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Impact of genotype and soil fertility on wheat rhizosphere microbiota under the trans-gangetic plain

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The effects of genotypes (HD3086 and PBW343) and soil physicochemical properties on the wheat rhizospheric bacterial communities along the trans Indo-Gangetic plains were studied. The trans-Indo-Gangetic Plains of India are one of the areas in the country where wheat is grown the most. Despite the agricultural significance of this region, extensive studies on the rhizosphere microbial abundance and community structure related to wheat cultivation in this area are still lacking. To address this knowledge gap, the present study was undertaken to characterize the rhizosphere microbiome using full-length 16 S rRNA–based metagenomic profiling, implementing universal primers, tailed with PacBio Sequel II barcode sequences, providing new insights into microbial dynamics across this major wheat-producing landscape. Statistical analysis revealed significant differences in both abundance and diversity among the different soil samples and wheat genotypes. Four phyla exhibited significant differences in relative abundance between the genotypes ($p < 0.05$): Proteobacteria ($p = 0.002$), Planctomycetes ($p = 0.000$), Verrucomicrobia ($p = 0.000$), and Firmicutes ($p = 0.030$). The number of genera identified in genotype HD3086 across all locations was 421, while it was 322 for genotype PBW343. There were 251 genera found common, with 170 genera exclusively present in HD3086 and 71 in PBW343. Significant differences were observed in the relative abundance of eighteen genera ($p < 0.05$) between the genotypes; some of them include *Luteolibacter*, *Gemmata*, *Pseudomonas*, *Stenotrophobacter*, *Pseudarthrobacter*, *Devosia*, *Lacibacter*, *Gaiella*, *Luteimonas*, and *Nitrosospira*. Correlation analysis indicated significant associations between microbial diversity and soil parameters like pH, total and available nitrogen, potassium, phosphorus, iron, and organic carbon for both varieties. Core taxa analysis revealed 27 core taxa across both genotypes. The study highlights significant genotype effects on rhizosphere microbiomes, with implications for soil health and crop management strategies.

Keywords Core microbiota, Rhizospheric bacterial diversity, Trans indo gangetic plains, Wheat genotypes

Globally, wheat (*Triticum aestivum*) is considered the most consumed crop. The microbiome of wheat rhizosphere holds immense potential for revolutionizing agriculture[1]. These microscopic communities of bacteria, fungi, and other microorganisms that inhabit the soil surrounding wheat roots play a critical role in plant health, nutrient uptake, and resilience to environmental stressors[1]. Certain microbes within the wheat rhizosphere can enhance the availability of essential nutrients; additionally, some of these microbes have been identified as providing resistance against common wheat diseases[2]. Healthy soil is the foundation of sustainable agriculture, and the microbiome is a key contributor to soil health. Microbes play essential roles in organic matter decomposition and cycling of nutrients, thereby maintaining soil fertility and productivity over the time[3]. Different wheat cultivars and development stages possess distinct root exudate profiles, which serve as a food source and signaling mechanism for soil microbes. As a result, certain cultivars at different growth stages may attract specific bacterial taxa that are beneficial for their growth and development. Understanding these cultivar-specific interactions can help breeders select or develop wheat varieties with enhanced microbial associations[4,5].

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By harnessing the complex interactions between plants and soil microbes at their interface, researchers can develop innovative strategies to enhance agricultural sustainability, productivity, and resilience[6]. Furthermore, only a handful of studies investigated how the soil characteristics affect the wheat rhizospheric microbiota[7,8]. The composition of microbiota in the rhizosphere is thought to result from interactions among the plants, soil properties, environmental conditions, and microbial relationships, and therefore can be greatly inconsistent along with the distributions[9,10]. To develop management practices that consistently influence soil communities, it is essential to thoroughly understand the microbial components associated with plants across different locations[11]. Studies indicate that a range of plant species share similarities in the compositions of rhizospheric microbiota at broader taxonomic levels up to family, particularly when explored under natural field situations[12].

Plants can recruit a core microbiome of rhizspheric symbionts that are specifically adapted to the rhizosphere[13]. It is suggested that certain core taxa and their host plants have co-evolved, enabling them to coexist effectively in the rhizospheres of specific plant species or genotypes[14]. As a result, these core taxa or consortia are crucial for plant health and are attractive targets for efforts to manipulate the plant microbiome or engineer synthetic consortia[14]. The ability of these microbes to enhance plant growth and health stems from evolutionary mechanisms that select for microbes possessing genes crucial to the plant's vitality[15]. Although core bacteria inhabit the wheat rhizosphere, their abundance varies among different wheat cultivars[7]. Studies have shown that the choice of wheat variety or cultivar significantly influences the composition of the rhizospheric prokaryotic community[7,16]. Although the Indo-Gangetic Plain is one of India's principal wheat-producing regions, there remains a clear knowledge gap in understanding how wheat genotype and soil fertility interact to shape rhizosphere microbial communities at a large geographic scale: regional studies have begun to describe core bacterial taxa from parts of the Indo-Gangetic Plain but are spatially limited or use short-read 16 S regions (e.g., V3–V4), providing useful baselines but not a comprehensive, high-resolution picture across multiple districts.

In this study, we examined the rhizospheric microbiota of two contrasting wheat varieties, HD3086 and PBW343, by characterizing the prokaryotic communities using 16 S rRNA gene amplicon sequencing (V3–V4 region). Rhizosphere soil samples were collected from eight geographically distinct locations across the trans-Indo-Gangetic Plain, including Punjab and Uttar Pradesh. The objectives were to assess the influence of wheat genotype and agricultural management (cropping systems) on the composition, diversity, and community structure of the rhizosphere microbiota. We also aimed to identify a core rhizospheric microbiota shared across soils, locations, and wheat varieties, and to evaluate the relationships between soil physicochemical properties and microbial assemblages. We hypothesized that, despite environmental and management variability, a conserved set of microbial taxa would consistently associate with wheat roots, and that genotype-specific differences would be reflected in the rhizosphere community structure. This study provides novel insights into genotype–microbiome–soil interactions under real field conditions, contributing to the development of microbiome-informed strategies for wheat improvement and sustainable crop management in the Indo-Gangetic Plains.

Materials and methods

Soil sampling and seed sowing

In November 2022, soil samples were collected from the fields prepared for wheat sowing, from seven districts including Mansa, Ludhiana, Faridkot, Jind, Amritsar, Ambala, and Hoshiarpur from the state of Punjab, as well as one district in Uttar Pradesh, Saharanpur (Table 1). Soils from each district were collected using a sterile soil auger at a depth of 15 to 25 cm near the root. To capture local heterogeneity, three separate soil subsamples were taken from each area within a radius of around 50 to 100 m. These soil samples were subsequently transported to the laboratory under chilled conditions and filled into 24 cm height and 20 cm diameter pots, while small portions were kept at 4 °C for soil analysis and DNA isolation. Two wheat varieties, HD3086 and PBW343, were selected for the study. They were sown in pots with soil samples collected from each of the eight locations. For each genotype, three replications were maintained for each of the soil samples in a completely randomized design. The pots were maintained under controlled environmental conditions with a day/night temperature of 22 °C/18°C, a photoperiod of 16 h/8 hours, and a relative humidity of 60%–65%. Pots were irrigated with water at 1-week interval with a fixed amount of sterile water, although during the experimental period, no fertilizer or manure was added to the pots. Rhizospheric soil samples were collected 45 days after sowing. From each of the replicates, three to five plants were uprooted, and soil around the root zone was carefully collected and combined

District	Location	Longitude/latitude
Mansa	Budhlada, Punjab, India	29.90 N, 75.61E
Ludhiana	Ajnod, Punjab, India	30.79 N, 75.97E
Faridkoot	Kotkapura, Punjab, India	30.62 N, 74.80E
Jind	Hatho-Gurthali Link Rd, Punjab, India	29.61 N, 76.18E
Amritsar	Manwala Kalan, Punjab, India	31.58 N, 74.98E
Saharanpur	Ismailpur, Uttar Pradesh, India	29.98 N, 77.49E
Ambala	Naraingarh, Punjab, India	30.49 N, 76.79E
Hosiyarpur	Nasrala Kapporthala, Punjab, India	31.49 N, 75.82E

Table 1. Sampling site details with location and GPS coordinates.

to form a composite sample. Samples were dried and sieved through a 4.0 mm sieve and divided into two parts-one for physicochemical analysis, and another is refrigerated for DNA isolation and amplicon sequencing.

