# Different Soil Salinity Imparts Clear Alteration In Rhizospheric Bacterial Community Dynamics In Rice And Peanut.

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**Research Article**

**Keywords:** Microbial ecology, rhizosphere, microbial community, metagenome, environmental factor, soil salinity

**Posted Date:** June 25th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-646600/v1

# Abstract

The rhizospheric microbiome is capable of changing the physio-chemical properties of its own micro-environment and found to be indispensable in overall health of the host-plant. The interplay between the rhizospheric environment and the microbiota residing therein tune the physiology of the associated plant. In this study, we have determined how the soil properties and the host-plant remains as an important parameter for microbial community-dynamics in the rhizosphere of rice and peanut. In addition to check the physio-chemical parameters of the rhizospheric soil, we have also prepared the metagenomic DNA from each rhizospheric soil followed by high-throughput sequencing and sequence-analysis to predict the OTUs that represents the community structure. The alpha-diversity of the bacterial community in the RRN sample was highest, while the lowest was in PRS sample. Actinobacteria is the most predominant phylum in PRN, PRS and RRN whereas Acidobacteria in RRS. We found a clear shift in bacterial community over the rice and peanut rhizosphere and also over these host-rhizospheres from normal and high saline region. The rhizospheric bacterial community composition found to be affected by the close-by environmental factors. Thus, the rhizospheric bacterial community-structure is related to both the adjoining soil characters and the type of the hosts.


# Introduction

The root-adjoining soil where the plant has much higher influence through secretion of their different root-exudates, mucilage and sloughed-cells is the rhizospheric region. The phytochemicals secreted in the rhizosphere are important for the establishment of the microbial community around it[1]. Rhizospheric region of the plant is a complicated and heterogeneous environment which dictates the type of allowed microbes therein. Molecular studies particularly the metagenomics suggest that the soil is the habitat of highest biodiversity which approximated as 1,000 Gbp of microbial genome sequence in each gram of soil[2]. The advantageous role of these rhizospheric microbes can be demonstrated through their direct role in plant-growth promotion, plant protection from phytopathogen and stress management of the plants[3]. By virtue of having various microbes, the rhizosphere remains as the chemical factory where a complex physical, chemical and biochemical interaction are operating which in turn influence the nutrient-cycling, nitrogen-fixation, phosphate mobilization and solubilization, nutrient uptake, water uptake, production of plant growth regulators, seed germination and early plant growth promotion, development in soil structure and competing with plant pathogens[4]. Thus, the diversity of microorganism plays fundamental role in maintenance of soil-fertility and nutrient-cycling. Mostly such rhizospheric microbiome is having beneficial effect on plant, however in few cases the rhizospheric community might have representatives which are either neutral or detrimental for the host plant.

The plant root exudates chose the type of microbes it will attract or repel from the rhizospheric region, but this is not the only parameter. The physiochemical composition of the soil remains as another important criterion for building the rhizospheric community. Thus, it is in general the reciprocal action of the plant root and the soil which establishes a particular type of microbiota. The physiochemical characteristics of

the soil are ever-changing mostly by various physical, chemical and biological means. The soil microbial community is influenced by crop rotation, use of fertilizers and tillage which in turn change the soil physiochemical parameters[5]. The compounds present in root-exudates influence the rhizospheric microbial community structure and is different at each developmental stage[6]. The microbial community-assisted nitrogen, phosphorous and other nutrients cycle are more rapid and dynamic in rhizospheric soil compared to the bulk soil[4]. Management of agriculture build up soil physicochemical properties which control microbial community composition and nutrient cycling. Furthermore, the microbial diversity and heterogeneity in bulk soil are increased by organic fertilizer[7]. Bacterial community compositions are different in organically-managed agricultural system than the conventional system[8]. Simultaneous analysis showed that the ecological interaction regulating structure, function and potential resilience of soil microbial communities [9,10]. Plant root systems are strong operator of microbial community congregation that build rhizosphere communities and are taxonomically and functionally different from bulk soil microbial community [11,12]. The rhizosphere effect and plant selection are evident in observation of microbiomes across different field-environment[13]. Agricultural management and plant relation with microbial community structure establish taxonomy to network structure. Rhizosphere network often smaller, less densely connected and less complex than bulk soil microbial community[4].

The abundance, diversity and composition of bacteria in the rice rhizosphere have been widely investigated. Bacterial population in rice rhizosphere were double to those of bulk soil[13,14]. The structures of bacterial communities in the rice rhizosphere are diverse and dynamic which are related to soil type, geographical location and rice genotype[15]. Metagenomic studies have indicated that the bacterial communities in rice rhizosphere are broadly inhabited by Proteobacteria, mainly Alpha-, Beta- and Delta-proteobacteria classes, Acidobacteria, Actinobacteria and Chloroflexi phyla[16]. Alpha-, Beta-proteobacteria classes are abundant in rice rhizosphere and essential for ecosystem functioning[17].

In this study, we have chosen the rhizospheric sample of peanut (dicotyledon) and rice (monocotyledon) from different geographical locations (Fig. 1) in order to analyze the microbiota present therein. Here we have undertaken characterization of rhizospheric soil of rice and peanut, grown in different salinity condition, using targeted 16SrRNA genes through a metagenomic approach. We further analyzed the sequence of 16SrRNA variable region and highlighted the following prediction: the microbial community is notably different in rice rhizosphere grown in normal soil (RRN) than that of the saline soil growing rice (RRS) and rhizosphere of peanut grown in normal soil (PRN) over that of saline soil (PRS), and also from monocotyledons to dicotyledon grown in normal and saline soil growing.

# Results

### Physical and chemical characteristics of the rhizospheric soil

The physical and chemical characteristics of all the rhizospheric soil were analysed in order to corelate with the rhizospheric microbiota (Table 1). pH analysis of the soil collected from the PRS and RRS

showed 7.91 ± 0.01 and 7.84 ± 0.02, respectively, whereas soil sample from PRN and RRN showed 6.48 ± 0.04 and 6.75 ± 0.03. Thus, the PRS and RRS soil sample has been found to be slight alkaline but near neutral in case of PRN and RRN. PRS and RRS showed significantly higher electrical conductivity (EC) than RRN and PRN. The organic carbon and organic matter present in these rhizospheric soils were found to be in a range between 0.64 to 2.79%. The salinity of PRS and RRS ranging between 1.5–1.9 mg/L and was found to be higher than PRN and RRN which were 0.2–0.3 mg/L. The rhizospheric soil samples showed higher silt and clay texture (34.19–38.25%) compared to the fine and coarse sand (5.83–15.84%). The metal analyses of the soil samples indicated prevalence of Fe and Al. The prevalence Cd in these sample was found to be the least. All the soil samples have shown presence of micro and macro nutrient (Cd, Co, Cr, Cu, Ni, Pb and Zn) which in general facilitated the growth of the microorganism.

Table 1
Characteristics of different rhizosphere soils and its elemental composition.

| Soil characteristic | RRN | RRS | PRN | PRS |
|---|---|---|---|---|
| pH | 6.75 ± 0.03 | 7.84 ± 0.02 | 6.48 ± 0.04 | 7.91 ± 0.01 |
| EC (µS/cm) | 118.2 ± 9.39 | 311 ± 10.21 | 163.5 ± 2.26 | 315 ± 11.56 |
| Organic C (%) | 1.62 ± 0.12 | 1.04 ± 0.21 | 0.64 ± 0.23 | 1.15 ± 0.39 |
| Organic matter (%) | 2.79 ± 0.19 | 1.79 ± 0.11 | 1.1 ± 0.08 | 1.65 ± 0.16 |
| Salinity (mg/L) | 0.3 ± 0.02 | 1.5 ± 0.27 | 0.2 ± 0.09 | 1.9 ± 0.14 |
| *Soil texture* | | | | |
| Coarse Sand (%) | 5.83 ± 0.03 | 10.84 ± 0.01 | 8.69 ± 0.02 | 11.35 ± 0.01 |
| Fine Sand (%) | 12.25 ± 0.05 | 15.25 ± 0.02 | 15.84 ± 0.06 | 15.32 ± 0.07 |
| Silt (%) | 38.25 ± 0.01 | 35.63 ± 0.03 | 34.19 ± 0.02 | 34.5 ± 0.01 |
| Clay (%) | 43.67 ± 0.26 | 38.28 ± 0.12 | 41.28 ± 0.59 | 38.83 ± 0.42 |
| Soil profile | silt clay loam | clay loam | clay loam | clay loam |
| *Metal concentrations (mg/kg)* | | | | |
| Al | 23241.66 ± 1259.23 | 47520.12 ± 2156.12 | 12146.21 ± 1429.12 | 59235.39 ± 1782.50 |
| Cd | 0.12 ± 0.02 | 0.094 ± 0.02 | 0.19 ± 0.03 | 0.089 ± 0.01 |
| Co | 10.16 ± 0.65 | 13.22 ± 0.87 | 11.56 ± 0.94 | 17.67 ± 0.45 |
| Cr | 1.23 ± 0.03 | 64.25 ± 3.39 | 2.39 ± 0.02 | 72.26 ± 4.20 |
| Cu | 83.18 ± 0.85 | 78.52 ± 4.22 | 91.23 ± 0.45 | 70.98 ± 5.12 |
| Fe | 4201.55 ± 1654.24 | 55012.87 ± 1278.24 | 10231 ± 985.76 | 40029.46 ± 3254.95 |
| Mn | 332.23 ± 20.25 | 590.24 ± 39.56 | 410.23 ± 12.22 | 502.45 ± 56.23 |
| Ni | 17.16 ± 5.75 | 54.22 ± 2.21 | 10.32 ± 1.97 | 69.67 ± 4.23 |
| Pb | 7.13 ± 2.93 | 15.67 ± 4.19 | 2.25 ± 1.89 | 13.23 ± 2.11 |
| Zn | 82 ± 3.87 | 70.13 ± 4.26 | 75.29 ± 5.76 | 59.53 ± 3.26 |
| Ca | 3.23 ± 2.31 | 1.62 ± 0.32 | 5.22 ± 1.56 | 2.12 ± 1.33 |
| Na | 7.79 ± 2.89 | 4.23 ± 1.12 | 9.13 ± 1.45 | 2.21 ± 1.45 |
| Mg | 12.43 ± 5.67 | 3.02 ± 0.25 | 18.27 ± 2.58 | 10.12 ± 1.29 |

