Discovery

Comparative metagenomic analysis of rhizosphere soil associated bacterial community structure and function from *Zea mays* and *Brassica nigra*

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ABSTRACT

Background: The rhizosphere soil is the hotspot for diverse plant-microbe interactions, which is beneficial for plant productivity. Two economically significant and Indigenous (Malda, India) crops, *Zea mays*, and *Brassica nigra*, were analyzed in this study using 16SrRNA metagenomic sequencing to explore the structure and function of rhizomicrobiome with advantageous interaction with the plants. *Methods:* Rhizosphere soils were collected, analyzed for soil physicochemical properties, and subjected to DNA extraction, PCR amplification, followed by sequencing using Illumina MiSeq. The metagenomic sequences were demultiplexed for quality control, chimera removal, OTU abundance in QIIME2, and taxonomic assignment using Kraken2/Bracken against Silva/Greengenes/NCBI/KEGG databases. *Results:* Proteobacteria and Planctomycetes were the two most abundant phyla, *Adurb.Bin063-1* sp. predominated *Zea mays* and *Brassica nigra*. Alpha and beta diversity differed insignificantly between *Zea mays* and *Brassica nigra*. The two rhizomicrobiomes showed a significant positive correlation at the genus level. *Ellin6067* sp., *Nitrosomonas* sp., *MND1* sp., *oc32* sp., and *mle1-7* sp. from the *Nitrosomonadaceae* predominated the rhizomicrobiomes. Enrichment of nitrogen-fixation, nitrite-reduction, and ammonia-oxidation occurred in *Zea mays* and *Brassica nigra*. Expression of pEA3 plasmid was detected in *Zea mays* with reports of putative nitrogen fixation properties. Antimicrobial resistance gene analysis revealed aminocoumarin resistance in *Zea mays*. Abundances of mobile genetic element vs bacterial genera, and functional characteristics describing *Zea mays* vs *Brassica nigra* were insignificantly associated. *Conclusion:* Rhizomicrobiomes of *Zea mays* and *Brassica nigra* revealed shared taxonomic and metabolic profiles related to the nitrogen cycle. Deciphering the structural and functional diversity of rhizomicrobiomes are essential for understanding advantageous plant-microbe interactions.

Keywords: Bacteria, rhizomicrobiome, metagenomics, taxa, function, diversity, mobile genetic elements

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1. INTRODUCTION

Maize, also called corn (*Zea mays* L.; family: *Poaceae*), is a widely cultivated food crop in the world. Maize is recognized as an essential annual cereal crop worldwide, which comes after wheat and rice. It is a staple food and a potential source of nutrition, and in India, maize ranks fourth in terms of area and seventh in terms of production (Rouf et al., 2016). Mustard (*Brassica nigra* L.; family: *Brassicaceae/Cruciferae*) is a vital oil crop plant, and is helpful as vegetable displaying anticancer activities (De-Zoysa and Waisundara, 2020). Because of its food and medicinal values, this crop can play a significant role in health and wellness as well as in agriculture and horticulture, globally (De-Zoysa and Waisundara, 2020). The metagenomic study of microorganisms involves the sequencing of entire nucleic acids in natural environmental samples without the need for laboratory cultivation and is valuable for uncovering the structural and functional information of microbes (Srivastava et al., 2019).

There are reports of rhizospheric soil mainly enriched with Bacteroidetes and Proteobacteria involved in nitrogen fixation and denitrification (Ling et al., 2022). Various beneficial bacterial genera, such as *Bacillus*, *Gemmatimonas*, *Rhodanobacter*, *Pseudolabrys*, and *Sphingomonas*, were found to be associated with the rhizosphere of tomato and pepper (Yan et al., 2024). Bochalya et al., (2016) reported the isolation of bacterial strains belonging to the genus, including, *Bacillus*, *Rhizobium*, *Pseudomonas*, *Arthrobacter*, and *Micrococcus* from the mustard rhizosphere. Chaudhary et al., (2022) reported three different bacterial strains, namely, *Burkholderia*, *Enterobacter*, and *Klebsiella* isolated from the mustard rhizosphere, having the capability of improving sulfur and nitrogen uptake essential for soil health and agronomic practices. The metagenomic profile of the mustard rhizosphere demonstrated bacterial phyla, such as Proteobacteria, Actinobacteria, Verrucomicrobia, and Planctomycetes, with diverse abundances (Liu et al., 2021).

On the other hand, the maize rhizosphere contained copiotrophic bacteria (*Rhizobiales*, *Sphingomonadales*, *Xanthomonadales*, and *Burkholderiales*), as reported by Navarro-Noya et al., (2022), through metagenomic analysis. The authors noted a high abundance of *Halomonas*, *Pseudomonas*, *DA101* and *Bacillus* in the rhizosphere soil samples. The abundance and diversity of microorganisms in maize rhizosphere-bearing functionalities suitable for plant health management and sustainability have been reported earlier (Navarro-Noya et al., 2022; Ling et al., 2022). As most microbes colonize the rhizosphere, the critical site of plant-microbe interactions, it exhibits an array of biological activities that determine plant health status (Navarro-Noya et al., 2022; Ling et al., 2022). The combined taxonomic and functional analysis of the rhizosphere delivers a comprehensive image of agricultural crop rhizomicrobiomes (Navarro-Noya et al., 2022; Ling et al., 2022).

As explained above, information is available, from different parts of the world, on microbial profiles of rhizospheres of mustard and maize, demonstrated using laboratory culture methods and metagenomic analysis. In our part of the globe (Malda, India), both *Brassica nigra* and *Zea mays* have been cultivated, respectively, as food crops and oilseed crops with financial advantages (Bhowmik and Roy, 2021). However, report on the rhizosphere bacteria associated with *Zea mays* compared to *Brassica nigra*, from this part of the globe is very rare. Given, the diverse ways with which rhizomicrobiome thrive and interact with host plant roots, we have undertaken the current study to decipher the taxonomical and functional features by metagenomic analysis of rhizospheric soils of *Brassica nigra* and *Zea mays*, grown locally, having enormous economic importance, towards sustainable agricultural practices.

2. MATERIALS AND METHODS

Collection of rhizospheric soil samples and analysis of soil physicochemical properties

Two indigenous crops, including *Zea mays* (Yuvraj Gold variety) and *Brassica nigra* (B9 variety) cultivated at Phulbarea, Malda (coordinates 25.0108 °N, 88.1411 °E), West Bengal, India, were chosen for the comparative analysis of the bacterial community structure and function of rhizomicrobiomes of the two crops. The rhizosphere soil samples were collected aseptically at a depth of approximately 0.30 m by uprooting the plants gently and brushing the soil on the root surface. The rhizosphere soil samples were stored at −40 oC until further analysis. We analyzed the associated physicochemical properties of the rhizosphere soil, including pH, electrical conductivity (EC), organic carbon, available Nitrogen, Phosphate, and Potassium (Black, 1965).

DNA extraction from rhizospheric soil for 16S rRNA gene amplification and metagenomic sequencing

The total genomic DNA from rhizospheric soil was extracted using high-salt lysis buffer, according to the method of (Zhou et al., 1996). The PCR amplification of bacterial 16S rRNA gene of soil metagenome was performed by targeting its V3-V4 hyper-variable region, using primers (Biokart Pvt Ltd, India): V13F (5′ AGAGTTTGATGMTGGCTCAG3′ and V13R (5′ TTACCGCGGCMGCSGGCAC3′), 10 pM of each, with high-fidelity DNA polymerase, 0.5 mM dNTPs, and 3.2 mM MgCl2 in PCR enzyme buffer (Shakya et al., 2013). The sequencing libraries from soil DNA amplicons were constructed by purification with Ampure beads and eight additional cycles of PCR, in Illumina MiSeq sequencing platform (Biokart Pvt Ltd, India) using a 2x300PE v3 sequencing kit (Shakya et al., 2013).