Physicochemical and nutritional properties of soil

Specific analysis techniques were implemented to determine the physicochemical parameters of the soil. The soil samples were analyzed for total nitrogen, total phosphorus, total potassium, total iron, total sulphur, available nitrogen, available phosphorus, available potassium, available iron, available sulphur, pH, electrical conductivity (EC), and organic carbon. The methodologies used for each of the soil parameter analyses is tabulated (Supplementary Table 1).

Soil DNA isolation, amplification of 16 S rRNA gene, and amplicon sequencing

DNA extraction was performed using FastDNA™ SPIN Soil kit (MP Biomedicals, USA). The concentration of isolated gDNA was quantified using Qubit 4.0 fluorometer (Thermofisher Scientific, Massachusetts, USA), and the integrity of DNA was visualized on 1% agarose gel. Full-length 16 S rRNA gene (V1-V9 region, 1.5 kb) was amplified using universal primer 27 F and 1492R, in a thermal cycler (peqSTAR, Germany), tailed with PacBio barcode sequences. Amplification was carried out with initial denaturation at 95 °C for three minutes, followed by 25 cycles with denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 30 s, and a final extension at 72 °C for five minutes. The quality and integrity of the amplicons were visualized in a 1.5% agarose gel. Amplicons were pooled in equimolar concentration and purified using AMPure PB beads (Pacific Biosciences, California, USA). The library was created following the manufacturer's instructions using the SMRTbell Express Template Preparation Kit 2.0 (Pacific Biosciences, California, USA). After preparation, the library was purified with AMPure PB beads (Pacific Biosciences, California, USA). Subsequently, primer annealing and polymerase binding were performed using the Sequel II Binding Kit 2.1 to form the bound complex. Then the sample was sequenced on PacBio Sequel II platform.

Data processing, community profiling, and diversity analyses

All analyses were conducted using R version 4.2.0. For quality control, read filtering, denoising, read merging, chimera removal, and taxonomy assignment, we utilized the DADA2 package (v1.24.0) with the Silva database. The identified taxa were aggregated at various taxonomic levels to create bar plots and tables using the phyloseq package (v1.40.0) in R. Diversity analysis of the operational taxonomic units (OTUs) was carried out with PAST version 4.5 (Martin-Laurent et al., 2001). To compare microbial communities across all samples and different cropping systems, Principal Component Analysis (PCoA) was performed using STAMP (version 2.1.3). The sequence data generated was submitted to the NCBI database with project number (Project number: PRJNA1128054; SRA ID: SRR29548375 to SRR29548382).

Core microbiota and network analyses

To identify the core bacterial microbiota in the wheat rhizosphere, we focused on the ubiquitous taxa found across all 20 study sites. To investigate microbe-microbe interactions, we constructed co-occurrence networks based on the prevalence of OTUs, using the MetagenoNets pipeline. Rare taxa were excluded from the analysis by setting a prevalence threshold of 0.02%. The SparCC method was applied to calculate correlations, with a cutoff based on Critical-R and a p-value of less than 0.05 to identify significant microbial associations.

Statistical analysis

Three replicates were employed to evaluate soil parameters, with the data subjected to a one-way analysis of variance (ANOVA) using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). The results were reported as means ± standard error (SE), and Duncan's multiple range test was used for mean comparisons. T-tests were performed in Microsoft Excel to examine statistical differences in genera between genotypes and across locations. Pearson's correlation analysis was conducted using PAST to assess correlations among soil parameters and diversity indices. To assess the influence of agricultural practices on the rhizosphere microbiome of wheat information on the cropping system was collected at the time of sampling. Out of eight samples from eight districts, six samples were from the rice-wheat cropping system, one from cotton-wheat system and one from sugarcane-wheat system. To find out statistical differences among rhizospheric microbial communities in different cropping systems, a principal coordinate analysis was performed. Venn diagrams were generated with Venny 2.1[18], and heat maps were created using ClustVis[19].

Results

Soil characteristics

The pH of the soil ranged from 6.72 to 7.44 in genotype HD3086, while it ranged from 6.72 to 7.38 in genotype PBW343. Electrical conductivity ranged from 69 to 168 (µsiemens/cm) for HD3086 and 65–177 (µsiemens/cm) for PBW343. Organic carbon ranged from 0.556 to 0.865% and 0.562–0.756%, respectively, in genotype HD3086 and PBW343 (Table 2). Available N, P, K, Fe, S ranged 137.2-495.2 mg/kg, 38.2–99.8 mg/kg, 66.3–213 mg/kg, 4.15–8.88 mg/kg and 477.5–1165 mg/kg respectively, in the samples of HD3086, while it ranged 144.8-252.8 mg/kg, 38.2–99.8 mg/kg, 120-197.3 mg/kg, 5.15–6.88 mg/kg and 655.4–1043 mg/kg respectively, in the samples of PWB343 (Table 3). Similarly total N, P, K, Fe, S ranged 1523–4676 mg/kg, 355.6–688 mg/kg, 2286–7287 mg/kg, 113.8–278 mg/kg and 1768–3788 mg/kg respectively, in the samples of HD3086, while it ranged 2392–3611 mg/kg, 355.6–688 mg/kg, 2286–5487 mg/kg, 107.8-231.3 mg/kg and 1807–3497 mg/kg respectively, in the samples of PWB343 (Supplementary Table 2).

Location	EC (Mosiemens/cm)		pH		Organic carbon (%)	
	HD3086	PBW343	HD3086	PBW343	HD3086	PBW343
Mansa	168 ± 4.4	177 ± 12.3	7.12 ± 0.3	7.15 ± 0.2	0.641 ± 0.02	0.641 ± 0.03
Ludhiana	69.0 ± 3.7	65.0 ± 3.7	6.98 ± 0.2	6.93 ± 0.4	0.767 ± 0.03	0.607 ± 0.03
Faridkot	107 ± 7.2	103 ± 5.9	6.72 ± 0.3	6.72 ± 0.5	0.562 ± 0.01	0.562 ± 0.01
Jind	135 ± 6.3	134 ± 7.8	7.05 ± 0.2	7.02 ± 0.2	0.724 ± 0.05	0.624 ± 0.04
Amritsar	112 ± 5.1	107 ± 4.3	7.03 ± 0.2	7.38 ± 0.2	0.556 ± 0.03	0.756 ± 0.02
Saharanpur	93.1 ± 2.9	102 ± 6.3	7.18 ± 0.3	7.31 ± 0.4	0.653 ± 0.02	0.753 ± 0.02
Ambala	103 ± 8.2	109 ± 8.8	7.32 ± 0.2	7.25 ± 0.4	0.857 ± 0.01	0.657 ± 0.05
Hoshiarpur	155 ± 7.3	153 ± 11.0	7.44 ± 0.4	7.25 ± 0.5	0.865 ± 0.04	0.665 ± 0.03

Table 2. Electrical conductivity, pH and organic carbon content of rhizospheric soil of genotype HD3086 and PBW343. The data are mean of three replicates ± standard error.