| Soil characteristic | RRN | RRS | PRN | PRS |
|---|---|---|---|---|
| K | 2.56 ± 1.95 | 0.152 ± 0.03 | 3.25 ± 1.56 | 0.75 ± 0.06 |

### Estimation of culturable bacteria present in rhizospheric soils

The 0.1 gm suspension of RRN plated on LA and ISP-2 media which upon estimation turned out to be $6x10^6$ and $1.8x10^4$ bacteria, respectively. Likewise, the RRS, PRN and PRS showed $4.8x10^6$ and $2.7x10^4$, $2.6x10^6$ and $1.5x10^5$ $3.5x10^6$ and $2.1x10^4$ cells, respectively (Supplementary Table S1). The number of bacteria appeared higher on LA than on ISP-2, as ISP-2 is generally supposed to be specific for isolation of actinobacteria.

### General characteristics of the amplicons and sequencing data

Four amplicon samples from Illumina Miseq sequencing analysis of four different salinity regions were successfully sequenced (Table 2). The V3 region of PRN, PRS, RRN and RRS samples produced 1934241, 677182, 1840904 and 671840 pair-end sequences, respectively. After quality filtering of these sequences, the obtained reads of PRN, PRS, RRN and RRS were 1891967, 668518, 1805567 and 653697, respectively with an average length range of 250 to 300 bp. Sequences were clustered into rarefaction curves of operational taxonomic units (OTUs) of four different samples of PRN, PRS, RRN and RRS with 7353, 7172, 9038 and 6391, respectively based on ≥ 97% similarity. The Shannon index of PRN, PRS, RRN and RRS were 8.05, 7.86, 9.23 and 7.88, respectively. The Shannon index was higher in rhizospheric soil of both monocot and dicot growing in normal saline soil with a high diversity. Simpson index also indicates the alpha-diversity and its increase is directly proportional to the magnitude of the Simpson indices. Simpson index of PRN, PRS, RRN and RRS were 0.984, 0.974, 0.993 and 0.983, respectively. Chou1 indices, which indicates specie-richness, are low in sample with higher salinity. Chou 1 of PRN, PRS, RRN and RRS were 7995.66, 7646.33, 9246.82 and 7174.52, respectively (Table 2). Beta-diversity indices show random distribution of bacterial population in these rhizospheres.

**Table 2:** Raw data summary of the Illumina Miseq sequences of the V3 region of 16S DNA of microbial communities in different hosts in different seasons.

| Samples | 16SrDNA amplicon reads | | | No. of OTU | Chou1 index | Shannon (H) index | Simpson (D) index |
|---|---|---|---|---|---|---|---|
| | Total | Filtered | | | | | |
| | | total | percentage | | | | |
| PRN | 1934241 | 1891967 | 97.8% | 7353 | 7995.66 | 8.05 | 0.984 |
| RRN | 1840904 | 1805567 | 98.0% | 9038 | 9246.82 | 9.23 | 0.993 |
| RRS | 671840 | 653697 | 97.29% | 6391 | 7174.52 | 7.88 | 0.983 |
| PRS | 677182 | 668518 | 98.72% | 7172 | 7646.33 | 7.86 | 0.974 |

## Microbial taxonomic analysis at the phylum and class level

Classification of the high-quality sequences also demonstrated differences in the bacterial communities among the different samples at the phylum, class, family and genus level (Fig. 2). A total of 29 phyla were identified in all samples out of which 7 phyla showed dominance in their relative abundance (> 1% of relative abundance in at least one sample) ($p$ value < 0.05) (Fig. 2a). Actinobacteria was the most dominant phylum (> 20% relative abundance) across all samples, accounting for 20.4–59.23%. Acidobacteria, Chloroflexi, Cyanobacteria, Fermicutes, Gemmatimonadetes and Proteobacteria were the subdominant phyla with > 1% relative abundance in at least one sample (Supplementary Table S2). The other 22 phyla had much lower abundances (less than 1% relative abundance in all samples).

A total of 85 bacterial classes were identified across all samples. There were twelve classes with a relative abundance of higher than 1% in at least one sample ($p$ value < 0.05) (Fig. 2b). Among these twelve classes, the significantly dominant class (> 20% relative abundance) was Actinobacteria with 20.31% – 59.01% of the total high-quality sequences. The class of Acidobacteria was also dominant in RRS sample with 35.99% relative abundance. The subdominant classes were Thermoleophilia, Anaerolineae, Chloroflexia, Bacilli, Clostridia, Gemmatimonadetes, Alpha-proteobacteria, Beta-proteobacteria, Delta-proteobacteria, Gamma-proteobacteria with relative abundance of greater than 1% in at least one sample (Supplementary Table S3). In the sample of PRN, Alpha-proteobacteria, Bacilli, Clostridia, Gemmatimonadates, Beta-proteobacteria, Delta-proteobacteria, Gamma-proteobacteria, Acidobacteria were dominant with relative abundance ranging from 1–16%. The class Proteobacteria was co-dominantly inhabited with Actinobacteria in the sample of PRN with 16.26% relative abundance. In the sample of PRS Acidobacteria, Anaerolineae, Clostridia, Alpha-proteobacteria were codominant with relative abundance of greater than 1%. Actinobacteria was also dominant in PRS sample like PRN with 48.64% relative abundance. Next to Actinobacteria, Anaerolineae was dominant in the sample of PRS with 19.96% relative abundance. Relative abundance of Alpha-proteobacteria was drastically reduced from 16–4% in PRS sample than the PRN. In the sample of RRN Alpha-proteiobacteria was also codominant with 8.54% relative abundance next to Actinobacteria. Only class Gemmatimonadetes was lesser than 1% relative abundance (0.06%) in RRN. In RRS, the relative abundance of Acidobacteria was dominant over others classes with 35.99% relative abundance. RRS was the only sample where Actinobacteria (20.31%) was not a dominant taxon. Class Anerolineae and Alpha-proteobacteria were co-dominantly present in RRS with relative abundance of 6.27% and 8.04%, respectively. Comparative relative abundance among four samples was also represented by heatmap analysis ($p$ value < 0.05) (Fig. 3). Phylum Acidobacteria was dominated in sample RRS. Actinobacteria was dominated in the low salt rhizospheric region of samples in both PRN and RRN. Phylum Chloroflexi was dominated in PRS (Fig. 3a). Relative abundance of rhizospheric bacteria at the class level also reflected the same abundance like phylum. The class Acidobacteria was also dominant in RRS. The class actinobacteria were also showed the dominant relative abundance in both PRN and RRN. Alpha-proteobacteria, Beta-protobacteria, Delta-protobacteria and Gamma-protobacteria were also dominant in normal salt soil of both PRN and RRN (Fig. 3b). This dynamic behaviour of bacteria, at phylum and class level in rhizospheric soils of

monocot/dicot growing in different saline-soil, was very important to maintain the plant growth promotion.

