P-starved PBW725 further suggests that elite breeding may have influenced the responsiveness of rhizosphere microbiomes to nutrient stress. In contrast, the landrace C306 may possess a more stable microbial association under varying phosphorus conditions, potentially reflecting adaptation to historically low-input agricultural environments. Such genotype-specific microbial assembly under phosphorus stress supports the concept that host genetic factors play a significant role in microbiome recruitment and may contribute to differential phosphorus-use efficiency among wheat genotypes.Collectively, these findings indicate that phosphorus availability modulates rhizospheric bacterial community structure in a genotype-dependent manner, supporting the hypothesis that plant genotype and nutrient status interact to shape the wheat rhizosphere microbiome. The elite genotype PBW725 exhibited a larger microbiome shift in response to phosphorus starvation than the landrace C306, suggesting greater plasticity of rhizospheric bacterial recruitment under nutrient stress. Conversely, the comparatively constrained response of C306 may indicate a more stable rhizosphere microbiome, potentially reflecting adaptation to nutrient-limited environments. These findings highlight the importance of considering genotype-dependent microbiome assembly when developing wheat cultivars with enhanced phosphorus-use efficiency and reduced fertilizer dependence.

The integration of predicted KO term profiles provides a detailed perspective on the functional landscape of soil microbial communities. The observed differentiation in KO composition among samples suggests that microbial functional potential is highly responsive to environmental and spatial heterogeneity. This

aligns with previous studies highlighting the impact of soil physicochemical properties and land management on microbial metabolism (Langille et al 2013; Knight et al 2018). The PCA-based clustering and relative abundance profiles demonstrate that despite some overlap in core metabolic functions, the microbial communities possess distinct auxiliary capabilities. Notably, transport-related genes and redox enzymes were among the most variable KOs, implying differing selective pressures or ecological functions, such as heavy metal resistance, nutrient acquisition, or organic matter decomposition (Kanehisa and Goto 2000). These functional differences can be ecologically meaningful in soil systems, where microbial activity drives key ecosystem processes such as nitrogen cycling, organic matter turnover, and plant-soil interactions. Future studies could enhance these insights by integrating metagenomic or transcriptomic data to validate gene expression and metabolic activity, or by associating functional profiles with soil metadata (e.g., pH, carbon content).

Clearly, there is inter-play of many factors which include but are not limited to cropping system, tillage practices, etc (Hartman et al 2018). Also, it is important to note that microbial community studies were carried out in different geographical areas where soil characteristics varied markedly. Simonini et al 2020 reported the soil type was the main driver in rhizospheric community structure. They said that 57% of variability was explained by it and only 2% variability by the genotype. They reported a core microbiome of 177 taxa. Similar results were shown by Cordero Elvia et al., 2021. One study using different types of P fertilizers, polyphosphate (poly-P) and orthophosphate (ortho-P) fertilizers revealed that type of P had little influence compared to the influence of growth stage. However, type of P influenced chlorophyll content, photosystem I activity, shoot biomass (Bourak et al 2023)

The findings of our study must be seen in the light of some limitations. The effect estimates are based on comparison of a landrace (C306) and only one elite genotype (PBW 725) which is a timely sown irrigated variety hugely popular in Indo-Ganetic plains. To compensate for it, we estimated the bacterial community composition from multiple samples. There are fewer previous studies on *bacterial community at tillering stage of wheat* grown in this part of the world which makes generalization of related research reports complex. Also, we did not look objectively at the root structure architecture (RSA). Genotype differences manifest themselves through RSA.

Conclusions

The Green Revolution breeding efforts focused on improving above ground traits, which led to disruption of beneficial belowground plant-microbe symbioses. Increased use of inorganic fertilizers during the Green Revolution reduced the diversity and abundance of beneficial rhizosphere microbes. The disruption of plant-microbe symbioses and reduction in rhizosphere microbial diversity have had negative impacts on soil health. Also, the soil physical and chemical characteristics have deteriorated. A potential remedy is selecting varieties on the basis of their nutrient use efficiency and associated rhizospheric microbiome assembly structure. We have shown that adaptive ability of Firmicutes and Proteobacteria is evident in their dominant presence in both the locally adapted landrace and elite genotype and under both conditions- P amended and P-starved at nascent development stage of wheat-

tillering stage. Future work to determine the stage at which rhizospheric community gets established under the impact of anthropogenic interventions and weather conditions would include a broader spectrum of genotype to include those which are grown in geographically distant areas and different growth stages for longer observations periods to better understand the dynamics of bacterial communities. This is useful information for strategizing rhizospheric engineering for greater profusion of communities having direct role in nutrient cycling.