Location	Available *N* (mg/kg)		Available *P* (mg/kg)		Available K (mg/kg)		Available Fe (mg/kg)		Available S (mg/kg)	
	HD3086	PBW343	HD3086	PBW343	HD3086	PBW343	HD3086	PBW343	HD3086	PBW343
Mansa	204.4 ± 7.4	188.8 ± 8.3	56.2 ± 3.9	56.2 ± 3.2	161.5 ± 11.6	131.5 ± 8.5	6.42 ± 0.3	6.42 ± 0.3	1062.6 ± 43.2	918.6 ± 27.3
Ludhiana	173.8 ± 5.6	144.8 ± 6.5	64.4 ± 4.2	43.7 ± 2.7	180.6 ± 8.4	120.4 ± 2.4	4.85 ± 0.2	5.15 ± 0.2	733.94 ± 44.7	712.4 ± 18.7
Faridkoot	137.2 ± 2.9	149.5 ± 5.2	38.2 ± 1.9	38.2 ± 1.6	73.1 ± 5.2	123.4 ± 9.4	4.15 ± 0.4	5.36 ± 0.2	655.4 ± 31.6	655.4 ± 37.2
Jind	166.2 ± 7.9	197.6 ± 12.7	43.7 ± 3.6	64.4 ± 4.3	66.3 ± 3.2	139.5 ± 7.4	4.36 ± 0.2	5.85 ± 0.2	817.9 ± 46.3	773.4 ± 56.7
Amritsar	181.2 ± 6.3	241.5 ± 13.9	68.8 ± 2.5	99.8 ± 6.6	122.3 ± 7.5	197.3 ± 11.2	5.88 ± 0.3	6.88 ± 0.4	477.5 ± 13.7	498.1 ± 13.2
Saharanpur	260.4 ± 13.2	233.9 ± 8.2	61.2 ± 5.9	92.8 ± 3.4	140.4 ± 2.8	190.4 ± 8.6	5.12 ± 0.5	6.62 ± 0.4	529.6 ± 28.4	554.1 ± 31.8
Ambala	434.1 ± 19.8	252.8 ± 7.5	92.8 ± 6.4	68.8 ± 4.1	213.2 ± 6.2	181.2 ± 9.7	6.75 ± 0.2	6.65 ± 0.3	918.6 ± 53.8	943.5 ± 57.2
Hosiyarpur	495.2 ± 12.4	249.4 ± 15.6	99.8 ± 6.5	61.3 ± 5.2	186.2 ± 9.5	166.2 ± 11.5	8.88 ± 0.6	6.38 ± 0.5	1165 ± 67.4	1043 ± 32.6

Table 3. Available N, P, K, Fe and S of rhizospheric soil of genotype HD3086 and PBW343. The data are mean of three replicates ± standard error.

Effect of genotypes and location on the abundance and diversity of the wheat rhizosphere microbiota

OTU counts varied from 6760 to 10,708 in the rhizospheric soil collected from genotype HD3086, and it ranged from 2687 to 13,571, in the rhizospheric soil collected from the genotype PBW343 (Supplementary Table 4). The t-test revealed a significant difference ($p < 0.05$) in the abundance (Chao-1) and diversity (Shannon Index) between the genotype and among the different locations (Fig. 1a-b). The abundance and diversity were highest in the soils collected from Hoshiarpur, Punjab, and Ambala Punjab respectively. The abundance and the diversity were lowest in the soil collected from the Faridkoot, Punjab, and Ludhiana, Punjab, respectively (Fig. 1a-b).

Effect of wheat genotypes on the rhizosphere Microbiome at the phyla and genera level

We observed significant effects of the two genotypes on the wheat rhizosphere microbiome in the study area. Out of the top 10 most abundant phyla (together constituting 95% of the relative abundance), a significant difference was observed in the relative abundance of four phyla between the genotypes. They include Proteobacteria, Verrucomicrobia, Firmicutes, and Planctomyces - the first three were found to have higher relative abundance in the genotype HD3086, while the relative abundance of Planctomyces was higher in PBW343 (Supplementary Table 4; Fig. 2a-b). Among the top 50 genera, 18 showed a significant difference ($p < 0.05$) in their mean relative abundance between the genotypes (Table 4). These genera include *UTCFX1*, *Luteolibacter*, *Terrimonas*, *Pseudomonas*, *AKYG587*, *Gemmata*, *Altererythrobacter*, *Stenotrophobacter*, *Devosia*, *Pseudarthrobacter*, *Algoriphagus*, *Noviherbaspirillum*, *Mariniflexile*, *Gaiella*, *Lacibacter*, *Nitrosospira*, *Luteimonas*, *and Aridibacter*. Among these 18 genera, 10 recorded a higher relative abundance in the rhizosphere of HD3086, and eight recorded a higher relative abundance in PBW343 (Supplementary Table 5; Fig. 3).

Rhizosphere microbial taxa distribution across the genotypes and locations

The number of genera recorded was 492, and the number of genera varied in relation to both genotypes and locations. A total of 421 genera were recorded in the rhizosphere of HD3086, while the rhizosphere of PBW343 recorded 321 genera. Of the total 492 genera recorded 251 (51%) were recorded in the rhizosphere of both the genotypes; 170 (34.6%) were recorded only in the rhizosphere of HD3086 and 71 (14.4%) were recorded only in the rhizosphere of PBW343 (Fig. 4). Across the location, number of genera recorded in the rhizosphere of both the genotypes were 92 (47.4%), 68 (37.4%), 56 (35%), 90 (39.3%), 114 (39%), 110 (46%), 92 (36.8%) and 79 (34.3%), respectively in the soil samples collected from the Mansa, Ludhiana, Faridkot, Jind, Amritsar, Saharanpur, Ambala and Hoshiarpur. Number of genera exclusively recorded in the rhizosphere of HD3086, under the eight locations in order above respectively were, 68 (35.1%), 88 (48.4%), 81 (50.6%), 102 (44.5%),