## Microbial taxonomic analysis at the family and genus level

The top 20 classified families with greater than 1% relative abundance atleast in one sample, was represented in a bar diagram (Fig. 2c, Supplementary Table S4). The distributions of the families differed greatly across the samples (p value < 0.05). Actinomycetales, Pseudonocardiaceae and Sphingomonadaceae were the dominant families in PRN among which Pseudonocardiaceae was the most dominant family (17.511 % relative abundance). In the sample PRS, Anaerolineaceae and Nocardioidaceae were the dominant families with 19.97% and 33.99% relative abundance, respectively. Actinomycetales and Acidobacteria was the most dominant families in the RRN and RRS, respectively. At family level bacterial dynamic community structures were significant. Total 35 genera were selected by the greater than 1% relative abundance in atleast one samples, for comparative abundance studies (Fig. 2d, Supplementary Table S5). Actinopolysporaceae, Bacteroidetes, Hydrogenedens, Nitriliruptor, Pseudonocardiaceae, Sphingopyxis were dominant in PRN. Whereas in the sample PRS Actinopolysporaceae, Bacteroidetes, Bellilinea, Longilinea, Nocardioides, Nocardioides were the enriched one. Actinopolysporaceae group of genera was the most dominant bacteria in the sample PRN. *Nocardioides* was the enriched bacteria in the sample PRS. *Actinocorallia*, Actinomycetales, Actinopolysporaceae, Bacteroidetes, *Hydrogenedens*, *Nitriliruptor*, *Nocardioides*, Streptomycetaceae were the most dominant genus present in the sample of RRN. Actinopolysporaceae, *Koribacter* and *Telmatobacter* were the most dominant in RRS sample. *Telmatobacter* was the most dominant genus with 25.285% relative abundance in the RRS. Comparative relative abundance of family and genus level was also represented by heatmap (p value < 0.05) (Fig. 3c, d). The family Acidobacteria was dominant in rice rhizosphere growing in saline soil. Actinomycetales was also dominant in PRN, RRN than the high-salt growing rice peanut rhizosphere PRS and RRS.

## Comparative fold-shift of families and genera between low and high-salt rhizosphere

We have tried to highlight the dominance of different bacterial group present in the rhizosphere of both the host growing in normal and saline soil between samples in phylum, class, family and genus-level (Fig. 4, 5, Supplementary Fig. S1, S2). We have also compared the relative abundance of bacterial families in peanut and rice rhizosphere. It is striking to note that there are many taxa which has considerable increase or decrease in relative abundance over the variation in plant and the soil they grow. In order to highlight the shift in community dynamics in family-level, we have estimated the fold increase or decrease within eight major families associated with each of the sample. The family Xanthomonadaceae has 387.46-fold higher abundance in PRN than PRS, whereas Anaerolineaceae, Chloroflexi, Nocardioidaceae are dominant in PRS than in PRN (Fig. 4a, b). Family Anaerolinaceae has 44.14-fold higher abundance in PRS over PRN (Supplementary Table S6). Likewise, the abundance of Chloroflexi, Nocardioidaceae, Solirubrobacteraceae, Streptomycetaceae, Thermoleophilaceae, Thermomonosporaceae families in RRN are much higher than in RRS (Fig. 4c). Thermoleophilaceae has

18.62-fold higher abundance in RRN than in RRS. Acetobacter and Gematimonadaceae have the highest fold increase in their abundance in RRS over RRN (Fig. 4d, Supplementary Table S6).

Similarly, we found that the genus *Actinocorallia*, Hydrogenedens, *Kaistia*, *Kibdelosporangium*, Koribacter, *Marmoricola*, *Nakamurella*, *Nitriliruptor*, *Plantactinospora*, *Prauserella*, Pseudonocardiaceae, Solirubrobacterales, *Sphingomonas*, *Sphingopyxis*, *Stella*, Xanthomonadales are many folds higher abundant in PRN over PRS (Fig. 5a, Supplementary Table S7). Relative abundance of Xanthomonadales is higher in the PRN rhizospheric soil than in the PRS. Xanthomonadales found to have the highest fold increase (710.89). *Bellilinea*, Chloroflexaceae, *Longilinea*, *Nocardioides*, *Nocardiopsis*, *Pelolinea* are shown to have a dominant fold increase in PRS than that of the PRN (Fig. 5b). *Longilinea* has the highest fold increase in abundance in PRS sample. *Actinocorallia*, Aminicenantes, Chloroflexaceae, *Marmoricola*, *Nocardioides*, *Nocardiopsis*, *Plantactinospora*, *Prauserella*, Solirubrobacterales, Streptomycetaceae, *Thermoleophilum*, were found to have higher fold increase in RRN over RRS (Fig. 5c). *Hydrogenedens*, *Koribacter*, *Paludibaculum*, *Telmatobacter* are having a higher fold increase in RRS than the same of RRN (Fig. 5d, Supplementary Table S7). We have further confirmed the bacterial community dynamics among tested sample in phylum level through circus plot as well as venn diagram. Both these analyses not only confirm the above results but also clarify the shift in abundance of certain bacterial taxa. The circus plot represents the switching in number of OTUs in phylum-level (Fig. 6a) and family level (Supplementary Fig. 3) among low and high salt grown peanut and rice rhizosphere. The same pattern has also been found when a total of 61 abundant genera present in all samples were plotted in a venn diagram (Fig. 6b). There were 8.6% of the total genera present in all rhizosphere while 20%, 5.7%, 22.9% and 5.7% genera were found exclusively in PRN, PRS, RRN and RRS, respectively (Fig. 6b). The details distribution of major genera present in all kind of rhizosphere has been mentioned in Fig. 6b.

## Analysis of the relationship between environmental factors and the rhizospheric microbial community

A beta-diversity analysis based on PCoA plot was performed to compare the bacterial compositions among the four different samples ($p$ value $< 0.05$). PRN and RRN were clustered into same group. Although PRS and RRS were situated distantly (Fig. 7). Canonical correspondence analysis (CCA) was used to established the relationship between the environmental factors and the bacterial phylum ($p$ value $< 0.05$) (Fig. 8). CCA plot was carried out using $OUT_S$ data together with environmental factors (pH, EC, salinity, coarse sand, Al, Cd, Cr, Fe, Ni. Pb and K), which might influence the bacterial community structure, to highlight relative abundance of different phyla among these samples. According to Tukey's post-hoc test (999 permutations) the significant relationship between environmental variables and canonical axes were analyzed by using the paleontological statistics package version 3.01 (PAST Software 1). Based on Tukey's post-hoc test, the bacterial phylum was significantly linked ($p \leq 0.05$) to the rhizosphere environmental factors (Fig. 8). Factors corresponding to the differences in the bacterial phylum were pH, EC, salinity of the sample, and presence of coarse sand, Al, Cd, Cr, Fe, Ni. Pb and K along with the first axis CCA1 explaining 69.2% and the second axis CCA2 (28.57%) of the variation (Fig. 8). The plot obtained by CCA visualizing the different habitat preferences of the phyla. The pH, EC, salinity, coarse sand, Al, Cr, Fe, Ni. Pb were the most important environmental factors to influence the rhizosphere bacterial phylum

abundance, and were positively correlated with CCA1 axis (Table 3). CCA2 axis had a positive correlation with pH, EC, salinity, coarse sand, Al, Cr, Fe, Ni. Pb but was negatively correlated with Cd and K.

Table 3
Correlation matrix of phylum, sampling sites and environment factors with CCA axis.

| **Phylum** | **Axis 1** | **Axis 2** | **Axis 3** |
|---|---|---|---|
| Acidobacteria | 2.48559 | 0.060397 | -0.23246 |
| Actinobacteria | -0.51468 | -0.09792 | -0.34334 |
| Chloroflexi | -0.22366 | 2.3638 | 1.08999 |
| Cyanobacteria | -0.94508 | 1.99688 | -0.85971 |
| Firmicutes | -0.03565 | -0.87308 | 0.076458 |
| Gemmatimonadetes | 0.0104 | -2.70731 | 6.84472 |
| Proteobacteria | -0.15786 | -1.11075 | -0.1061 |
| *Sampling sites* | | | |
| RRN | -0.31307 | -0.03629 | -0.15341 |
| RRS | 1.00534 | 0.022587 | -0.00477 |
| PRN | -0.25591 | -0.44394 | 0.086508 |
| PRS | -0.3098 | 0.527683 | 0.07222 |
| *Environmental factors* | | | |
| pH | 0.517672 | 0.820354 | 0.226273 |
| EC | 0.545552 | 0.665969 | 0.514376 |
| Salinity | 0.391096 | 0.851018 | 0.357954 |
| Coarse Sand | 0.443493 | 0.506949 | 0.748845 |
| Al | 0.344927 | 0.913177 | 0.212671 |
| Cd | -0.38441 | -0.86764 | 0.256688 |
| Cr | 0.490065 | 0.782779 | 0.386734 |
| Fe | 0.753271 | 0.545006 | 0.3578 |
| Ni | 0.360087 | 0.879326 | 0.31856 |
| Pb | 0.646596 | 0.746688 | -0.01185 |
| K | -0.6742 | -0.71621 | -0.12223 |

# Discussion

Rhizosphere is the region which is biologically active and enriched with microbes. The chemical, biological and physical characteristics of this area have great significance on the plant roots. We noticed that rhizosphere region has significant effect on soil physico-chemical properties and rhizospheric bacterial dynamicity and diversity. Previous study shown that soil bacterial communities have great contribution in nutrient cycling. Carbon metabolites secreted by plant roots promote growth and activities of microbes inhabited in the soil surrounding the roots[14]. Parallelly rhizosphere microbes benefit plants by supplying nutrient and growth stimulating hormones phytopathogen and enhancing tolerance to environmental stress[1]. Physical and chemical properties indicate the health of the agricultural soil. Soil collected from the RRS and PRS indicates slight alkaline in nature due to prevalence of the soil belt in the intertidal region of Sundarbans, India, but the soil samples from RRN and PRN are neutral. The experimental results are in good agreement with previously published results observed by different researcher [18,19,20]. The high conductivity, organic carbon content and fair distribution of the metals like Ca, Na, Mg and K for the entire soil sample gives us insight into the availability of the essential nutrient in the rhizosphere environment which helps in proliferation of microbial population in this area[19]. The salinity of the PRS and RRS was found to be higher than PRN and RRN, which indicates the presence of the soluble salt which essentially increases the conductivity of the soil. Clay content for all the soil was found to be higher with respect to the coarse sand, fine sand and silt that in turn might also provide better condition for the microbial growth in the rhizospheric region[21].