Bioinformatic analysis of raw metagenome sequences from rhizospheric soil

The raw metagenomic sequence data obtained as paired-end fastq files were further analyzed using bioinformatics tools by applying quality control with FastQC, removal of adapters and low-quality reads with Trim Galore. The paired-end reads were merged, followed by chimera removal, and estimation of OTU abundance in QIIME2 Bolyen et al., (2019) using databases Silva Quast et al., (2013) and Greengenes (DeSantis et al., 2006). For the taxonomic assignment of metagenomics sequencing reads, we used Kraken 2 and Bracken Lu et al., (2022) and classified based on % coverage and identity.

We applied the FAPROTAX Louca et al., (2016), KEGG Kanehisa et al., (2023) and NCBI Sayers et al., (2022) annotations for predicting putative metabolic and ecologically relevant functional profiles based on the conversion of bacterial and archaeal taxonomic community profiles in the form of OTU table derived from 16S rRNA amplicon sequencing. We used QIIME2 Bolyen et al., (2019) for the phylogenetic analysis of the bacterial sequences, based on MAFFT alignment and neighbor-joining algorithm, classified by the Greengenes database (DeSantis et al., 2006). The mobile genetic elements were detected using Silva Quast et al., (2013) and Kraken 2 (Lu et al., 2022). We applied the comprehensive antibiotic resistance database (CARD) of resistance genes and associated phenotypes for the identification of resistance genes (McArthur et al., 2013).

Statistical analysis of the metagenome samples from rhizosphere soil and data visualization

We analyzed the rhizosphere soil metagenomic sequences, statistically, using the Shannon α-diversity index, Bray-Curtis β-diversity, principal component (PC) analysis, clustering analysis based on Ward algorithm with Euclidean distance, the details of which we have described earlier (Mandal and Mandal, 2022). Tukey pairwise multiple comparisons, ANOVA, paired t-test, and linear regression analysis, at $p < 0.05$ level of significance, were applied to evaluate the taxonomical and functional features of *Zea mays* and *Brassica nigra*.

We applied the Venn diagram tool as described earlier Mandal and Mandal, (2022) to visualize the commonness of bacterial community structural features at the genus level between Zea mays and Brassica nigra rhizomicrobiomes. We utilized the interactive tree of life Ciccarelli et al., (2006) for the annotation, management, and displaying of the phylogenetic relationship between *Zea mays* and *Brassica nigra* rhizomicrobiomes. We applied the R packages Maindonald and Braun, (2010), factoextra, and FactoMineR, for PC analysis and visualization. Further, we used pheatmap R package Maindonald and Braun, (2010) for clustering analysis and construction of the heatmap.

3. RESULTS AND DISCUSSION

Microorganisms are natural inhabitants of different environments including plant rhizosphere that possess huge functional capacities modulating various mechanisms in plants related to plant productivity as well as offering defense under adverse external conditions (Navarro-Noya et al., 2022; Ling et al., 2022). Hence, it is essential to investigate the assemblage of bacteria associated with plant rhizosphere and decipher the functional diversity implicated in plant health towards the advancement of farming practices.

The current study, the first of its kind from our part of the globe (Malda, West Bengal, India), investigated the bacterial composition and their associated functional profiles in the rhizosphere soil of two Indigenous crops, including *Zea mays* (Yuvraj Gold variety) and *Brassica nigra* (B9 variety), using next-generation sequencing techniques. The soil physicochemical properties, including pH, EC, OC, N, phosphate, and potassium are represented for *Zea mays* and *Brassica nigra* rhizospheres (Table 1).

Table 1 Rhizospheric soil physicochemical properties of *Zea mays* and *Brassica nigra*.

Crop	Variety	Type	pH	EC (ds/m)	OC (%/gm)	Nitrogen (Kg/ha)	Phosphate (Kg/ha)	Potassium (Kg/ha)
Brassica nigra	B9	Rabi	6.9	0.34	0.37	138	420	241
Zea mays	Yuvraj Gold	Kharif	7.7	0.16	0.12	44	130	268

EC: Electrical conductivity, OC: Organic carbon

Metagenomic sequencing of the PCR-amplified V3-V4 region of 16S rRNA revealed the GC content for each sequence of *Zea mays* and *Brassica nigra* of about 11%. The number of Kraken2 assigned sequences (against the Silva database) of *Zea mays* and *Brassica nigra*, were 2276 and 897, respectively. Of the 2276 *Zea mays* sequences, 2235 (98.20%) sequences were classified, and 41 (1.80%) sequences were unclassified, while in *Brassica nigra*, out of 897 sequences, 882 (98.33%) sequences were classified, and 15 (1.67%) sequences remained unclassified.

The superkingdom Bacteria constituted 11% and 12% of the Root in *Zea mays* and *Brassica nigra*, respectively, while Archaea comprised 3% and 4% of the Root in *Zea mays* and *Brassica nigra*, respectively (Figure 1, 2, 3). Among Archaea, Euryarchaeota was the topmost phylum occupying respectively 3% and 2% of the root in *Zea mays* (Figure 3A) and *Brassica nigra* (Figure 3B). Proteobacteria (26.71%), Planctomycetes (11.34%), Acidobacteria (9.97%), Bacteroidetes (8.92%), Chloroflexi (8.04%), Patescibacteria (7.95%), Verrucomicrobia (5.1%), Firmicutes (4%), Actinobacteria (3.91%), and Gemmatimonadetes (2.94%) were the bacterial phyla prevailing in *Brassica nigra* (Figure 2).

The same group of bacteria were also prevalent in *Zea mays*, although with different abundances and included Proteobacteria (24.75%), Planctomycetes (10.93%), Bacteroidetes (10.59%), Chloroflexi (9.03%), Acidobacteria (8.92%), Patescibacteria (6.8%), Verrucomicrobia (4.91%), Firmicutes (4.24%), Actinobacteria (3.79%), and Gemmatimonadetes (3.01%) (Figure 1). The subsequent key taxononomical levels featuring *Zea mays were Gammaproteobacteria* (13.58%), *Betaproteobacteriales* (7.29%), and *Burkholderiaceae* (3.25%), while *Gammaproteobacteria* (12.93%), *Betaproteobacteriales* (7.36%), and *Pedosphaeraceae* (3.46%) dominated *Brassica nigra* (Figure 1, 2).

Overall, we detected a total of 265 genera, of which 220 and 130 features belonged to *Zea mays* and *Brassica nigra*, respectively. A 32.08% (85) of the genera present on *Zea mays* were also detected in the *Brassica nigra* microbiomes (Figure 4A). There were 50.94% (135) and 16.98% (45) of unique genera in *Zea mays* and *Brassica nigra*, respectively (Figure 4A). Nevertheless, the relative abundances of the genera differed across the two microbiomes (Figure 4B). The uncultured genera accounted for the similar fraction (23% approximately) of the total reads in both microbiomes. *Adurb.Bin063-1* sp. was the most abundant species in both *Zea mays* (3.34%) and *Brassica nigra* (3.77%). The following top five bacterial species prevalent in *Zea mays* were *MND1* sp. (2.69%), *Dongia* sp. (2.04%), *Sphingomonas* sp. (1.95%), *Bryobacter* sp. (1.77%), *Ellin6067* sp. (1.72%).