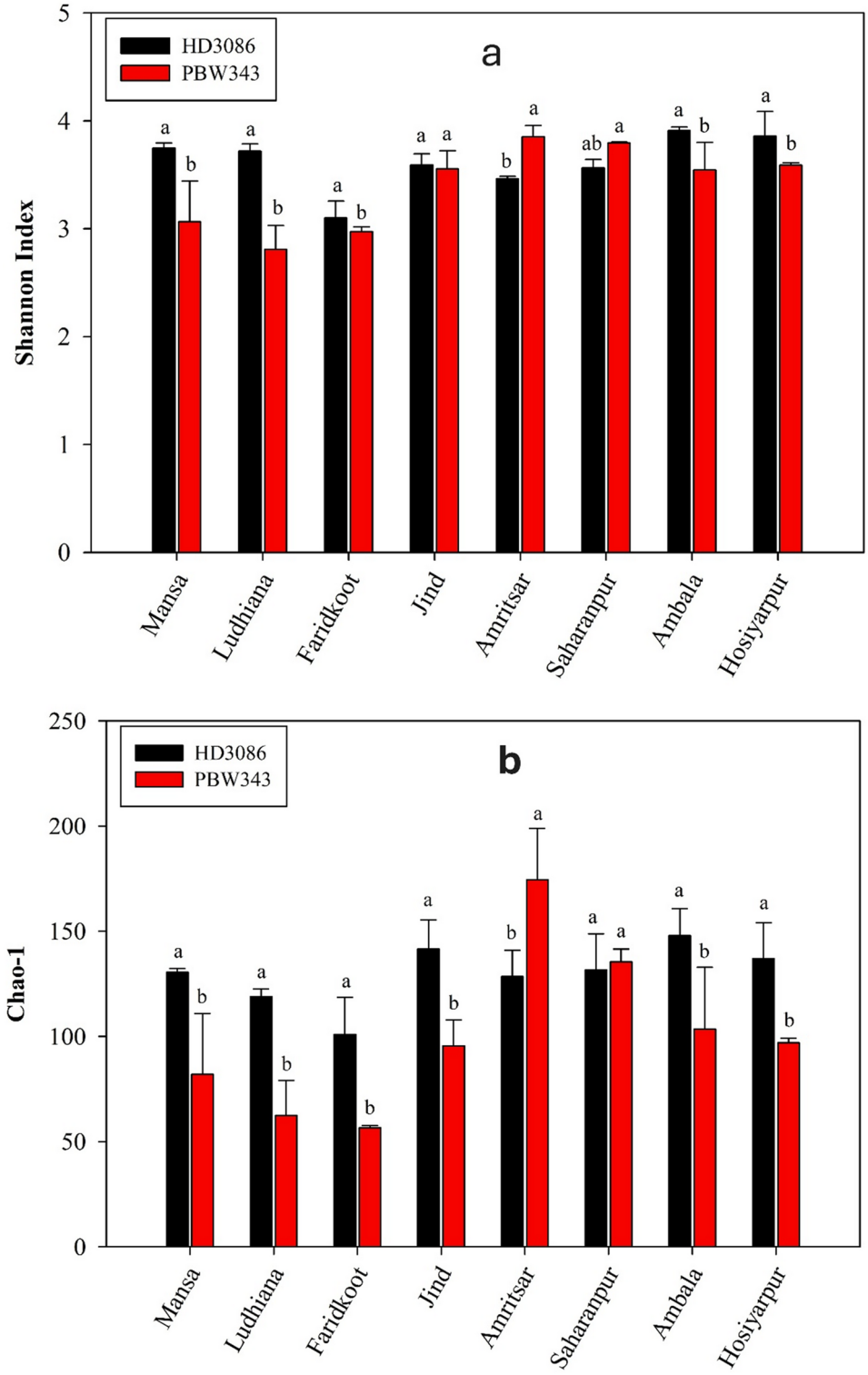

Fig. 1. **(a)** Alpha diversity indices in terms of Shannon Index in HD3086 and PBW343 rhizopsheric soil collected from eight districts of lower IGP. Data presents replicates mean ± standard error, values followed by the same letter are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test. **(b)** Alpha diversity indices in terms of Chao-1 in HD3086 and PBW343 rhizopsheric soil collected from eight districts of lower IGP. Details as mentioned in Fig. 1a.

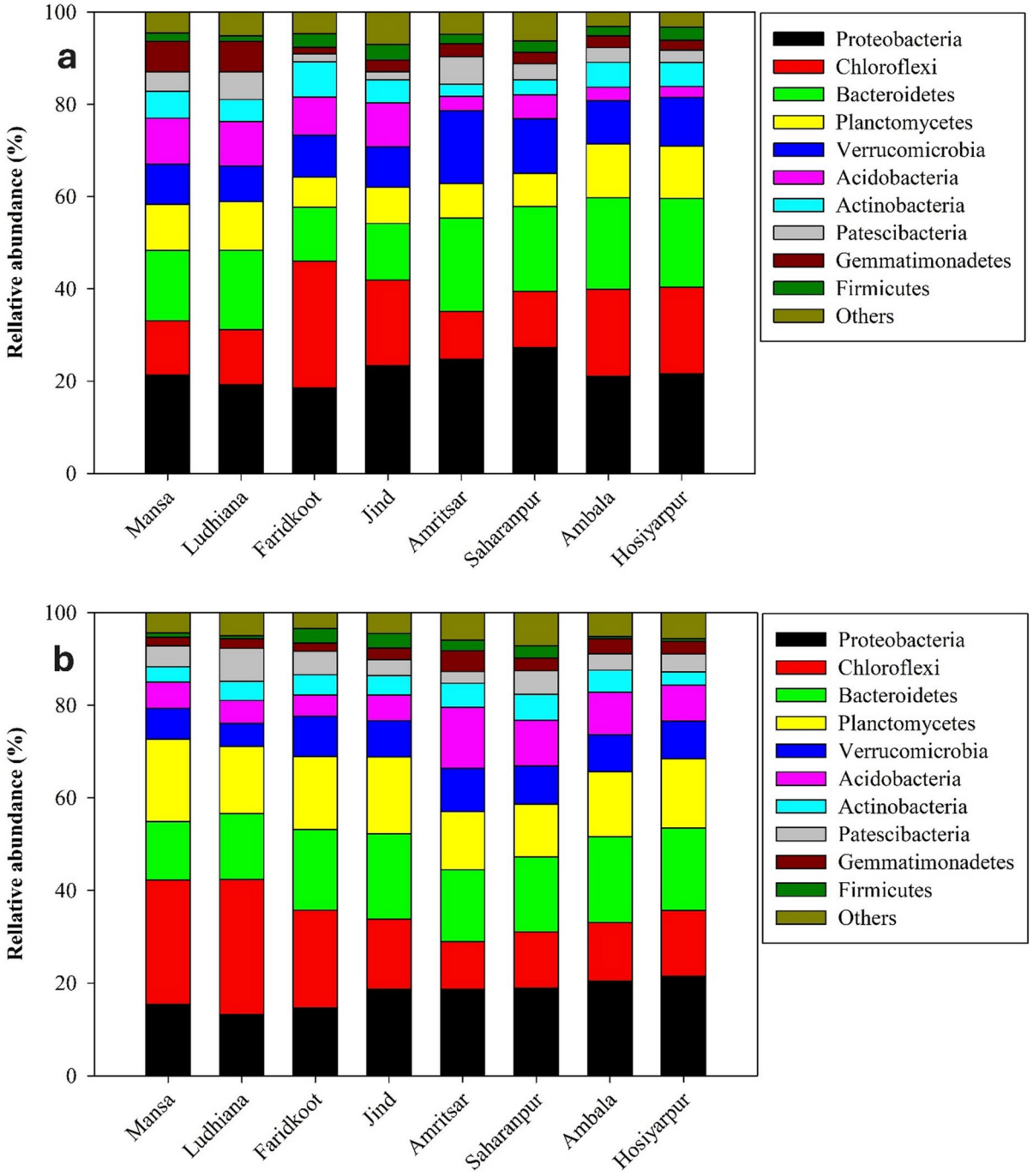

Fig. 2. **(a)** Relative abundance (%) of bacterial phyla present in HD3086 rhizopsheric soil collected from eight districts of lower IGP. **(b)** Relative abundance (%) of bacterial phyla present in the PBW343 rhizopsheric soil collected from eight districts of lower IGP.

60 (20.5%), 61 (25.5%), 99 (39.6%) and 105 (45.7%), while in the rhizosphere of PBW343, number of genera exclusively were 34 (17.5%), 26 (14.3%), 23 (14.4%), 37 (16.2%), 118 (40.4%), 68 (28.5%), 59 (23.6%) and 46 (20%) (Fig. 4).

The relative abundance of the top 10 microbial phyla (in terms of relative abundance) in the rhizospheres of HD3086 and PBW343 is presented in Supplementary Table 4. Among them, the 10 most abundant microbial phyla with their relative abundance in the rhizosphere of HD3086 included Proteobacteria (22.12%), Bacteroidetes (16.80%), Chloroflexi (16.23%), Verrucomicrobia (10.24%), Planctomycetes (9.03%), Acidobacteria (6.38%), Actinobacteria (4.94%), Patescibacteria (3.62%), Gemmatimonadetes (3.40%), and Firmicutes 2.35%. Similarly top 10 phyla recorded in the rhizosphere of PBW343 were Chloroflexi (17.70%), Proteobacteria (17.68%), Bacteroidetes (16.40%), Planctomycetes (14.70%), Verrucomicrobia (7.71%), Acidobacteria (7.59%),

Genera	Relative abundance %		*P* value
	HD3086	PBW343	
UTCFX1	13.9	19.6	0.025
Luteolibacter	8.49	4.71	0.032
Terrimonas	1.79	3.20	0.029
Pseudomonas	2.28	1.01	0.038
AKYG587	0.68	2.34	0.000
Gemmata	0.99	2.02	0.000
Altererythrobacter	1.73	1.11	0.003
Stenotrophobacter	0.68	1.13	0.032
Devosia	1.43	0.27	0.004
Pseudarthrobacter	1.03	0.61	0.046
Algoriphagus	1.27	0.00	0.016
Noviherbaspirillum	1.19	0.11	0.000
Mariniflexile	1.12	0.02	0.035
Gaiella	0.37	0.57	0.019
Lacibacter	0.07	0.85	0.000
Nitrosospira	0.80	0.12	0.007
Luteimonas	0.65	0.21	0.024
Aridibacter	0.18	0.52	0.006

Table 4. Relative abundance of genera that showed significant differences between the varieties.