The distribution of the metal content in the soil considerably varies as per the sampling locations. Few factors like- a) soil characteristics like grain size of the soil, organic carbon content, Mn or Fe oxygenated hydroxides, chelating agent as well as ligands[22]; b) substrate variation due to natural weathering of the soil[23]; and c) discharge of pollutions and anthropogenic pressure determines the distribution of the elements in the soil[24]. The gradient of distribution of elements indicates presence of high concentration of Fe and Al which generally occurs due to natural weathering or due to presence of basaltic and lateritic rocks present in the soil[25]. The enrich prevalence of heavy metals like Cd, Co, Cr, Cu, Ni, Pb and Zn might occur due to natural factors or anthropogenic pressure like uneven use of pesticides, insecticides, and chemical fertilizers[18]. The level of K might occur due to presence of granodiorites and granites in the soil[18]. High occurrence of Fe might occur due to hydrological fluctuation e.g., alternate period of wet and dry season in the crop cultivated agricultural land[26].

In our study, we have found total 29 phyla, 85 classes, 315 families and 639 genera from the tested samples (Supplementary Table S8-S11). The total OTUs of four different samples of PRN, PRS, RRN and RRS are 7353, 7172, 9038 and 6391, respectively. Rhizospheric bacterial alpha-diversity were higher in both monocot and dicot grown in normal soil than the saline soil. Beta-diversity shows random distribution of bacterial population in all the four samples. We have identified total 29 phyla in all four samples. Among them Actinobacteria, Acidobacteria, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadetes, and Proteobacteria were higher in abundance than other 22 phylum. Previous

research has shown that Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes and Firmicutes were dominant phyla in the rhizosphere soil of *Arabidopsis* and cotton[27,28]. Total 85 bacterial classes were identified across four samples. Actinobacteria, Acidobacteria, Acidobacteria, Thermoleophilia, Anaerolineae, Chloroflexia, Bacilli, Clostridia, Gemmatimonadetes, Alpha-proteobacteria, Betaproteobacteria, Deltaproteobacteria, Gamm-aproteobacteria were the higher relative abundance classes in four sampling sites. Different species of Actinobacteria were responsible for recycling of nutrient in a great extent of rhizosphere soil[29,17,30] and this Actinobacteria is the most abundant phylum in the rhizospheric soil PRN, PRS and RRN. However, the rhizospheric soil of RRS showed Acidobacteria as most abundant but they have quite high abundance of Actinobacteria as well. Previous study showed that Acidobacteria could grow in environment which is nutrient less and also showing higher abundance in poor soil[31]. Acidobacteria and Actinobacteria were found to be abundant in disease-suppressive soils and reported to be responsible to suppress disease-causing microbes and trigger enhancement of beneficial microbes that have potential to promote crop health[32]. Pseudonocardiaceae is the most dominant families in the sample PRN whereas Nocardioidaceae (33.99%) in PRS relative abundance. Actinomycetales is the most dominant in the RRN but Acidobacteria in the RRS sample. The relative abundances of the bacterial communities at the genus level in four rhizospheric soil were significantly different. Although Actinopolysporaceae group of genera was the most dominant bacteria in the sample PRN. *Nocardioides* was the enriched bacteria in the sample PRS. *Telmatobactor* was the most dominant genus in the sample RRS. Proteobacteria, Acidobacteria, Actinobacteria, Firmicutes and Planctomycetes were most dominant bacteria among all bacterial community found in the rice rhizosphere soil[30]. The Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi and Firmicutes are dominant taxonomic group in most of the soil samples. Actinobacteria and proteobacteria has also been reported dominant taxonomic group found in rhizospheres soil of Maize[26]. Decomposition of organic matter are performed by the Chloroflexi. PGPR activity like siderophore production, IAA production, nitrogen fixation and prevention of different phytopathogen are fascinated by the group of Firmicutes and Proteobacteria[33,34]

Analysis of the rhizospheric microbiome of peanut grown in normal and saline soil delineates that the Acetobacteraceae, Bacillaceae, Gaiellaceae, Gemmatimonadaceae, Micromonosporaceae, Mycobacteriaceae, Pseudonocardiaceae, Solirubrobacteraceae, Sphingomonadaceae, Thermomonosporaceae, Xanthomonadaceae are the most dominant family in PRN. And among these, Xanthomonadaceae shows higher fold shift in PRN than PRS whereas Anaerolineaceae, Chloroflexi, Nocardioidaceae are found to be the dominant family in PRS than in PRN. The genus of *Actinocorallia*, Hydrogenedens, *Kaistia*, *Kibdelosporangium*, Koribacter, *Marmoricola*, *Nakamurella*, *Nitriliruptor*, *Plantactinospora*, *Prauserella*, Pseudonocardiaceae, Solirubrobacterales, *Sphingomonas*, *Sphingopyxis*, *Stella*, Xanthomonadales were higher abundant in normal soil in respect to saline soil, as well as *Bellilinea*, Chloroflexaceae, *Longilinea*, *Nocardioides*, *Nocardiopsis*, *Pelolinea* were dominant genus in saline soil in compare to normal soil. Similarly, rhizospheric microbiome of rice between normal and saline soil condition, the families Chloroflexi, Nocardioidaceae, Solirubrobacteraceae, Streptomycetaceae, Thermoleophilaceae, Thermomonosporaceae were dominant fold increase values in normal rhizospheric soil than the saline condition, whereas Acetobacter and Gematimonadaceae were the highest fold

increase in saline rhizosphere soil compare to normal soil. The genus *Actinocorallia*, Aminicenantes, Chloroflexaceae, *Marmoricola*, *Nocardioides*, *Nocardiopsis*, *Plantactinospora*, *Prauserella*, Solirubrobacterales, Streptomycetaceae, *Thermoleophilum*, were the higher fold increase in normal rhizosphere than saline soil. *Hydrogenedens*, *Koribacter*, *Paludibaculum*, *Telmatobacter* were showed a higher fold increase in saline rhizosphere than the normal soil. Several studies reported Sphingomonadaceae, Chitinophagaceae, Nocardioidaceae, Solibacteraceae, Bacillaceace, Cytophagaceae and Methylobacteriaceae were predominant families (> 2% relative abundance) of *T. aestivum* L rhizosphere region and different bacterial genera like *Sphingomonas*, *Microvirga*, *Bacillus*, *Nocardioides*, *Marmoricola*, *Bryobacter*, *Flavisolibacter* were the dominant (> 1% relative abundance)[35,36]. Whereas *Bacillus nealsonii*, Rhodospirillales_ bacterium_WX36 and *Bacillus niacini* were prominent (> 0.5% relative abundance) bacterial species[36]. *Sphingomonas*, *Kaistia*, Xanthomonadales were unique genus belong to phylum Proteobacteria and *Nakamurella*, *Plantactinospora*, *Thermomonospora*, from Actinobacteria; genus *Stella* from Bacteroidetes present only in the rhizosphere of PRN, whereas genus *Pelolinea*, *Longilinea* belong to phylum Chloroflexi were found only in PRS. It was interesting that phylum Chloroflexi has been found only in the rhizosphere of PRS. Similarly, the genus *Thermoleophilum*, *Prausere*, Solirubrobacterales, *Rhodococcus*, *Marmoricola* from phylum Actinobacteria; *Methyloceanibacter*, *Rhodospirillales* from Proteobacteria and Aminicenantes has been found distinctively only in the rhizosphere RRN, whereas genus *Paludibaculum*, Koribacter from Acidobacteria were found only in the rhizosphere of RRS.

The influence of different environmental factors describing the microbial community is like the finding of beta diversity analysis due to differences of rhizospheric community to the monocot and dicot types (Fig. 7). Factors corresponding to the differences in the bacterial communities were pH, EC, Salinity, Coarse Sand, Al, Cd, Cr, Fe, Ni. Pb and K along with the first axis explaining 69.2% and the second axis (28.57%) of the variation (Fig. 8). CCA plot shows that pH, EC, Salinity, Coarse Sand, Al, Cr, Fe, Ni. Pb were the most important environmental factors to significantly influence the rhizosphere bacterial phylum abundance (Fig. 8). Previous study has shown bacterial community structure were influenced by pH, Cr, Sb, As, Zn and moisture content, but pH was the most dominant factor[37].