In *Brassica nigra*, *Diaphorobacter* sp. (3.54%), *Nitrospira* sp. (3.30%), *Aquabacterium* sp. (3.07%), *MND1* (2.95%), *Ellin6067* sp. (2.36%) constituted the genus in the top five relative abundances. Linear regression analysis of the taxonomical attributes at the genus level in *Zea mays* (IQR: 0.00279, Mean: 0.003589) and *Brassica nigra* (IQR: 0.00236, Mean: 0.00348) showed a significantly positive association between the two (R: 0.9736, p-value: 0, 95% CI: 1.0181,1.0781, t-Statistic: 68.7157) (Figure 4C). The evolutionary relationship of the 16S rRNA bacterial sequences in *Zea mays* and *Brassica nigra* rhizosphere classified from Greengenes database displayed *Aeromonas* sp. at the root of phylogenetic tree, while rest of the bacterial taxa clustered into several subclades (Figure 5).

The Shannon diversity index estimated in *Zea mays* and *Brassica nigra* were 1.81 and 1.64, respectively, which differed insignificantly (p-value: 0.9078), although microbiome composition of rhizosphere soils associated with *Zea mays* reflected relatively high diversity in comparison to *Brassica nigra*. The relative abundances of bacteria shared between two microbiomes examined using beta diversity based on the Bray-Curtis index (BCI) indicated indistinguishable dissimilarity (p-value: 0.2871) of the *Zea mays* (BCI: 0.2376) and *Brassica nigra* (BCI: 0.2587) rhizomicrobiome.

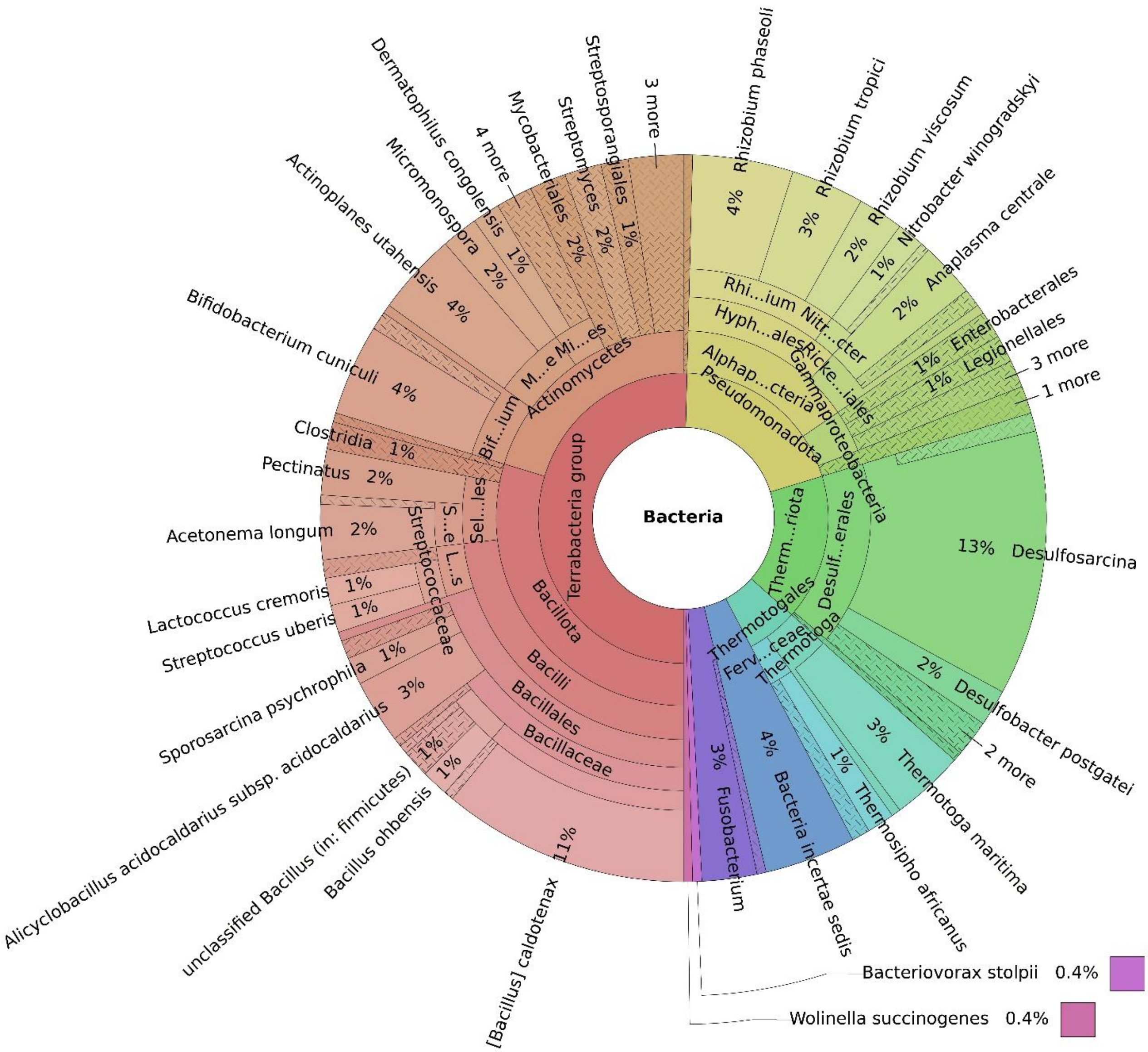

Figure 1 Kraken2 assigned taxonomic labels to 16S rRNA rhizomicrobiome of *Zea mays* using 250-mers against Silva database, representing superkindom Bacteria, accounting for 11% of Root and 256 bacterial genera.