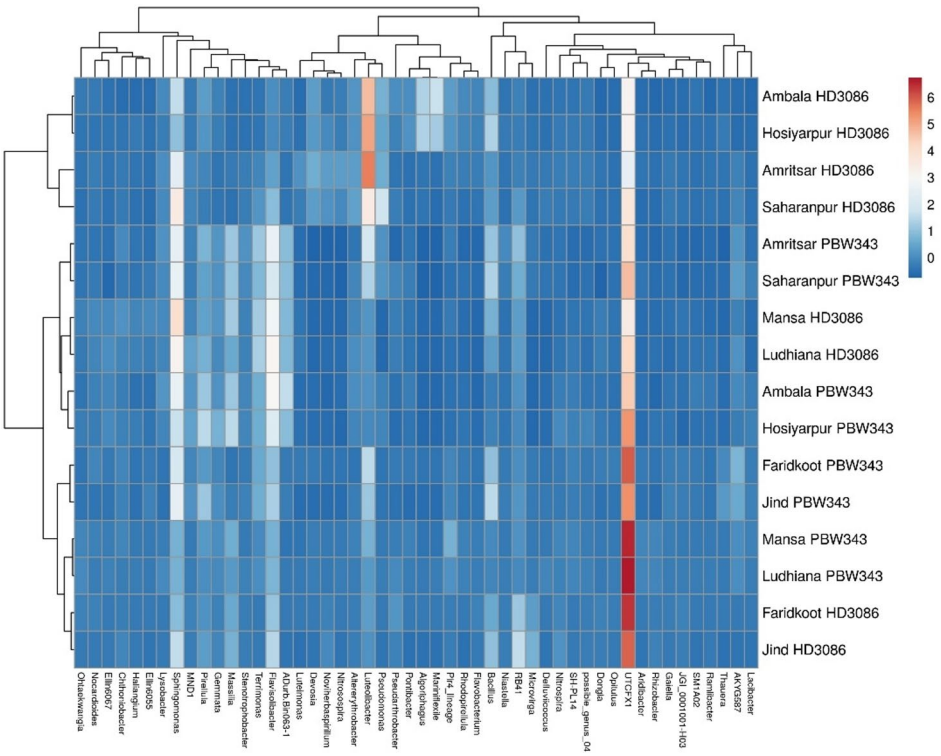

Fig. 3. Top 50 genera heat-map showing the relative abundance (%) present in HD3086 and PBW343 rhizopsheric soil collected from eight districts of lower IGP.

Patescibacteria (4.37%), Actinobacteria (4.32%), Gemmatimonadetes 2.70%), and Firmicutes (1.73%). The order of top 10 genera in the rhizosphere of HD3086 were *UTCFX1* (13.88%) > *Luteolibacter* (8.49%) > *Sphingomonas* (8.25%) > *Flavisolibacillus* (5.04%), *Bacillus* (3.76%) > *RB41* (2.93%) > *Massilia* (2.07%) > *Pirellula* (2.11%) > *Terrimonas* (1.79%) > and *Altererythrobacter* (1.73%), similarly in the rhizosphere of PBW343 this order was *UTCFX1* (19.64%) > *Sphingomonas* (7.79%) > *Flavisolibacter* (6.72%) > *Luteolibacter* (4.72%) > *Pirellula*

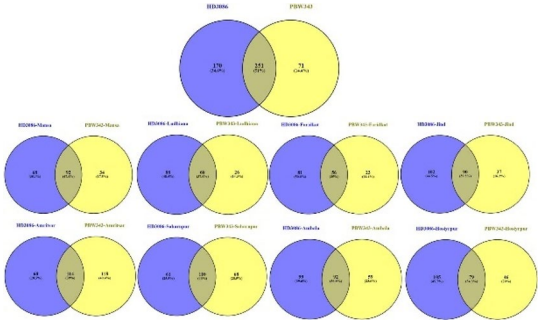

Fig. 4. Venn diagram showing distribution pattern of genera in HD3086 and PBW343 rhizopsheric soil collected from eight districts of lower IGP.

(3.67%) > *Massilia* (3.35%) > *Bacillus* (3.34%) > *Terrimonas* (3.21%) > *ADurb.Bin063-1* (2.56%) and *AKYG587* (2.35%) (Supplementary Table 5).

Effect of cropping system on rhizosphere microbial communities

The principal coordinates explained 71.9% (PC1) and 12.5% (PC2) of the variation in the microbial communities among the cropping systems. Group 1 comprises one sample of rice-wheat cropping system and one sample of cotton-wheat system; group 2 comprises two samples of rice-wheat system; group 3 comprises one sample of rice-wheat system, and group 4 comprises 2 samples of rice-wheat system and one sample of sugarcane-wheat system (Fig. 5a). To find out the statistical differences among the microbial communities in two genotypes and different cropping systems, a principal coordinate analysis was performed. The principal coordinates explained 54.4% (PC1) and 24.4% (PC2) of the variation in the microbial communities (Fig. 5b). The results of both analyses revealed cropping system does not have any significant control in selecting the microbial communities.

Core taxa and their abundance

Core taxa of the wheat rhizosphere microbiome were identified based on the criteria of the presence of the OTU in all the soil samples. This prevalence criterion enabled the identification of the wheat rhizosphere core microbiota across the location and wheat genotype. We observed that the number of core taxa was 27 across both genotypes (Supplementary Table 6). These represent 5.48% of the total taxa and 62% of the total relative abundance. The number of core taxa recorded in genotype HD3086 was 47 (Supplementary Table 7) while it was 37 in PBW343 (Supplementary Table 8); these represent 9.55% and 7.50% of total taxa and 69.6% and 73% of total relative abundance, respectively.

Correlation between soil parameters and microbial community diversity

Correlation analysis was performed among soil parameters and microbial community diversity in terms of the Shannon index for both varieties separately. In case of genotype HD3086, it was observed that the microbial diversity index was significantly correlated with soil parameters like pH ($P=0.002$), total N ($P=0.000$), available N ($P=0.023$), total K ($P=0.007$), available K ($P=0.016$), available P ($P=0.020$), total P ($P=0.013$), available Fe ($P=0.043$), and OC content ($P=0.003$), (Fig. 6a-b, supplementary Table 2). While in case of genotype PBW343 microbial diversity index was significantly correlated with soil parameters like pH ($P=0.002$), total N ($P=0.010$), available N ($P=0.001$), total K ($P=0.032$), available K ($P=0.003$), available P ($P=0.002$), total P ($P=0.032$), available Fe ($P=0.001$), and OC content ($P=0.010$), (Fig. 6a-b).

Co-occurrence network analysis

To further compare the interactions and associations of microbiomes in the rhizospheric soil of HD3086 and PBW343 from all the sites, we assessed the co-occurrence patterns of microbial communities and calculated microbial network parameters in terms of distance, including node and edge. Microbial network analysis had demonstrated that with respect to the sampling location nodes representing both varieties, the microbial abundance of the single location was very close to each other (Fig. 7).

Discussion

The primary goal of wheat crop breeding has historically been to identify wheat varieties that are tolerant to pests and diseases. However, root features and their ability to attract beneficial bacterial populations have received less attention[20]. Considering the crucial role that bacterial communities play in enhancing crop productivity[21], further investigation is needed to understand how beneficial bacteria are recruited in the wheat rhizosphere.