The clustering of PCoA and CCA analysis signify PRN and RRN were clustered into the same group, while PRS and RRS were situated distantly. The CCA result in our study indicate pH, EC, Salinity, Coarse Sand, Al, Cr, Fe, Ni. Pb is positively correlated with rhizospheric community structure. Thus, pH, EC, Salinity, Coarse Sand, Al, Cr, Fe, Ni. Pb appeared to be an important factor that influence microbial communities and dynamics.

# Conclusion

In summary, this study illustrated the rhizospheric bacterial community of rice and peanut growing in normal soil and saline soil of West Bengal, India. Along with comment on the relative abundance of bacterial taxa present in different rhizospheric soil of peanut and rice, we also highlighted the shift in community dynamics in phylum, class, family and genus level. The normal rhizospheric soil in both

monocot and dicot shows more diverse compare to saline rhizospheric soil in monocot and dicot. Alpha-diversity like Shannon Index was higher in both monocot and dicot normal rhizospheric soil with high diversity in respect to saline rhizospheric soil. Once again it has been proved that the rice being a monocot and the peanut being a dicot has their own choice to contain preferable bacterial representative around their roots. The decreased abundance and variation of bacterial population in the rice and peanut rhizosphere of high saline zone might be one of the reasons behind the significant reduction in the vigor and yield of the crop cultivated in high salt containing region. Lastly the shift in community dynamics has been shown as a factor of soil parameters as well as the choice of the associated plants.

# Methods

### Site description

The study was performed with samples of rhizospheric region associated with peanut and rice plant grown in normal and high saline soil condition collected from places of West Bengal, India (Fig. 1). Geographical location of the sample collection sites is Galsi (23°19'48.00"N, 87°42'.00"E) for rice rhzospheric soil with low salinity area (RRN). This is central plain areas of the district Burdwan of West Bengal, sometimes encounter heavy floods during rainy seasons. Next sample collection site is Egra (21°53'58.09"N, 87°32'16.58"E) for the peanut rhizospheric soil with low salinity area (PRN). Third and fourth sites are Gosaba (22°09'36.00"N, 88°47'60.00"E) both for rice and peanut rhizospheric soil with high salinity (RRS and PRS, respectively). This is one of the main deltaic islands in the Sundarban region.

### Sampling

Four samples of the peanut and rice rhizosphere having different soil-salinity were collected in May 2017. The owner of the all four sampling sites granted all necessary permits to access. On spot, surface soils around the plants were cleared of debris and unwanted pebbles and the soil sample were collected to an 8–10 cm depth from the surface on soil using auger with 10 cm diameter around the roots. Roots are gently shaken to remove the non-rhizospheric soil. The rhizospheric soil samples were collected using brush. Each of the soil sample that is free of debris kept into a labeled zipper storage bag in cool place for further analysis.

### Analysis of the physio-chemical parameters of the sample

Soil sample were air-dried and grounded. Soil pH and electrical conductivity were measured with Thermo Fisher Scientific Orion STAR A329 multi parameter, following sparks et al., (1996)[38]. Organic carbon and organic matter of the soil samples were estimated by rapid titrimetric method[39]. Salinity of the soil samples were measured by Mohr's method, often called the argentometric method[40]. Soil texture analysis were measured following ASTM standard method. The air-dried soil samples were ground finely with an agate mortar and pestle. The ground soil sample was digested with the help of di-acid digestion process. 400 mg of soil samples were digested with 8 ml of 1:1 $HNO_3$: HCl (EMSURE grade) in a Teflon vessel for 1 hour (modified USEPA 3051) at 200ºC. After cooling, the soil samples were diluted to 100 ml in a cleaned

volumetric flask using ultrapure water (18.2 ms $cm^{-1}$) and the solutes were filtered through Axiva 0.2 µ PTFE filter paper and stored at 4ºC in 50 ml polypropylene tubes. Then samples were analyzed for metal using an inductive–coupled plasma optical emission spectrometer (ICP-OES) (Thermo Fisher Scientific, Model iCAP 7000 Series). The plastic and glassware used during the experiment were washed thoroughly with 10% v/v $HNO_3$ acid solution. There after a series of washing was done using Mili-Q water and finally it was air dried. Merck standard solution IV and IX was used during the analysis. Coefficient of correlation for the calibrated curve was observed to be in between 0.9990–0.9996. The quality of the data generated in ICP-OES was assessed using standard reference material (Estuarine Sediment-SRM 1646a) purchased from NIST (National Institute of Standard and Technology). Accuracy of the of the analysis in the form percentage recovery ranging between 96.12–104.59 % was observed for all the metals, the precision of < 5% relative standard deviation was also obtained for all the metals. Concentration of metals was expressed in the form of mg/kg.

### Estimation culture dependent bacteria from the soil samples

In order to find the approximate number of culturable bacteria present in the soil sample, 1 gm of each of the PRN, PRS, RRN and RRS samples were homogenized in 10 ml of 0.9% NaCl solution. Two types of agar media, Luria agar or LA (Tryptone, 10 gm/litre; yeast extract, 5 gm/litre; NaCl, 10 gm/litre; and agar, 15 gm/litre in 1000 ml of MiliQ-grade water) and International Streptomyces Project-2 or ISP-2 (malt extract, 10 gm/litre; yeast extract, 4 gm/litre; dextrose, 4 gm/litre; and agar, 20 gm/litre in1000 ml of MiliQ-grade water) were used to get the total bacterial cfu/gm (colony forming unit/microliter) soil sample. The media pH was maintained between 7–8. Cycloheximide (50 µg/ml) was added in the media used as the anti-fungal agent to avoid the undesired growth of the fungi. This soil-suspensions were then serially diluted and 100 µl from different selected dilutions from each sample were then used as inoculum and spread onto LA and ISP-2 agar plates. LA plates were used for estimating the cfu of all general types of bacteria; while, the ISP-2 media were used for the estimation of cfu of actinobacteria. The LA and ISP-2 agar plates were then incubated at 37ºC and 30ºC for 2 and 5 days, respectively. Colonies were then counted to determine the cfu/ml from each sample.

### Metagenomic DNA extraction, 16SrDNA amplicon library generation and sequencing

Metagenomic DNA preparation, 16SrDNA library generation and Illumina MiSeq sequencing were done by AgriGenome Labs, India. Total genomic DNA of four different rhizospheric soil were prepared separately using the DNeasy Power Soil Kit (Qiagen, USA) following manufacturer's protocol. Concentration and purity of DNA were determined by Nanodrop 2000c spectrophotometer and 1% agarose gel electrophoresis. After DNA extraction, the total DNA of the rhizospheric samples was used as the template, and the bacterial V3 region-specific primers. (V3 Forward primer CCTACGGGNBGCASCAG and V3 Reverse primer GACTACNVGGGTATCTAATCC). 5 ng of amplified product was used for library preparation using NEBNext Ultra DNA library preparation kit. The library quantification and quality estimation were done in Agilent 2200 TapeStation. The prepared library was sequenced in Illumina MiSeq 2500 with 2*150 cycles chemistry.

### Sequence analysis

Paired-end FASTA sequences of four soil samples were analyzed using the standalone installation of QIIME 1.9.1[41] and USEARCH (v.11.0) on Linux platform (Ubuntu 20.04)[42]. Initially the raw sequences (in fastq format) were visualized using FastQC version 0.11.9[43]. PCR primer sequences, of V3 region of the respective samples were removed from the sequencing data using cutadapt (v.1.8.3)[44]. Sequence data in FastQ format was quality trimmed using sickle (v.1.33) in paired-end mode with default settings[45]. Forward and reverse read were merged into a single amplicon read using fastq-join allowing fragments with a length of 200–250 bp for V3[46]. A minimum Phred score of 38 were used for downstream analysis. The entire cleaned sequence data was concatenated into a single file. Minimum 2 nucleotides similarity parameter was set up for OTU selection. Duplicated and chimeric sequences were checked by USEARCH (v.11.0)[42]. Processed sequences were used for OTU picking with USEARCH using 97% sequence similarity against the silvagold database by the UCLUST algorithm (v.1.2.21)[42]. The OTUs were taxonomically annotated using the RDP classifier using RDP database (v 11.5)[47]. Taxon-by-sample abundance tables were created for all taxonomic levels from phylum to genus, as well as for OTUs. QIIME was used to determine the alpha diversity and rarefaction curve that respectively represents community diversity (Inverse Simpson and Shannon) and species richness (observed OTUs and Chao1). Beta diversity was calculated by weighted UniFrac distance matrix[46] and was visualized using the Principal Coordinate Analysis (PCoA) plot. To assess the stability of the PCoA plot was performed on the OTU table. Based on the relative abundance of genera from each sample Venny 2.1 software was used to construct the venn diagram[48] whereas the Circos-0.67-7 software has been used to construct the Circos diagram[49]. Associations among relative abundances of phylum in each sample and measured environmental factors (pH, EC, Salinity, Coarse Sand, Al, Cd, Cr, Fe, Ni. Pb and K) were determined using canonical correspondence analysis (CCA)[50].