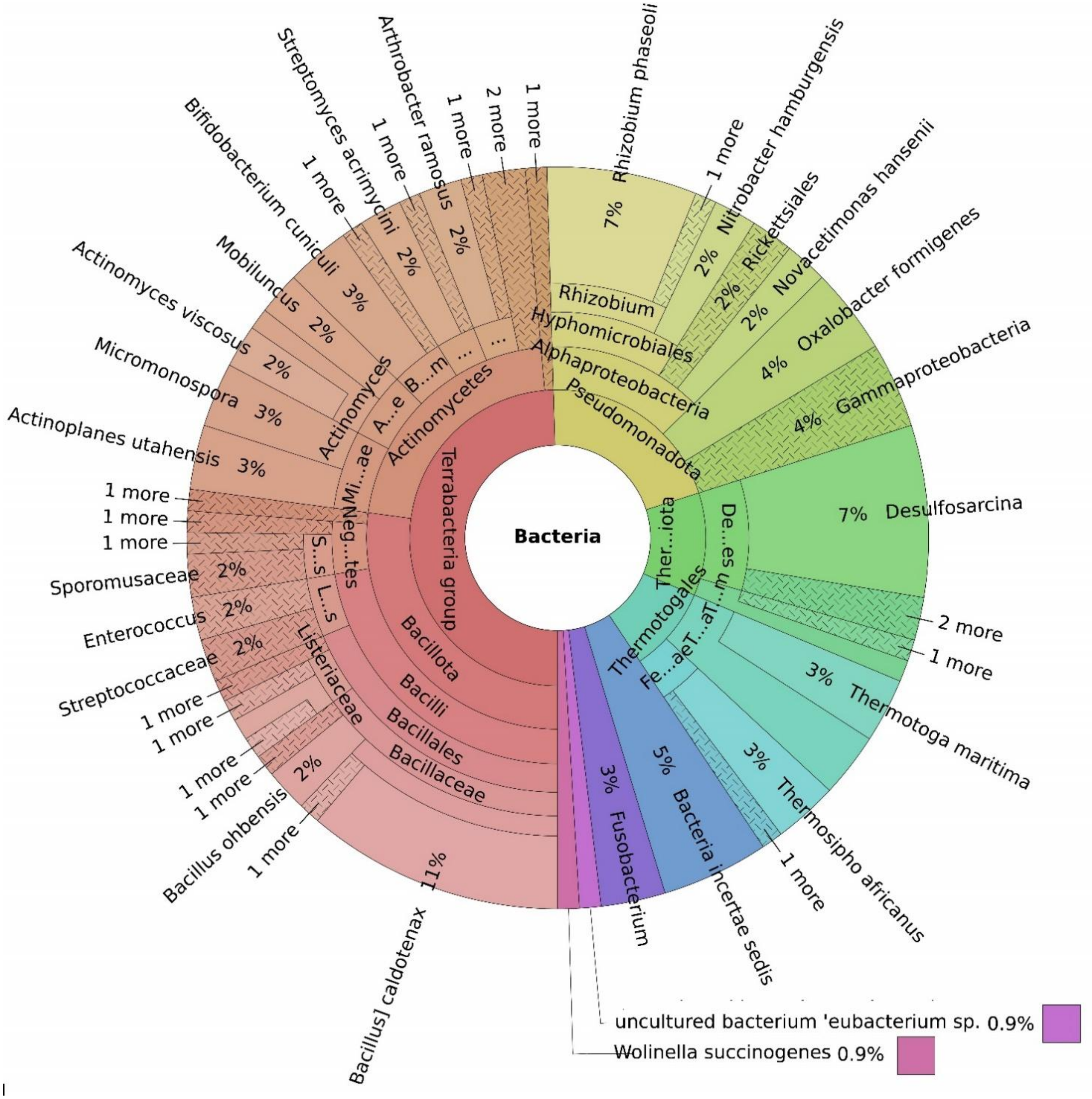

Figure 2 Kraken2 assigned taxonomic labels to 16S rRNA rhizomicrobiome of *Brassica nigra* using 250-mers against Silva database, representing superkindom Bacteria, accounting for 12% of Root and 152 bacterial genera.

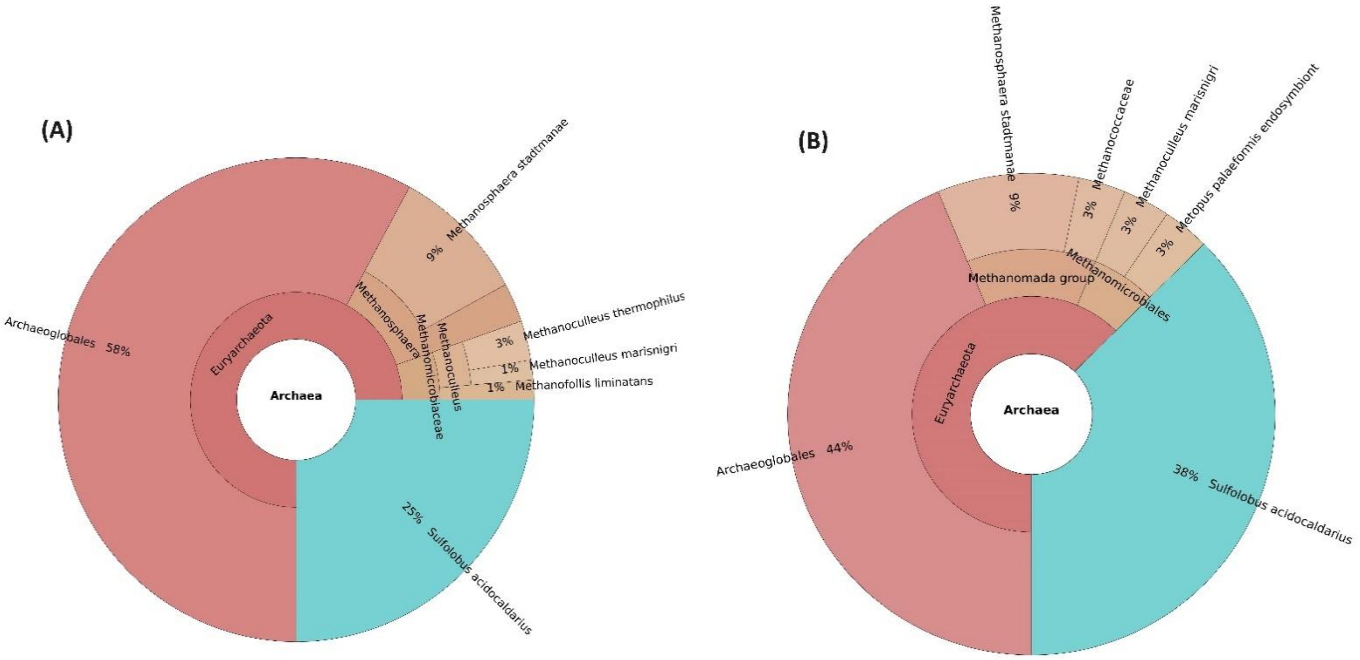

Figure 3 Kraken2 assigned taxonomy using 250-mers against Silva database representing superkindom Archaea, in 16S rRNA rhizomicrobiome of (A) *Zea mays* accounting for 3% of Root (B) *Brassica nigra* accounting for 4% of Root.