In this study, we employed an integrative approach to investigate the rhizosphere microbiome of two wheat varieties, HD3086 and PBW343. Our analysis included evaluating the comprehensive microbial diversity, encompassing bacteria, across soils collected from various locations in the trans Indo-Gangetic Plain (IGP). The abundance of microbial communities associated with plants generally depends on factors such as soil characteristics, plant growth stage, variety cultivated, weather conditions of the crop-growing area, and cropping history[22–24].

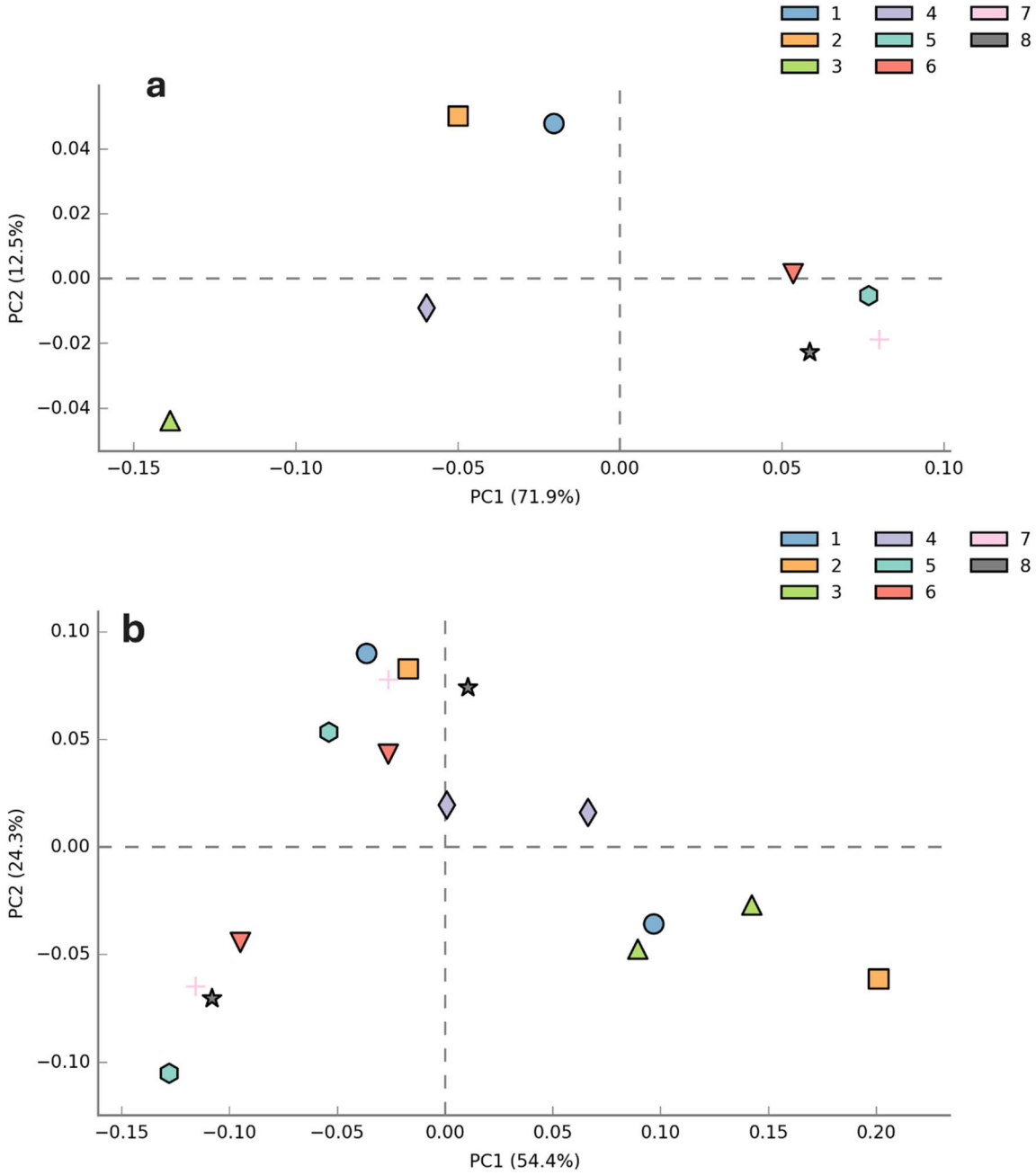

Fig. 5. **(a)**β diversity of bacteria associated with HD3086 and PBW343 wheat rhizosphere across eight districts of lower IGP based on weighted unifrac distances. **(b)**β diversity of bacteria associated with HD3086 and PBW343 wheat rhizosphere across eight districts of lower IGP based on weighted unifrac distances in replicates.

By growing the two wheat varieties in eight different soils, we found a significant varietal impact on diversity (Shannon Index) and abundance (Chao-1). The variability between the same locations under both genotypes was larger than the average variability of both genotypes. These results contrast with the findings of Simonin et al.[25], who did not find a significant varietal impact on the abundance and diversity of microbial populations in eight diverse soils cultivated under three different varieties. However, our findings align with previous reports indicating that wheat genotypes affect microbial abundance[4,26,27]. The observed varietal impact in our study may be highlighted by the differential recruitment of microbiota in the wheat rhizosphere concerning soil properties and wheat variety.

Principal Coordinates Analysis (PCoA) in our study did not show any consistent grouping patterns, with no fixed grouping based on soil location, wheat variety, or cropping system. These findings are parallel to our earlier reports where we did not find any fixed grouping pattern of soil across different locations[28]. However, the grouping pattern based on cropping systems was contrary to a previous report where we found separation based