Declarations

Author Contribution

SG- conceptualization of the experiments, conduct of experiments, data collection and analysis, manuscript writingPS- conceptualization of the experiments, conduct of experiment, manuscript drafting suggestions

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Data Availability

The sequence data generated during this study have been deposited in the NCBI BioProject database under accession number PRJNA1139961 and available at the following URL: https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1139961

Weblinks -www.ifpri.org/topic/food-security, www.oecd.org

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Figures

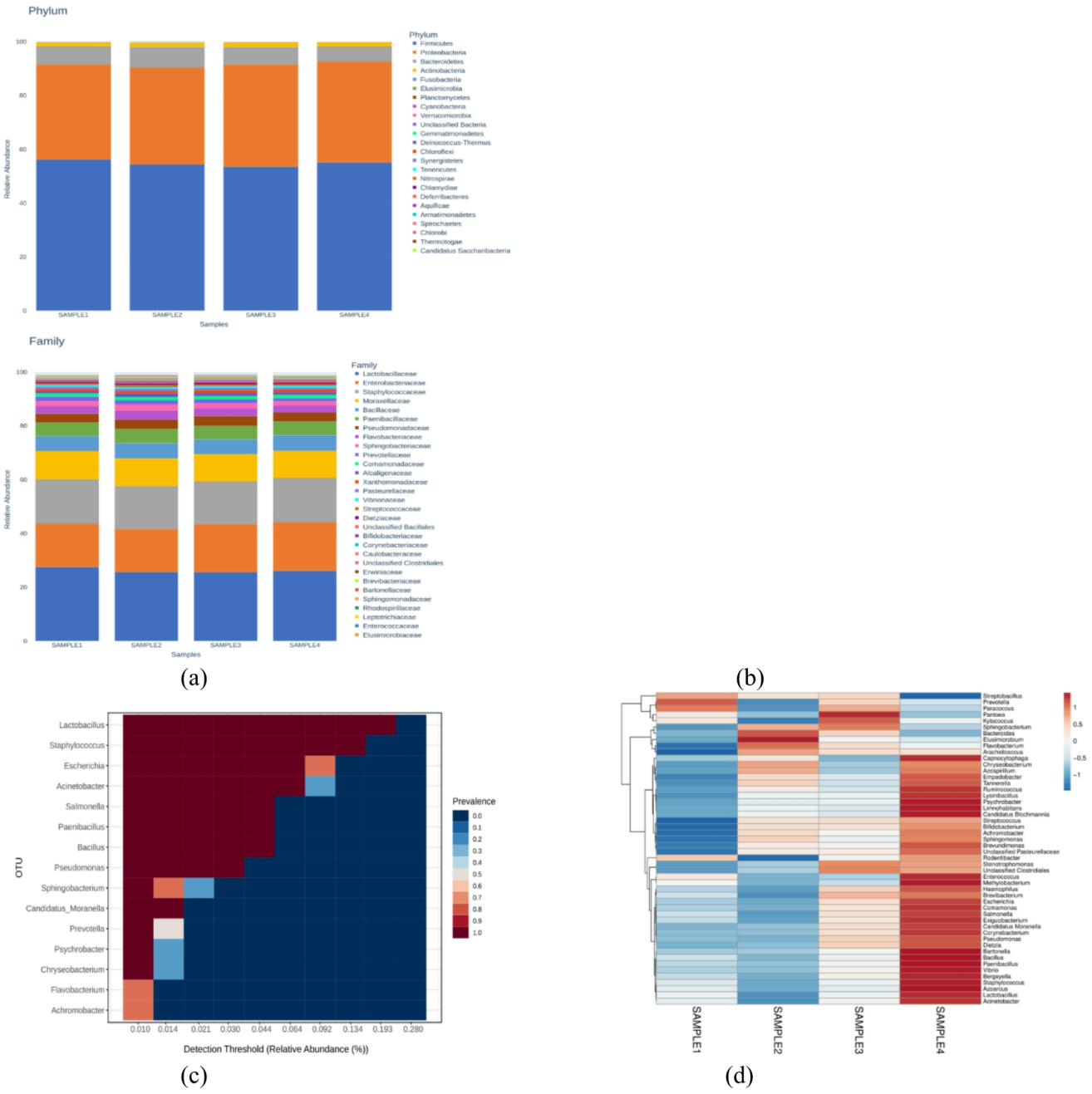
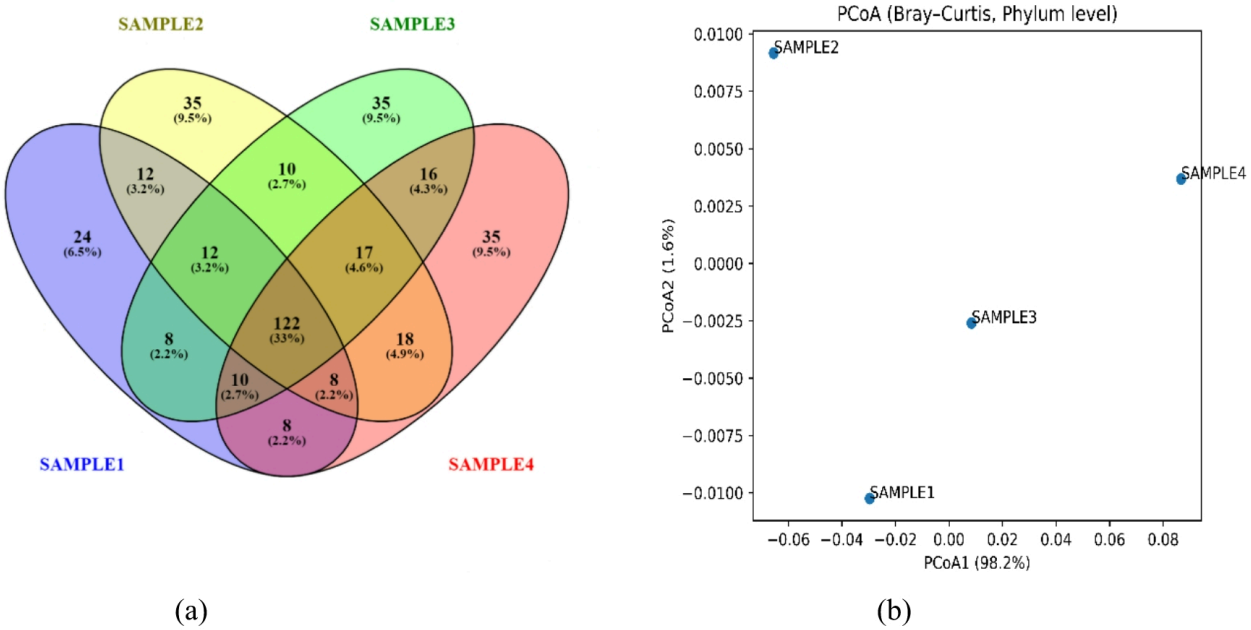


Figure 1

Representation of bacterial community structure and core microbiome across samples. (a) Phylum-level relative abundance of bacterial communities across four samples. (b) Family-level relative abundance profiles highlighting dominant and sub-dominant taxa. (c) Core microbiome analysis showing prevalence of dominant bacterial genera across increasing detection thresholds based on relative abundance. (d) Heatmap of normalized (z-score transformed) relative abundance of top 50 bacterial genera across samples with hierarchical clustering, illustrating differential taxon enrichment patterns (sample 1-P-