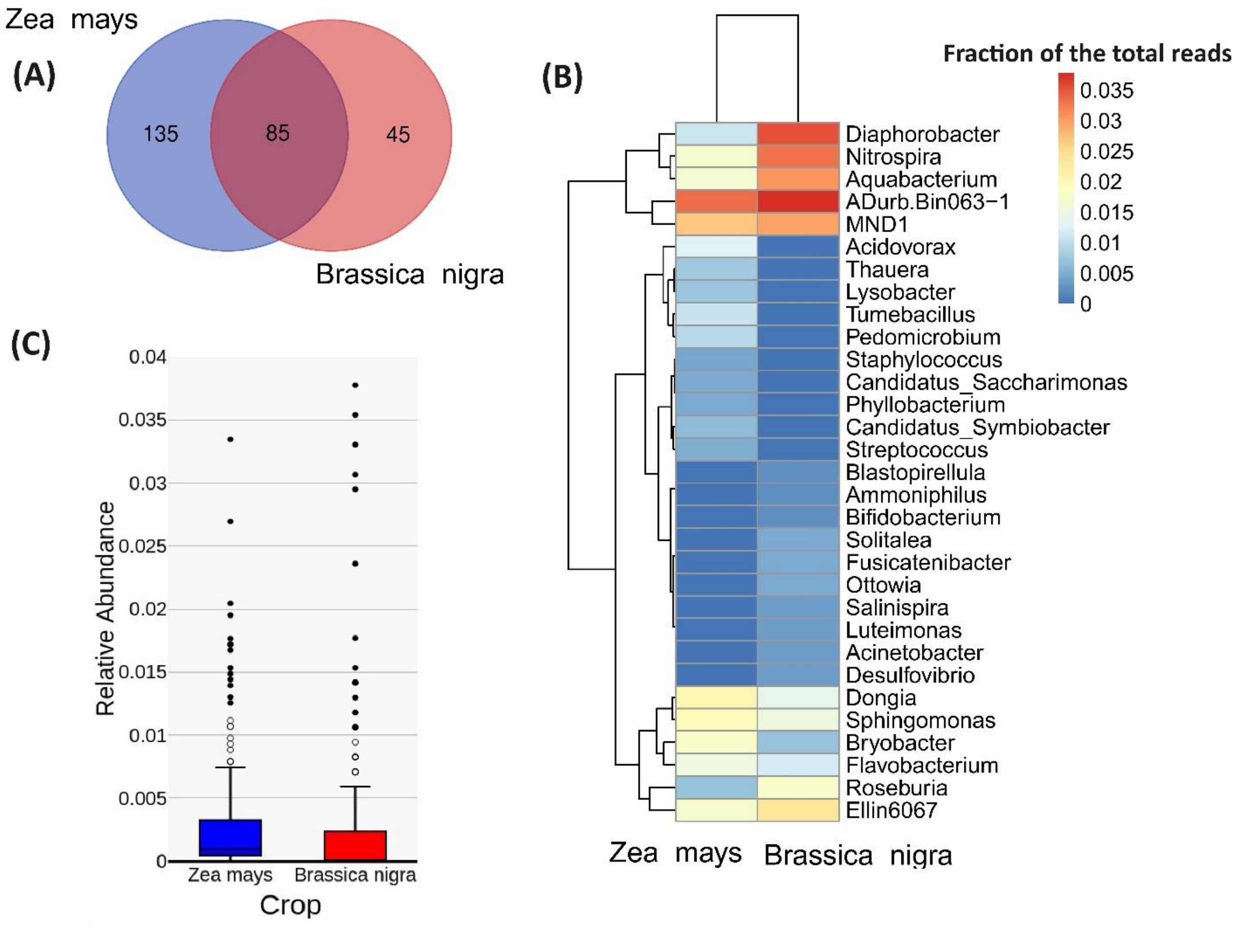

Figure 4 (A) Venn plot of specific and shared taxa at genera level in 16S rRNA rhizomicrobiome of *Zea mays* and *Brassica nigra,* (B) Euclidean distance based hierarchical clustering analysis of top 30 abundant bacterial genera from 16SrRNA metagenomes of *Zea mays* and *Brassica nigra* rhizospheres, (C) Box plot depicting significant correlation (R: 0.9736, p-value: 0, 95% CI: 1.0181,1.0781, t-Statistic: 68.7157) between *Zea mays* and *Brassica nigra*.

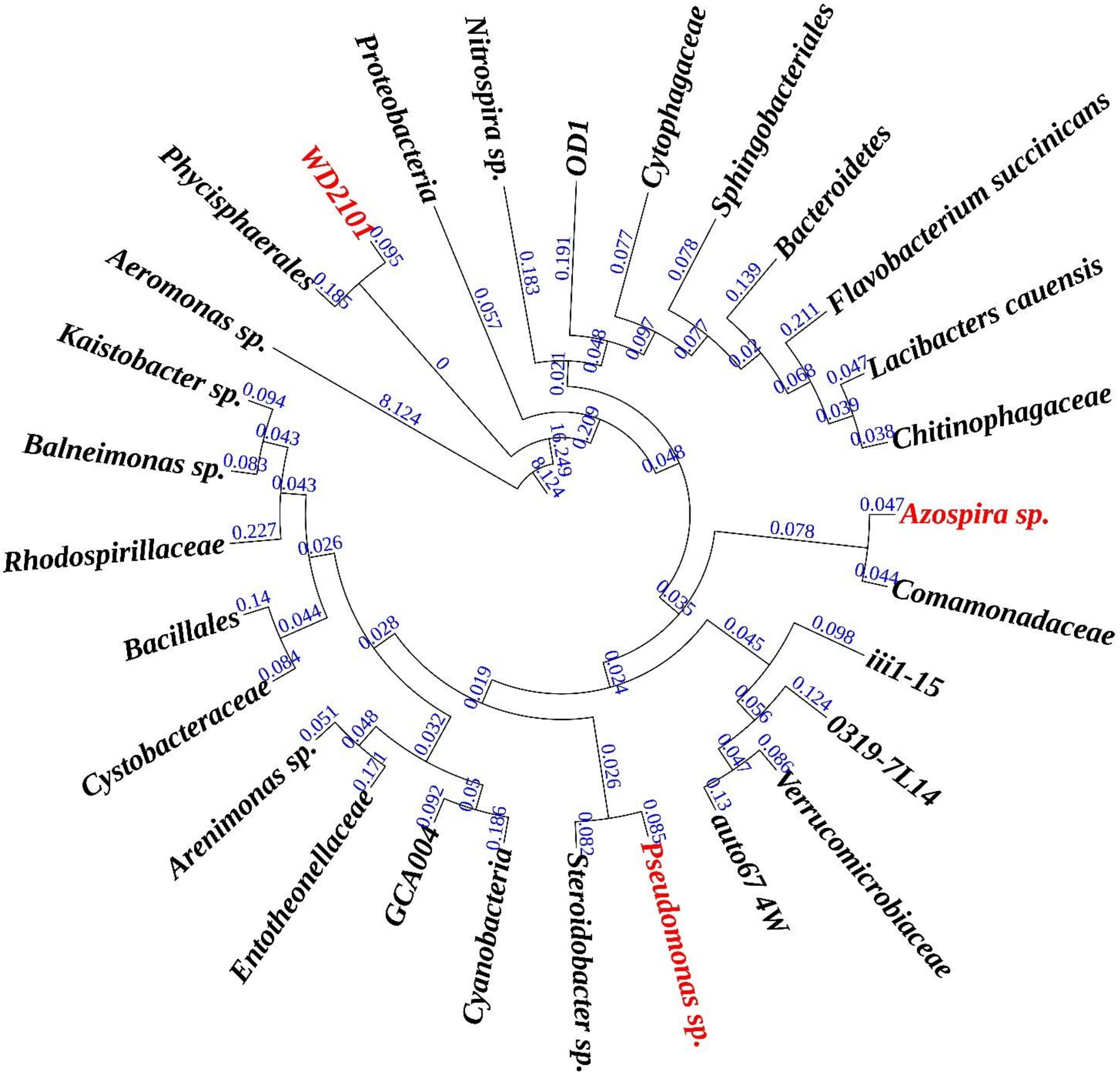

Figure 5 Phylogenetic tree representing the evolutionary relationship of bacterial 16S rRNA rhizomicrobiomes taxonomically classified using Greengenes database in *Zea mays* (in black) and *Brassica nigra* (in red) based on MAFFT alignment and neighbor-joining algorithm. The taxonomic identities have been indicated up to the assigned levels for the corresponding bacterial sequences.