### Statistical analysis

Statistical analyses were performed using the Paleontological Statistics package version 3.01 (PAST software1). All the data were subjected to one way ANOVA analysis followed by Tukey's post-hoc test. Differences were considered significant if $p \leq 0.05$ with multiple comparisons using 999 permutations.

### Data deposition

Raw reads of four samples were deposited in fastq format to the National Center for Biotechnology Information. Sequences were deposited under Bioproject PRJNA660114. The accession number of PRN, PRS, RRS, RRN were SAMN15920910, SAMN15920911, SAMN15920912 and SAMN15920913, respectively (Following links https://www.ncbi.nlm.nih.gov/biosample/15920910).

# Declarations

**Acknowledgements:** We are grateful to UGC UPE Phase II, University of Calcutta, for partial financial support used for this research. MI thankfully acknowledge the financial support in form of fellowship

(UGC Ref No-712/CSIR-UGC NET DEC.2017) by the University Grant Commission, New Delhi, India. BS is thankful to University of Calcutta for his fellowship.

**Authors' contributions:** MI, RB, BS, PKM, ShM designed and performed all the experiment and prepared the tables and figures; SM conceptualized the research, designed the experiments. SM, PC, SRB analysed and discussed the data. All the authors wrote and edited the main manuscript.

**Competing interests:** The authors declare no competing interests

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# Figures

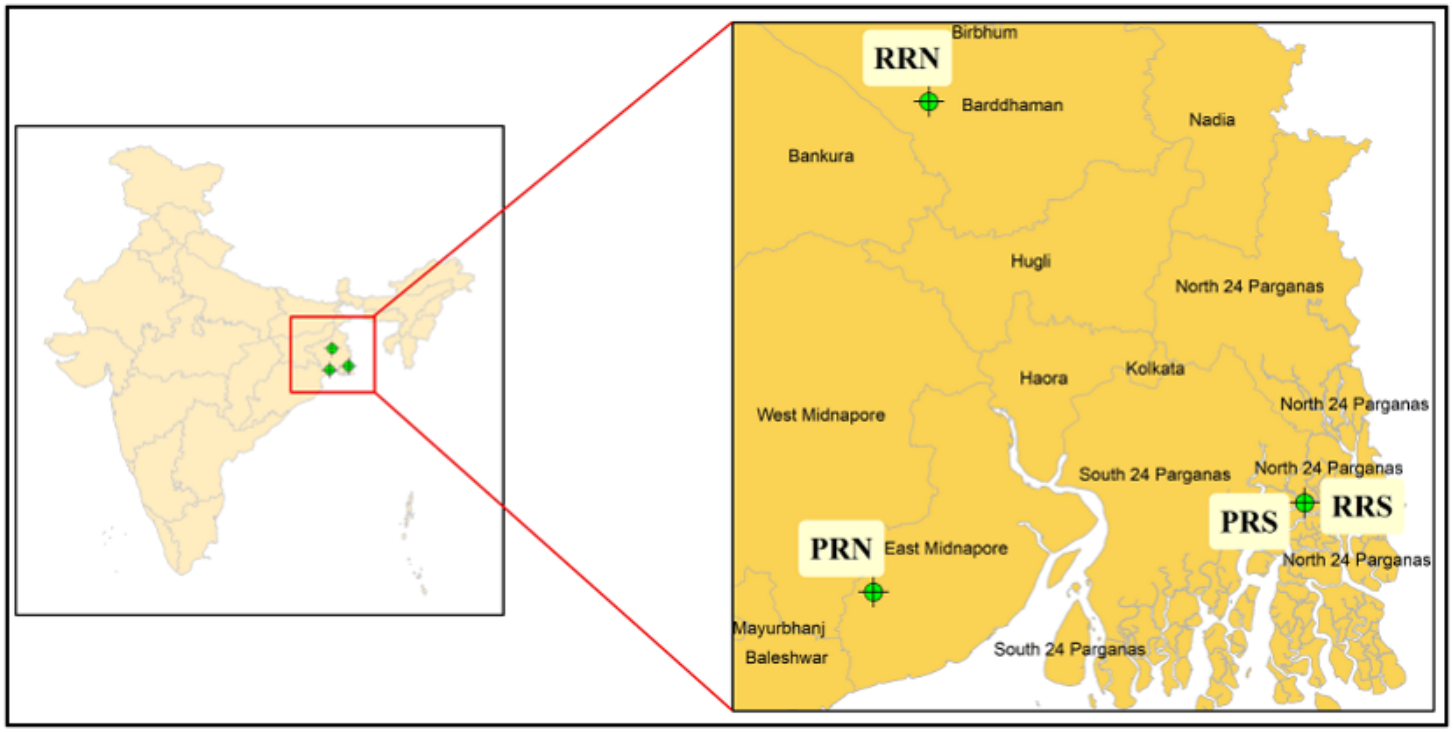
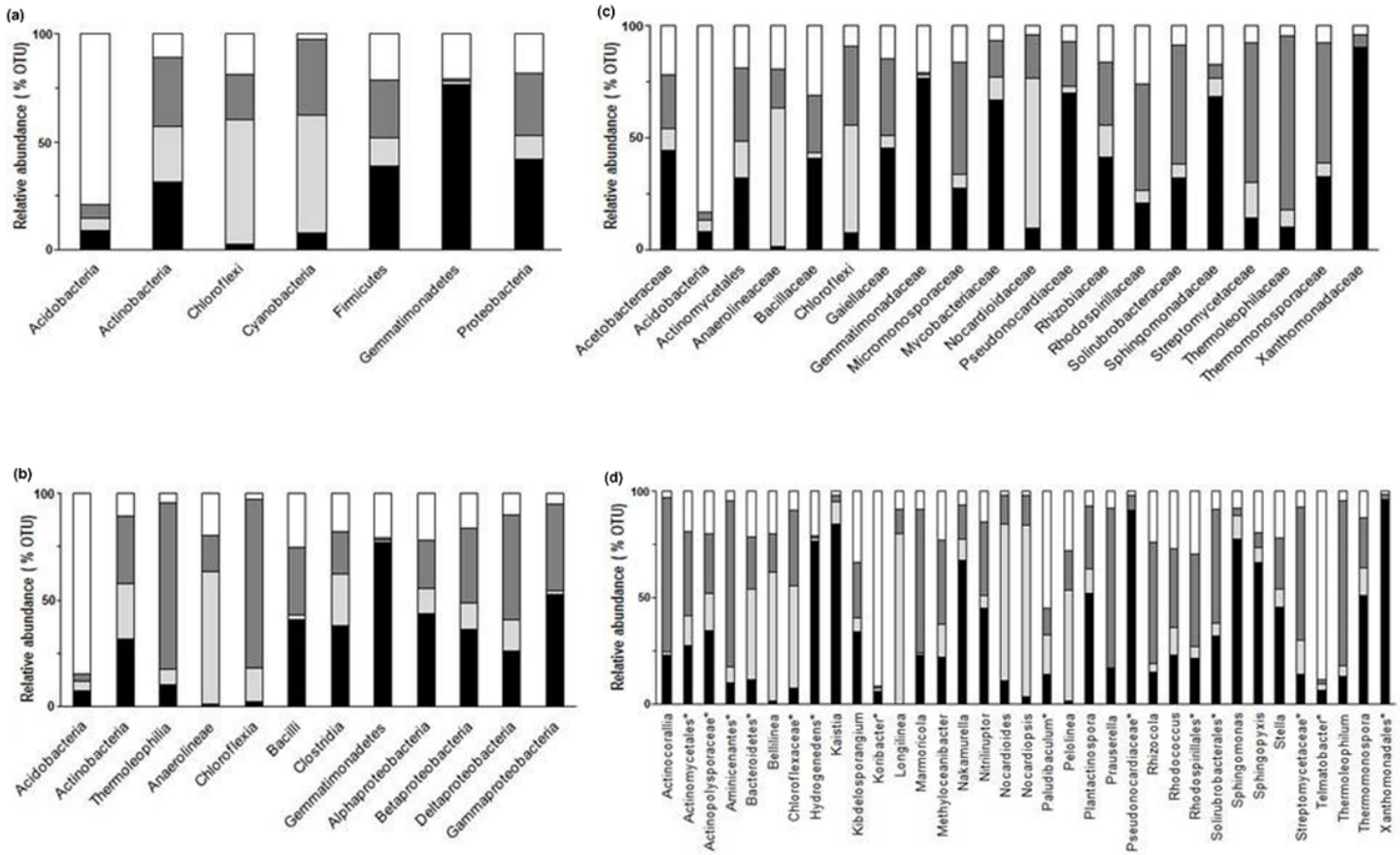