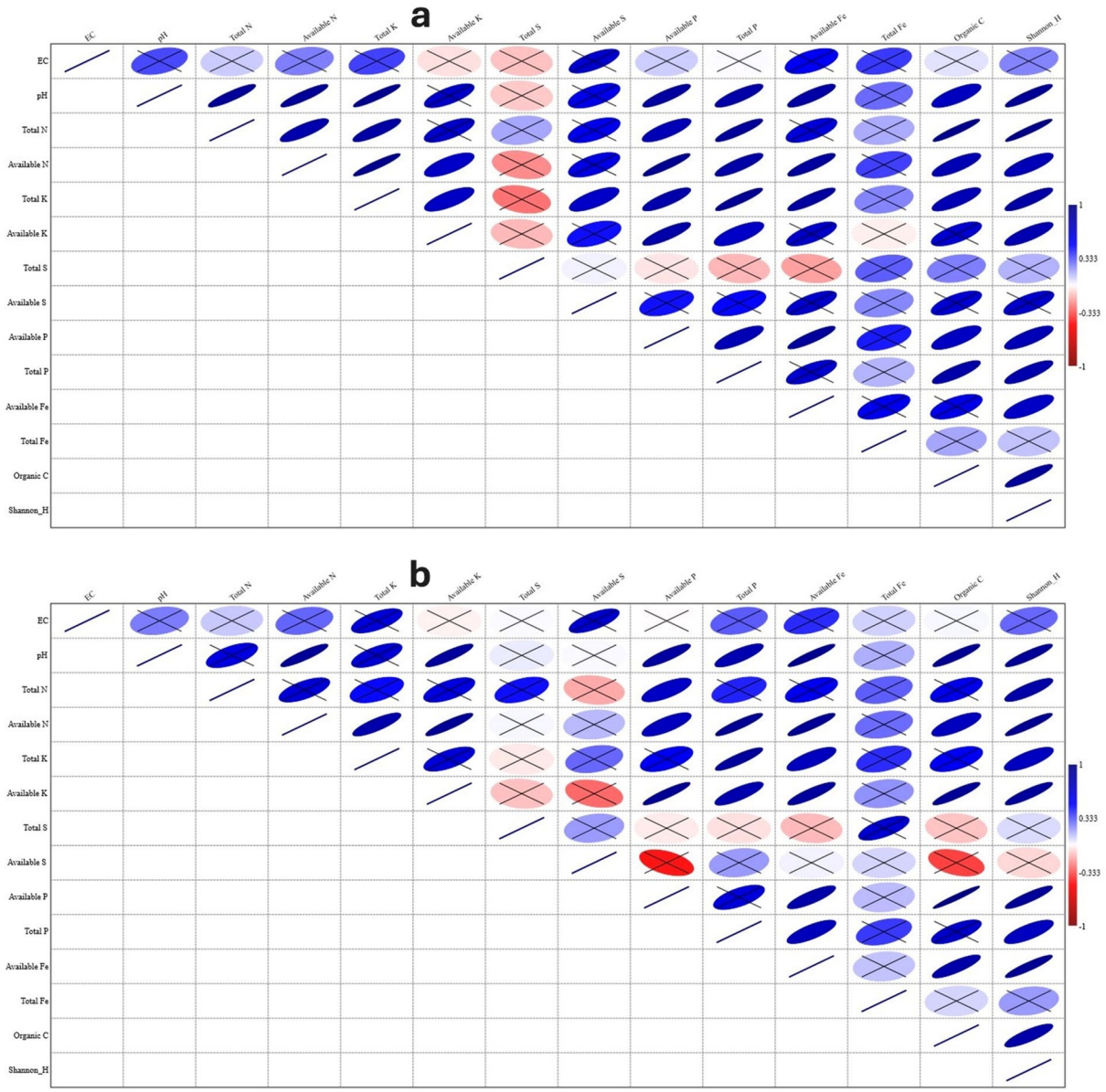

Fig. 6. **(a)** Pearson correlations in terms of the Shannon Index between the physicochemical characteristics of the soil and the bacterial diversity of the wheat rhizosphere in variety HD3086. **(b)** Pearson correlations in terms of the Shannon Index between the physicochemical characteristics of the soil and the bacterial diversity of the wheat rhizosphere in variety PBW343.

on cropping systems[29]. This discrepancy might be due to differences in the number of samples from cropping systems, as six soils were from the rice-wheat cropping system, while only one soil each was from the cotton-wheat and sugarcane-wheat cropping systems. Additionally, this segregation could be attributed to variations in soil parameters, particularly those identified as primary influencers of wheat microbiomes[8].

Plants continuously attract certain taxa, known as the core microbiome, which are essential for plant nutrition and health[14]. Furthermore, in the manipulation of plant-associated microbiomes, it is crucial to identify taxa that contribute beneficial functions to plants. We observed yet the distinct soils from multiple varieties, from diverse altitude and longitude showed significant effects on the microbial communities abundance and composition in the rhizosphere of wheat, it was constantly enriched with lesser number of microbial taxa within the phyla Proteobacteria, Chloroflexi, Bacteroidetes, Planctomycetes, Verrucomicrobia, Acidobacteria and Actinobacteria, which together comprises more than 80% phyla, paralleling results in other studies[7,27]. Proteobacteria predominated in both varieties (HD3086 and PBW343), a typical finding consistent with other reports in soil samples and diverse plant environments, particularly the rhizosphere, owing to the copiotrophic traits of many members within this phylum[30,31]. This phylum is vital in various biogeochemical cycles, notably those concerning carbon and nitrogen[32].

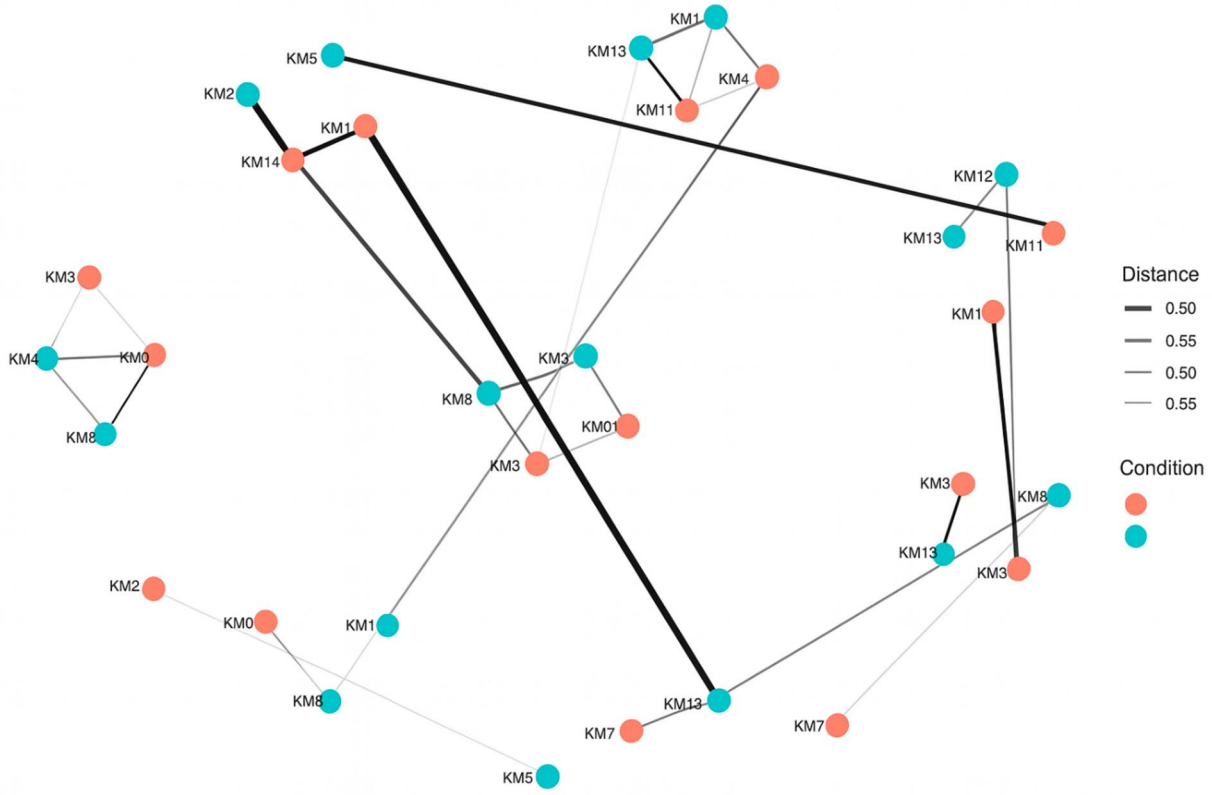

Fig. 7. Co-occurrence network graph, pink color nodes present variety HD3086 while green nodes presents variety PBW343. KM1, KM2, KM5, KM6, KM9, KM10, KM13, KM14, KM17, KM18, KM21, KM22, KM25, KM26, KM29, and KM30 presents Mansa, Ludhiana, Faridkot, Jind, Amritsar, Saharanpur, Ambala and Hoshiarpur in duplicates for variety HD3086 while KM3, KM4, KM7, KM8, KM1, KM12, KM15, KM16, KM19, KM20, KM23, KM24, KM27, KM28, KM31, and KM32 presents Mansa, Ludhiana, Faridkot, Jind, Amritsar, Saharanpur, Ambala and Hoshiarpur in duplicates for variety PBW343.