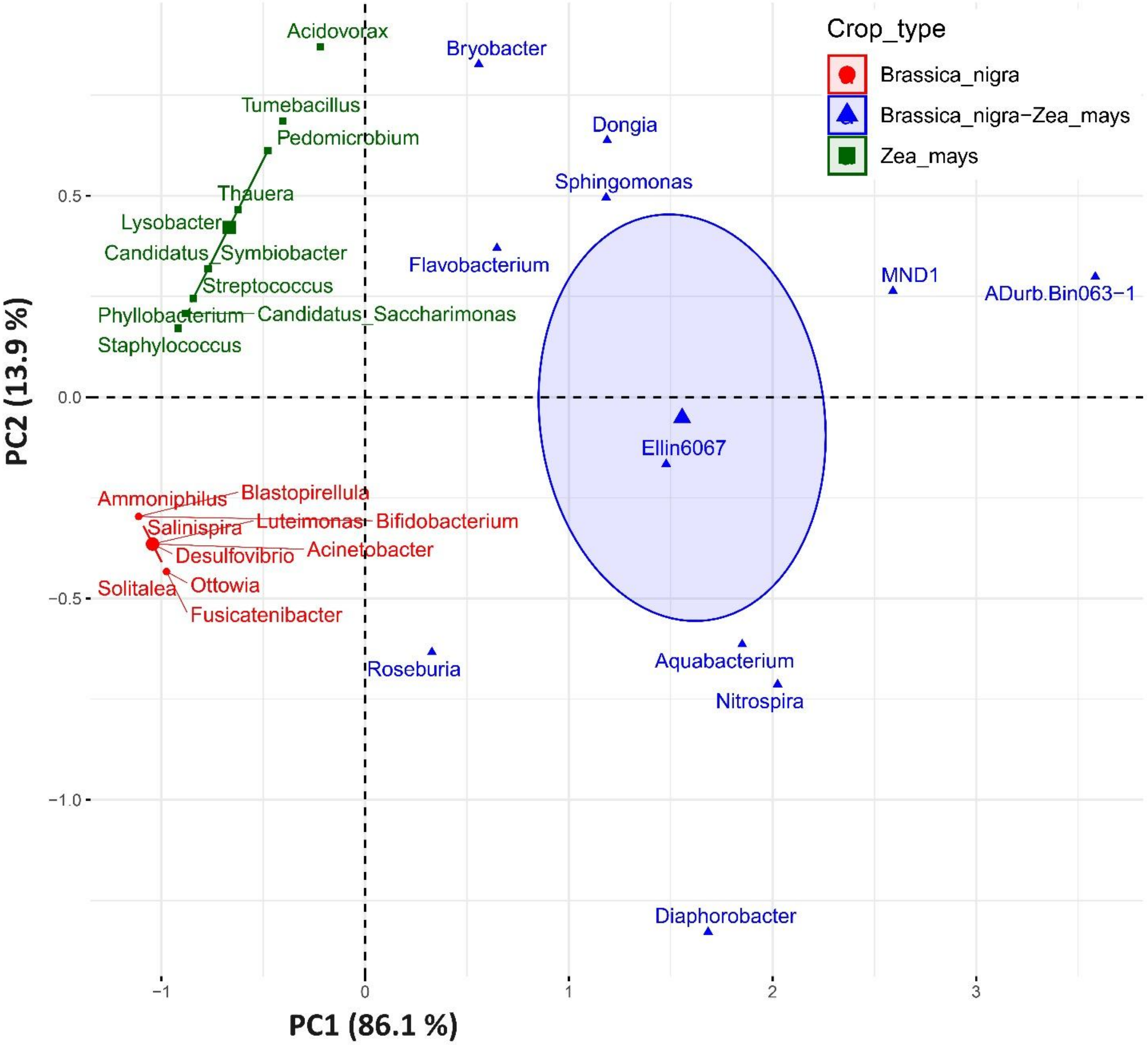

Figure 6 PCA of the genus level bacterial community structure in *Zea mays* and *Brassica nigra* rhizomicrobiomes.

The PCA score plot of the bacterial community structure showed 86.1 % and 13.9 % of the variance being explained by *Zea mays* and *Brassica nigra* rhizomicrobiomes, respectively (Figure 6). The relationships between all the bacterial taxa appearing in the two rhizomicrobiomes displayed distinct clustering of *Zea mays* and *Brassica nigra* rhizomicrobiomes where the correlated variables appeared contributing similar attribute (Figure 6). A single 95 % confidence ellipse was obtained under the shared category of the two metagenomes, where *Nitrosomonas* sp., *Ellin6067* sp., belonging to the *Nitrosomonadaceae* family, appeared as the most influential taxa.

Apart from this, several other representative genera of this family with putative taxa related to ammonia-oxidation appeared in the rhizosphere metagenome samples, including *MND1* sp., *Nitrosomonas* sp., *oc32* sp., and *mle1-7* sp. *Nitrosomonadaceae* abundance has been determined in the rhizosphere, which are mainly ammonia oxidizers with significant roles in the control of the nitrogen cycle (Prosser et al., 2014). Additionally, *Aquabacterium* sp. and *Diaphorobacter* sp. belonging to the *Burkholderiaceae* family co-occurred in *Zea mays* and *Brassica nigra* rhizomicrobiomes. The family *Burkholderiaceae* is a very heterogeneous group comprising soil bacteria, rhizobacteria, and some pathogens (Bothe et al., 2007). Some species of *Burkholderia* genus are associated with the bioremediation of recalcitrant xenobiotics Coenye, (2014*)*, nitrogen fixation, and nodulating leguminous plants in the rhizosphere of maize, rice, and coffee plants (Bothe et al., 2007).

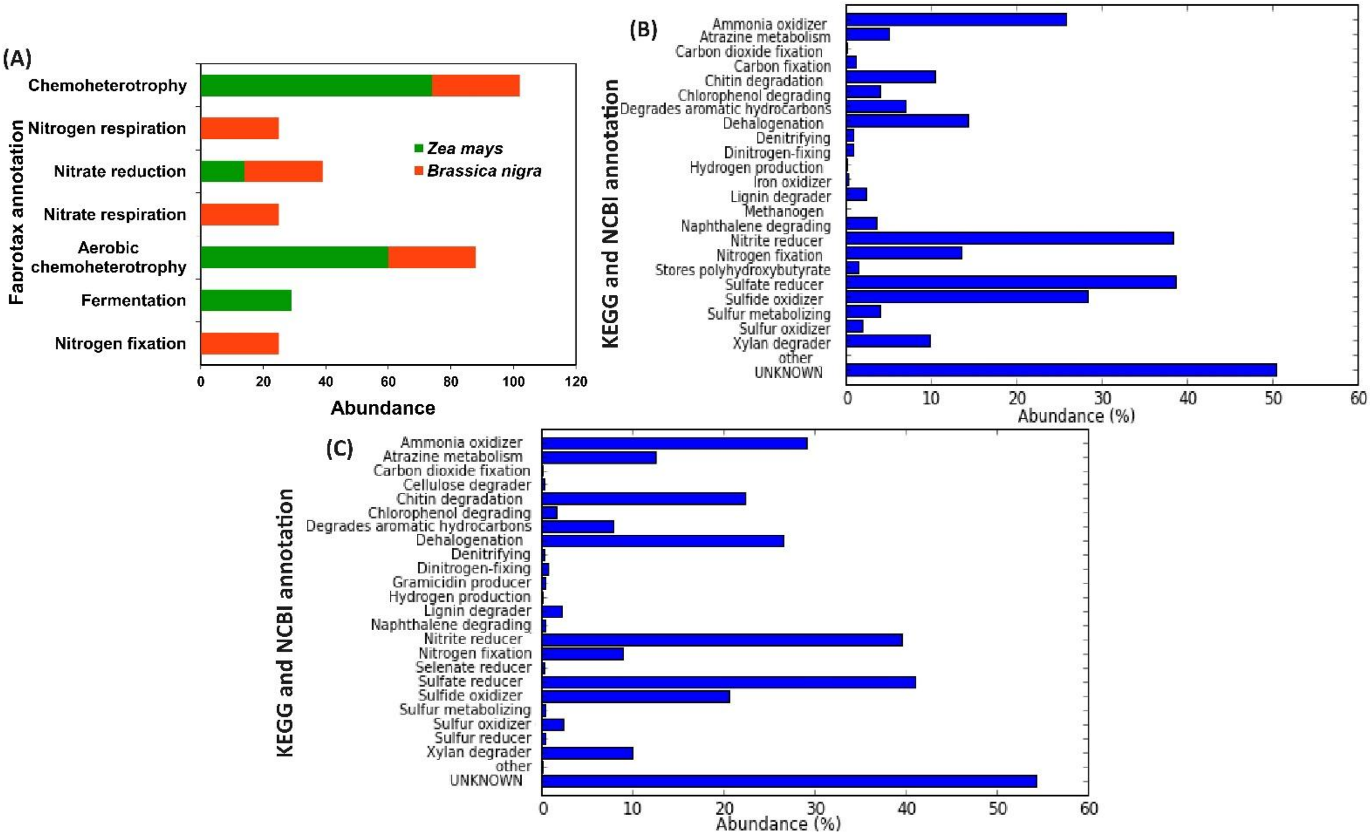

Figure 7 Functional interpretation of 16S rRNA marker gene in rhizomicrobiomes annotated by (A) Faprotax (B) KEGG and NCBI in *Zea mays* (C) KEGG and NCBI in *Brassica nigra*.