**Figure 1**

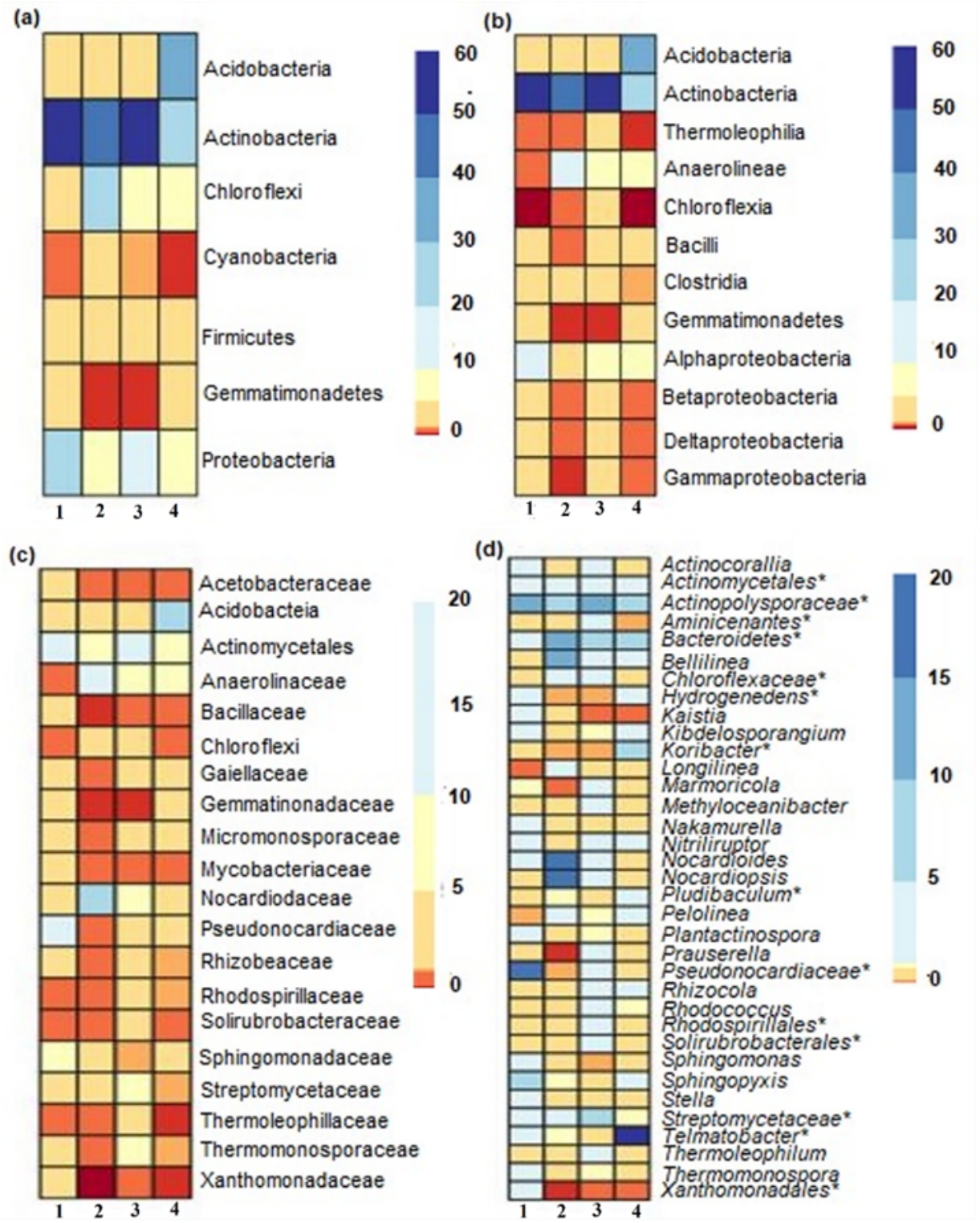
Map showing the geographical location of the sample collection site. Map pointed with peanut rhizosphere sample from normal soil (PRN) in 21°53'58.09"N, 87°32'16.58"E, peanut rhizosphere sample from saline soil (PRS) in 22°09'36.00"N, 88°47'60.00"E, rice rhizosphere sample from normal soil in 23°19'48.00"N, 87°42'0.00"E, and rice rhizosphere sample from normal soil in 22°09'36.00"N,

88°47'60.00"E. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.




## Figure 2

Relative abundance of bacteria at (a) phylum, (b) class, (c) family and (d) genus in different samples. Black, light grey, dark grey and white color indicates OTUs from PRN, PRS, RRN and RRS, respectively




## Figure 3

Heatmap of the bacterial distribution among four different samples. (a), (b), (c) and (d) represents phylum, class, family and genus level bacterial distribution, respectively whereas, 1, 2, 3 and 4 in each panel represents PRN, PRS, RRN, and RRS, respectively. * indicates unclassified genera.

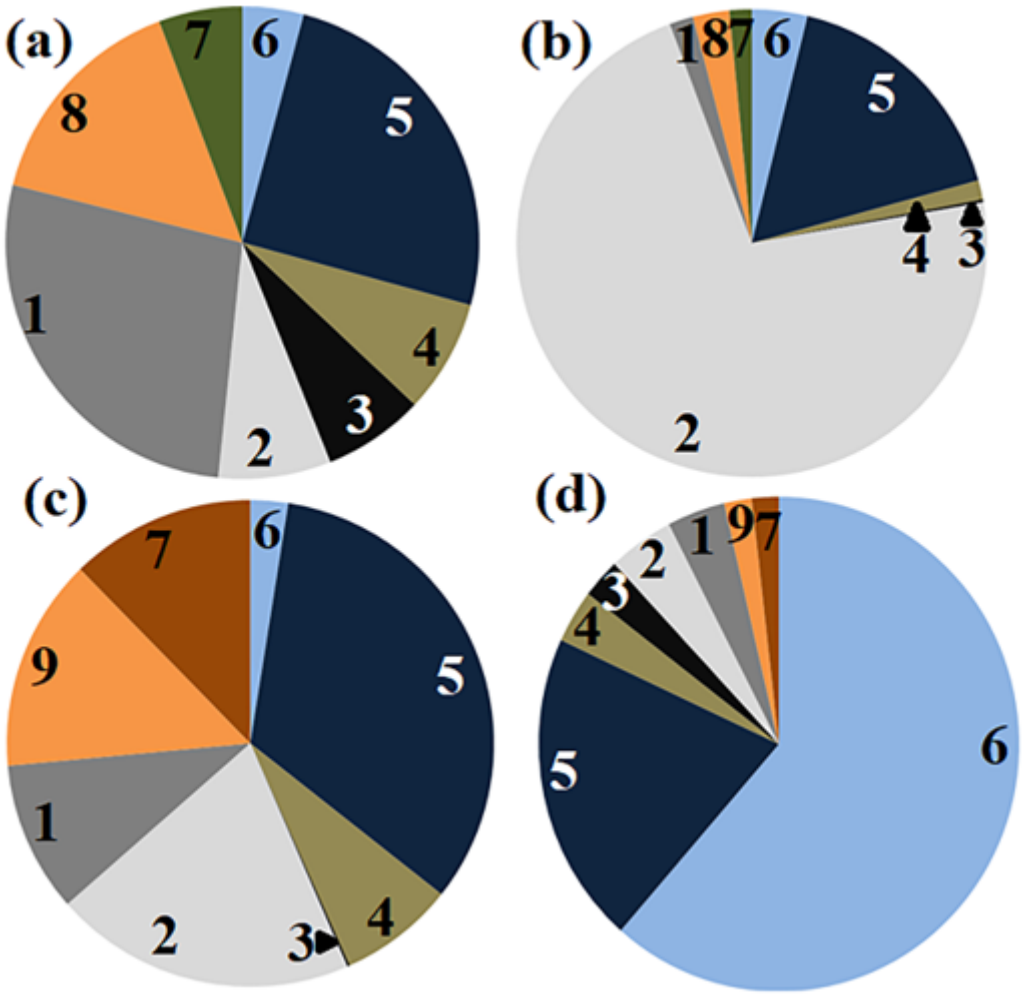
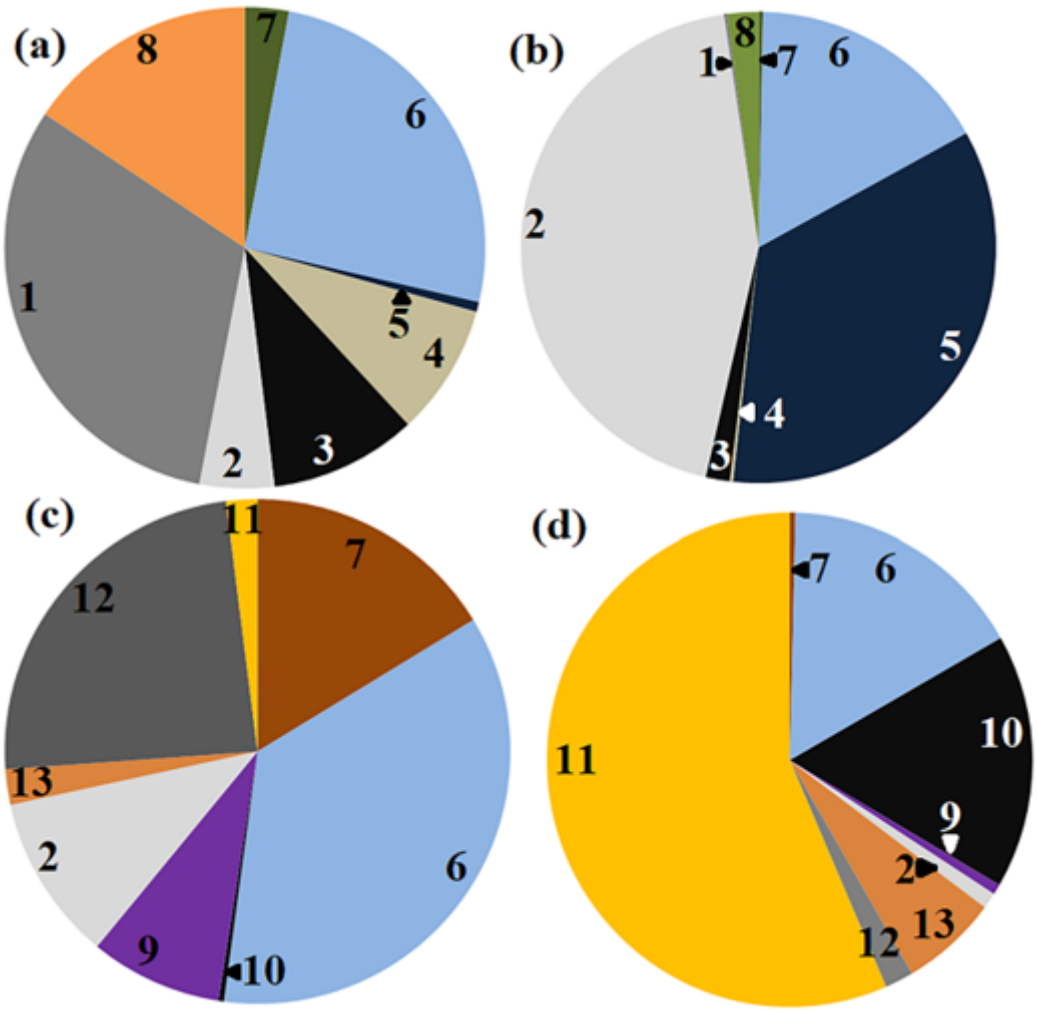