The presence of the phyla Chloroflexi, Bacteroidetes, Planctomycetes, Verrucomicrobia, Acidobacteria, and Actinobacteria, is consistent with earlier reports in the different crops[33,34]. Members of the Bacteroidetes phylum encode a unique constitutive alkaline phosphatase *PafA*, implying that their inclusion as core microbiota in crops could be significant for phosphorus mobilization. Conversely, Actinobacteria and Firmicutes emerge as the dominant phyla in the rhizosphere of different wheat varieties, characterized as K-strategists, a finding supported by several other studies[22,35]. In our current findings, we reported that important factors of the microbial community's population and abundance in the rhizosphere of wheat are the soil location and wheat variety, although, cropping system showed very little impacts. In eight diverse soils spanning the trans IGP region and two wheat varieties, we identified 47 prokaryotic taxa at the genus level consistently associated with the HD3086 wheat rhizosphere, 37 taxa with PBW343 wheat rhizosphere, and common core taxa that constitute the core microbiome of both varieties were 27, constituting a core microbiome. As our findings of varietal effects on microbial community, earlier reports also showed that the selection of a specific genotype of wheat for cultivation influence and regulates the population and abundance of bacterial community in the rhizosphere[36]. Dilla-Ermita et al.[4] recently reported field and growth chamber findings in which they utilized eight distinct cultivars of wheat under the same soil and found that the major factor which affects rhizospheric microbial communities was the cultivar used. The core taxa which had a significant effect on growth and productivity of crops are in general represents more than 50% of relative abundance of microbial population and it has host specific activity[12]. Our present findings facilitated the selection of a concise list of taxa for future wheat microbiome explorations. These will serve as important findings for culturomics, genomic, and synthetic community analyses, advancing microbiome engineering in agriculture.

Many factors that influence the rhizospheric soil bacterial community structure include physicochemical characteristics and the geographical location of the soil. Plant rhizosphere exudates and other elements interact with the soil's preexisting microbiota to recruit and alter the microbiome in the rhizosphere for the plants' benefit[37]. Many reports on multiple crops are available, which showed the soil characteristics of the rhizospheric microbiota[34,37]. In our current study, correlation analyses showed significant relationships between the diversity index (Shannon Index) and soil physicochemical properties, including soil pH, organic carbon content, and the availability of nutrients such as N, P, K, and Fe, as well as total N, P, and K, were significantly correlated in both the varieties, hence, these parameters drive the diversity of microbial population of wheat rhizosphere grown

in trans IGP. Our results supported the findings of Rascovan et al.[37], and Wu et al.[38], as they discussed how rhizospheric microbial communities are affected by pH, organic carbon concentration, and other soil nutrient variables. pH and OC are critical factors which determine the composition of microbial communities in the soil[38,39]. Our research clearly shows that the available nutrients, particularly N, P, and Fe, have a significant impact on bacterial diversity. The most significant primary nutrient that limits crop growth and output is nitrogen, which is regulated by the microbial populations in the soil. It is commonly known that nitrogen affects rhizospheric microbial populations[34,38,39]. The current study's substantial positive association between accessible nitrogen and diversity is consistent with these previous findings. Like nitrogen (N), phosphorus (P) is a vital soil element that limits crop growth by limiting the capacity of crop plants to use available P to increase productivity. Research has documented the reciprocal influence between available phosphorus and rhizosphere microbial communities in various crops[40,41]. The available P has a positive correlation with microbial abundance and uniformity throughout the wheat rhizosphere in the present study, which is consistent with previous findings on other crops in addition to wheat[39,42]. Additionally, we also recorded a significant relationship between diversity index and available Fe content, which is similar to the earlier findings of Wu et al.[39].

More exclusively, 27 genera have been identified as core taxa of the wheat rhizosphere of varieties HD3086 and PBW343, in all soil samples. These core taxa exhibited high prevalence and abundance, defying expectations since we did not establish a relative abundance threshold for their identification. Together, identified 27 taxa (5.50% of the identified taxa) represent 62% of the relative abundance, putting forward a significant presence of these bacterial populations in the rhizosphere. These results imply that wheat interacts with the same bacterial populations, suggesting that these microbes and wheat may have co-evolved. This finding aligns with a prior study on the wheat microbiome, which showed that cropping practices did not affect highly co-occurring taxa or possible core taxa[43]. The identified core taxa exhibit significant prevalence and abundance, indicating their ecological relevance.

Among the core 27 genera, genera with relative abundance more than 1% include *UTCFX1*, *Sphingomonas*, *Luteolibacter*, *Flavisolibacter*, *Bacillus*, *Pirellula*, *RB41*, *Terrimonas*, *Gemmata*, *MND1*, and *Pir4_lineage*. *UTCFX1* were the most abundant genera identified in the present study; similarly Chen et al.[44], also reported these genera as the most abundant in the multiple cropping system. Recently, Xia et al.[45], suggested the role of these genera in salt stress tolerance. The genus *Sphingomonas* not only promotes plant growth[29], but also increases plant resistance to pathogens. *Sphingomonas* have been shown to improve plant response to a variety of pathogens[46], including fungi and bacteria. The average relative abundance of this genera in the present study was 8%. The genera *Luteolibacter*, *Flavisolibacter*, and *Bacillus*, are the genera which are commonly known for their plant growth promoting properties for a long time[29]. Therefore, their abundance in the current findings suggests their positive contribution to wheat plant growth independent of the wheat variety. *MND1* comprises a cluster of ammonia-oxidizing bacteria (AOB) crucial for soil nitrogen cycling via nitrification and nitrogen fixation in agricultural soils[47]. *RB41* is widely acknowledged as a vital genera of soil microbiomes and agroecosystems, with the ability to selectively influence decomposition of soil organic matter and carbon cycling in agricultural soils[48]. The increase in these bacteria's abundance after the nitrogen fertiliser was reduced might have improved the soil's capacity to sequester carbon and nitrogen, which would have improved overall health through a variety of processes like nutrient cycling, soil structure, and biological interactions. Dai et al.[49], reported genera *Terrimonas*, was significantly accumulated in the rhizopheric soil of wheat, and their significance in disease resistance was evidenced. Similarly, we also noted the abundance of these genera in the rhizosphere of the wheat. The *Gemmata*, which was found in abundance in the present study, belongs to the phylum Planctomycetes and is universally disseminated in wide environments, and its diversity is sensitive to soil management history. The sole member of this phylum identified was *Gemmata*, a genus newly found in the rhizosphere of corn. Gemmata possesses the ability to perform heterotrophic nitrification and anaerobic ammonia oxidation (anammox)[50]. *Pir4 lineage* (Pirellulaceaea) anaerobic ammonia oxidizing bacteria were present in abundance in both varieties in the present study, suggesting their significant involvement in ammonia oxidation[51]. In addition to this, the genus has been well established for sulphur oxidation. The abundance of this genus in the wheat rhizosphere can be linked to the rich sulfur content in the rhizosphere of both wheat varieties[52].

Conclusion

In conclusion, this study provides a comprehensive characterization of the wheat rhizosphere microbiota across the trans-Indo-Gangetic Plain, detailing core microbial taxa. The rhizospheric soil exhibited distinct bacterial community compositions and diversity, which were influenced by variations in soil parameters. We extensively investigated the impacts of wheat variety and geographical location on these microbial communities. The number of shared and unique genera differed significantly between the two wheat varieties. These findings enhance our knowledge of bacterial diversity in intensive agricultural settings, offering insights for sustainable agriculture practices through biological management strategies. Future research should focus on culturing these wheat-associated microorganisms to unlock their potential in enhancing crop productivity and disease resistance sustainably. A holistic approach considering the plant holobiont, beyond bacteria alone, is essential for advancing these efforts.

Data availability

The sequence data generated was submitted to NCBI database with project number (Project number: PRJNA1128054; SRA ID: SRR29548375 to SRR29548382).

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Author contributions

MK: Conceptualization, Supervision, Writing - review & editing. WAA: Soil sampling, Investigation, Formal analysis, Visualization, Writing - original draft. ASi: Formal analysis, Writing - review & editing. HC: Writing - review & editing. SCK and MTZ: Soil biochemical analysis. MSF, AS, SS, and GKJ: did statistical analysis, bioinformatics analysis, and interpretation of the data. AKS: Resources, Writing - review & editing.

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Declarations

Ethics approval and consent to participate

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Consent for publication

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Competing interests

The authors declare no competing interests.

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