The metabolic and ecological interpretation of the 16S rRNA marker gene using Faprotax indicated an over-representation of putative functions related to the nitrogen cycle, including nitrogen fixation, nitrate respiration, and nitrogen respiration in the rhizosphere metagenome sample of *Brassica nigra*, while nitrate reduction function co-occurred in both the metagenomes with a higher incidence in *Brassica nigra* compared to *Zea mays* (Figure 7A). There was an insignificant difference between the functional features by Faprotax in *Zea mays* and *Brassica nigra* (p-value: 0.8542, 95% CI: −0.343,0.3989, t-Statistic: 0.1935). Functional annotation using KEGG and NCBI also showed enrichment of several functionalities connected to the biogeochemical cycle associated with nitrogen, such as nitrite reduction, ammonia oxidation, nitrogen fixation, dinitrogen fixation, and denitrification, in *Zea mays* and *Brassica nigra* (Figure 7B, 7C).

Annotation using CARD for the identification of the antimicrobial resistance gene analysis revealed aminocoumarin resistance in *Zea mays* (with 37.18% identity of the matching region of reference sequence) belonging to the type III ATP binding cassette antibiotic efflux pump (encoded by the gene clusters responsible for novobiocin synthesis) mediating novobiocin resistance. The MGE profiles observed in *Zea mays* and *Brassica nigra* displayed an insignificant negative relationship (R: −0.1869, p-value: 0.4725, 95% CI: −1.2741 to 0.6194, t-Statistic: −0.737). In PCA, the MGEs of *Zea mays* accounted for 59.3% of the variation. *Brassica nigra* explained 40.7% of the variation in the occurrence of MGEs (Figure 8). The PEX102, pFA3, and R144 were the topmost abundant plasmid shared between *Zea mays* and *Brassica nigra* genomes, while R65 and pBWH77 clustered inside the confidence ellipse (Figure 8, 9).

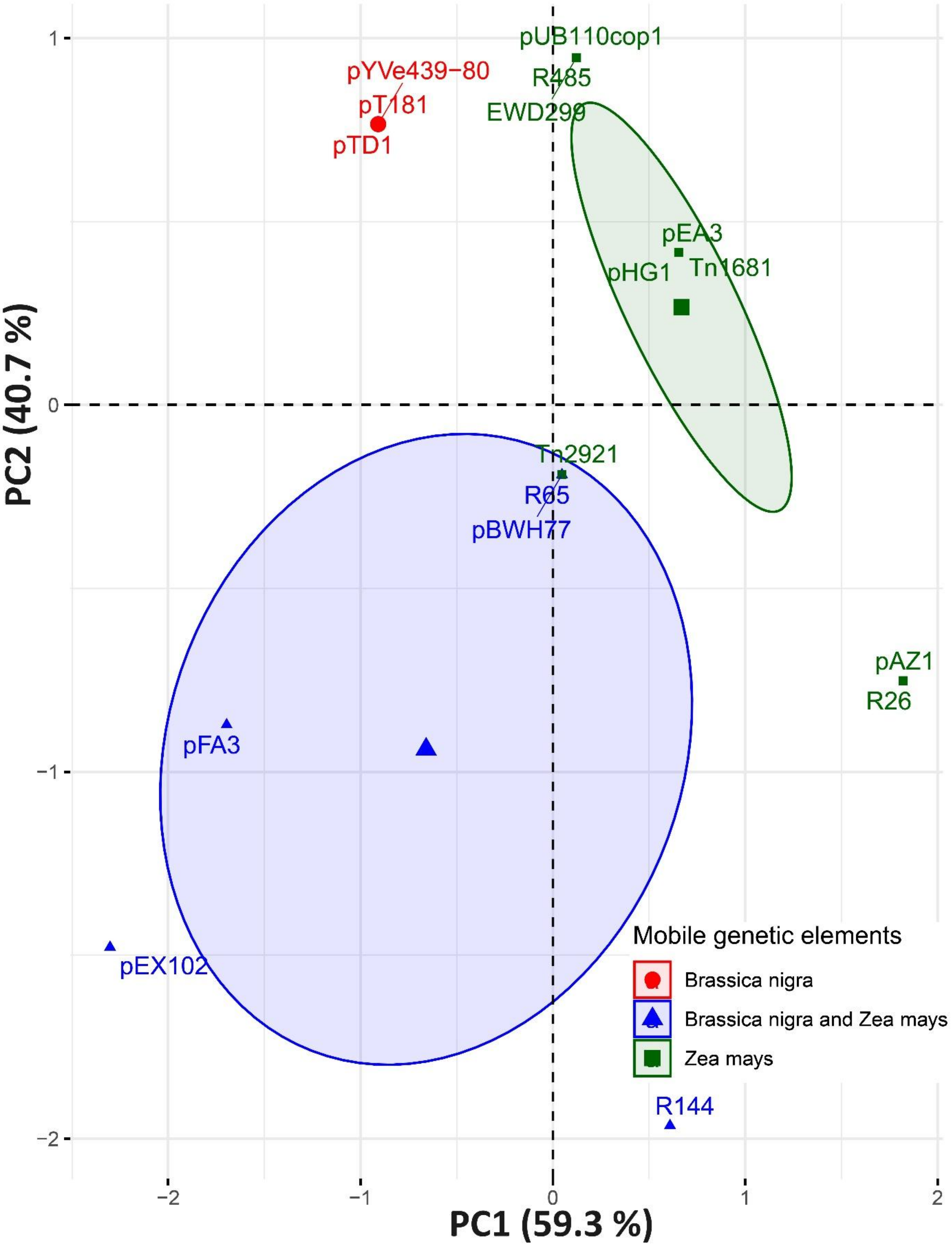

Figure 8 PCA of specific and shared mobile genetic elements among *Zea mays* and *Brassica nigra* rhizomicrobiomes.

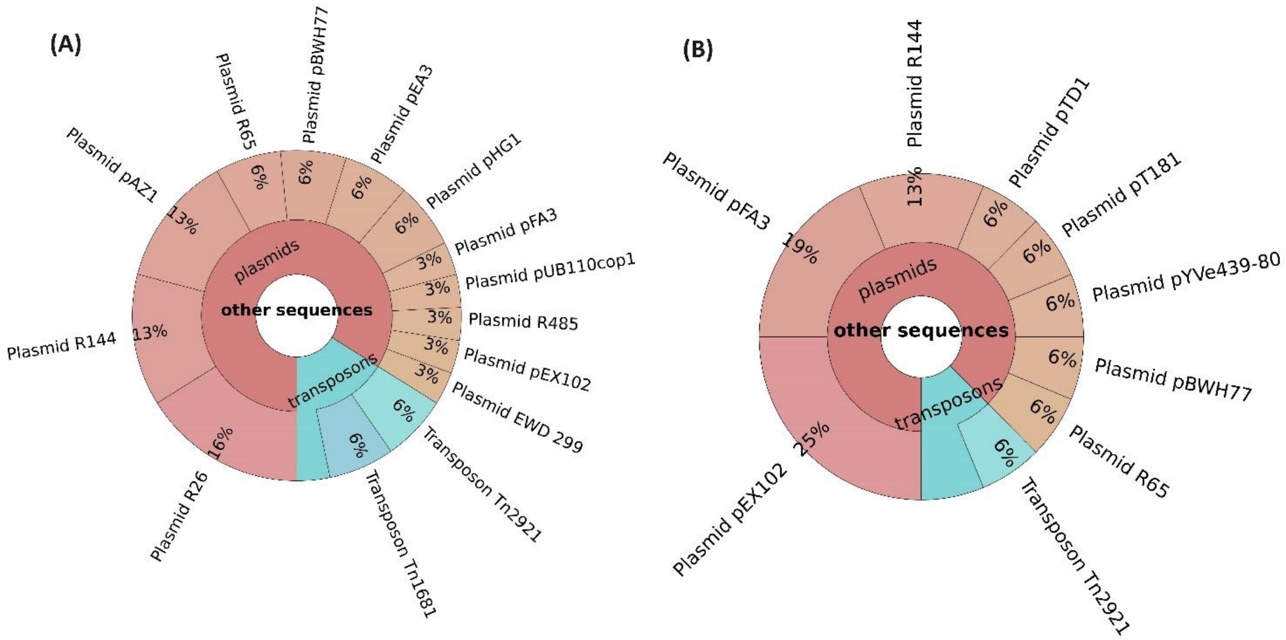

Figure 9 Abundance profile of Kraken2 assigned 250-mers mobile genetic elements (other sequences: NCBI taxon ID 28384) against Silva database in (A) *Zea mays* representing 1 % of Root (B) Brassica *nigra* representing 2 % of Root.