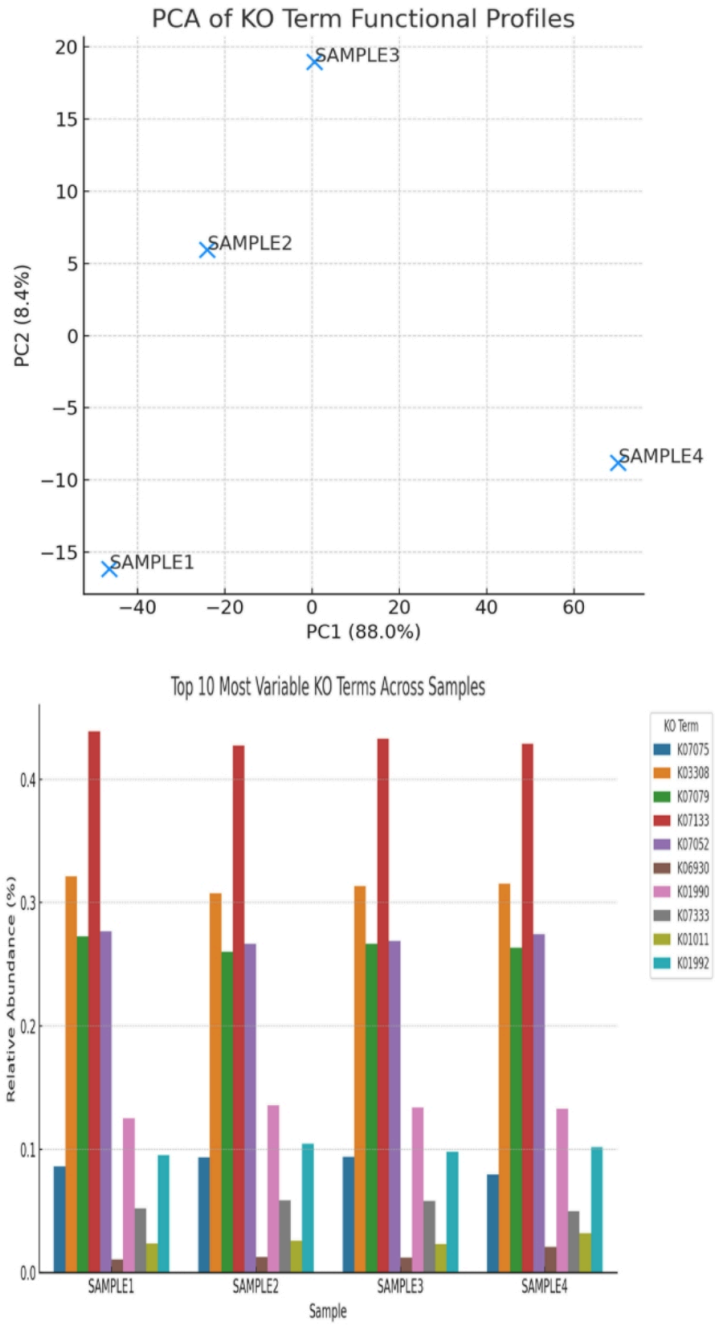
amended C306, sample 2- P-starved C306, sample 3- P-amended PBW 725 and sample 4- P-starved PBW725).



(a) (b)

Figure 2

Composite Figure showing shared and beta-diversity relationships among samples. (a) Venn diagram illustrating the number and percentage of shared and unique bacterial taxa across the four samples. (b) Principal Coordinates Analysis (PCoA) of rhizosphere bacterial communities at the phylum level based on Bray–Curtis dissimilarity (sample 1-P-amended C306, sample 2- P-starved C306, sample 3- P-amended PBW 725 and sample 4- P-starved PBW725).



(a) (b)

Figure 3

Functional profiling of microbial communities based on KEGG Orthology (KO) terms. (A) Principal component analysis (PCA) of KO term functional profiles across samples, showing separation of samples along the first two principal components. (B) Relative abundance of the top 10 most variable KO terms across samples, highlighting functional differences among microbial communities (sample 1-

P-amended C306, sample 2- P-starved C306, sample 3- P-amended PBW 725 and sample 4- P-starved PBW725).

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