Another confidence ellipse included pEA3, pHG1 plasmids and Tn1681 transposon in *Zea mays*. MGEs, including plasmids and transposons, aid in transmitting of antibiotic resistance by encoding genes conferring resistance to several antibiotics. Transposons disseminate among the plasmids, among the chromosomes, or across the chromosomes to the plasmid, thus spreading antibiotic-resistance genes in bacteria (Foxman, 2012*)*. Plasmids as extrachromosomal autonomous genetic elements encode several properties, including antibiotic resistance and xenobiotic degradation (Foxman, 2012). The pFA3, pT181, R65, R26, pUB110cop1, pAZ1, R485 plasmids, and Tn2921 transposon detected in this study were related to conferring resistance to beta-lactamases, tetracycline, chloramphenicol, neomycin, trimethoprim, sulfonamide, and fosfomycin respectively, while some virulence-associated MGEs included pEX102, pTD1, pYVe439-80, EWD299, and Tn168. Nevertheless, the pEA3 plasmid expressed in *Zea mays* linked with nitrogen-fixation property is indicated in (Table 2).

Tukey pairwise multiple comparisons between bacterial taxa, MGE, and faprotax functional annotation displayed insignificant differences between taxonomical features comparing *Zea mays* with *Brassica nigra* (−0.8402,0.8404), presence of MGE and taxonomical features in *Zea mays* vs *Brassica nigra* (−2.3319,2.4824), and functional characteristics describing *Zea mays* and *Brassica nigra* (−2.141,8.141), where figures in parentheses represent 95% Tukeys Interval (Table 3). On the other hand, a comparison of the community structural, functional, and MGE abundances in both *Zea mays* and *Brassica nigra* showed significant differences between each other (Table 3).

Table 2 Abundance of MGE (mobile genetic elements) in *Zea mays* compared to *Brassica nigra* rhizomicrobiomes and their reference host bacteria with characteristic features.

MGE derived from Silva database	**Abundance (% of the bacterial taxonomic Root)**		**Features of mobile genetic elements**	**Reference**
	Zea mays	***Brassica nigra***		
Plasmids				
pEX102	0.04	0.4	Virulence-associated proteins encoded by *Salmonella typhimurium*	Hovi et al., 1988
pFA3	0.04	0.3	TEM beta-lactamase production in *Neisseria*	Gilbride and Brunton, 1990

			gonorrhoeae	
pTD1	0	0.1	Cryptic plasmid from *Treponema denticola*	MacDougall et al., 1992
pT181	0	0.1	*Staphylococcus aureus* plasmid, encoding inducible resistance to tetracycline	Khan and Novick, 1983
pYVe439-80	0	0.1	Virulence plasmid from *Yersinia enterocolitica* 439-80 (serogroup 9)	Laroche et al., 1984
pAZ1	0.2	0	Trimethoprim and sulfonamide resistance	Fling et al., 1988
pEA3	0.09	0	*Enterobacter agglomerans* containing structural nitrogen-fixation (nif) genes	Singh and Klingmüller, 1986
pHG1	0.09	0	*Ralstonia eutropha* H16 megaplasmid encoding H2-based lithoautotrophy and anaerobiosis	Schwartz et al., 2003
pUB110cop1	0.04	0	Neomycin resistance	Valero-Rello et al., 2017
EWD299	0.04	0	Heat-labile enterotoxin subunit A	Pronk et al., 1985
Transposons				
Tn2921	0.09	0.1	Fosfomycin resistance transposon	Seoane et al., 2010
Tn1681	0.09	0	Heat-stable (ST) toxin	So and McCarthy, 1980

Table 3 Tukey pairwise multiple comparisons between the taxonomical, functional and MGE features in *Brassica nigra* and *Zea mays* rhizomicrobiomes.

SN	**Comparison**	**Absolute value of sample mean difference**	**95% Tukeys Interval**	**Difference (level of significance, alpha=0.05)**
1	μTaxa_BIS−μTaxa_ZMS	0.0001	(−0.8402,0.8404)	Not Significant
2	μMGE_ZMS−μTaxa_ZMS	0.0752	(−2.3319,2.4824)	Not Significant
3	μMGE_ZMS−μTaxa_BIS	0.0753	(−2.3318,2.4825)	Not Significant
4	μMGE_BIS−μTaxa_ZMS	0.0846	(−2.3225,2.4918)	Not Significant
5	μMGE_BIS−μTaxa_BIS	0.0848	(−2.3224,2.4919)	Not Significant
6	μMGE_BIS−μMGE_ZMS	0.0094	(−3.2895,3.3083)	Not Significant
7	μfaprotax_ZMS−μTaxa_ZMS	25.2821	(21.5987,28.9656)	Significant
8	μfaprotax_ZMS−μTaxa_BIS	25.2822	(21.5988,28.9657)	Significant
9	μfaprotax_ZMS−μMGE_ZMS	25.2069	(20.8876,29.5262)	Significant
10	μfaprotax_ZMS−μMGE_BIS	25.1975	(20.8782,29.5168)	Significant
11	μfaprotax_BIS−μTaxa_ZMS	22.2821	(18.5987,25.9656)	Significant
12	μfaprotax_BIS−μTaxa_BIS	22.2822	(18.5988,25.9657)	Significant
13	μfaprotax_BIS−μMGE_ZMS	22.2069	(17.8876,26.5262)	Significant
14	μfaprotax_BIS−μMGE_BIS	22.1975	(17.8782,26.5168)	Significant
15	μfaprotax_BIS−μfaprotax_ZMS	3	(−2.141,8.141)	Not Significant

μ: Sample mean; Taxa: Taxonomical feature; MGE: Mobile genetic element features; faprotax: Functional features by faprotax annotations; BIS: *Brassica nigra*; ZMS: *Zea mays*.

4. CONCLUSION

Overall, the current study revealed that the rhizomicrobiomes of *Zea mays* and *Brassica nigra* shared taxonomic and metabolic profiles related to interactions beneficial to plant growth, including the nitrogen cycle. Additionally, we detected the enrichment of plasmid-mediated nitrogen fixation and antibiotic-resistance in the rhizomicrobiome of *Zea mays*. Also, several antibiotic-resistance and virulence-associated MGEs were abundant in both crops, particularly in *Zea mays*. Thus, it is essential to gain an insight into the structural and functional diversity of the rhizomicrobiome. Moreover, this is crucial for identifying the advantageous aspect of plant-microbe interactions, and for monitoring the drug resistance in rhizomicrobiome.

Author's contribution

MM (Manisha Mandal) analyzed, interpreted the data, and wrote the manuscript. BG (Biswajit Ghosh) collected the samples for soil analysis and metagenomic sequencing. SM (Shyamapada Mandal) designed the study, analyzed, interpreted, discussed, wrote and edited the manuscript.

Informed consent

Not applicable.

Ethical approval

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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