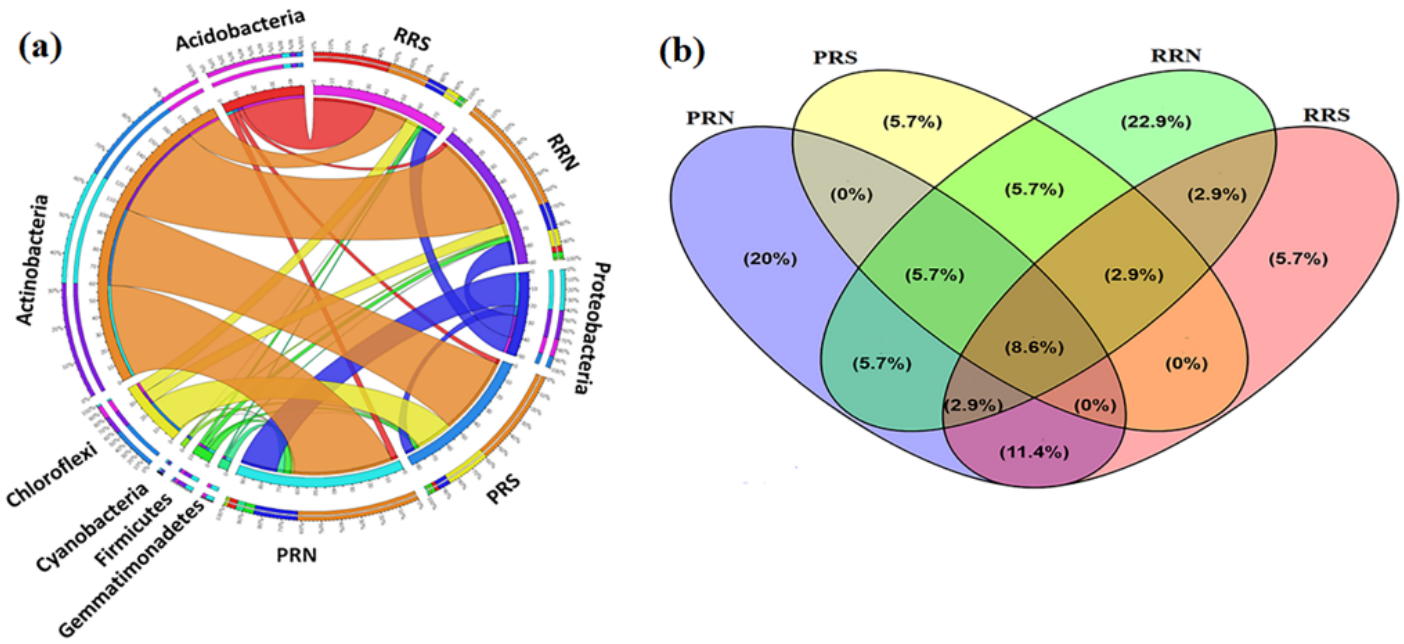

**Figure 4**

Pie chart representing the comparative abundance of the most common Family of sample PRN (a), PRS (b), RRN (c), RRS (d). 1. Pseudonocardiaceae, 2. Nocardiaceae, 3. Gemmatimonadaceae, 4. Gaiellaceae, 5. Actinomycetales, 6. Acidobacteria, 7. Thermomonosporaceae 8. Sphingomonadaceae 9. Streptomycetaceae



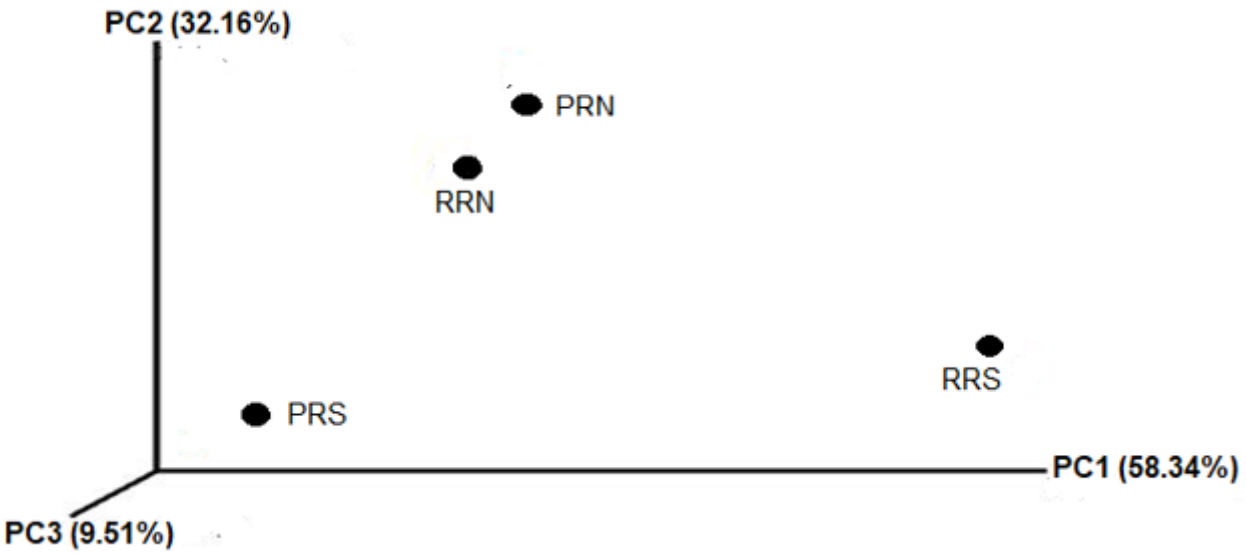

## Figure 5

Pie chart representing the comparative abundance of the most common Genera of sample PRN (a), PRS (b), RRN (c), RRS (d). 1. Pseudonocardiaceae 2. Nocardiodes 3. Nitriliruptor 4. Hydrogenedens 5. Bellilinea 6. Actinopolysporaceae 7. Actinocorallia 8. Sphingopyxis 9. Marmoricola 10. Koribacter 11. Telmatobactor 12. Streptomycetaceae 13. Paludibaculum




## Figure 6

Distribution of the bacterial community among rice and peanut rhizosphere. (a) Circos plot showing the distribution of bacterial communities present in different rhizospeheric soils. (b) Venn diagram of the percentages of shared and unique bacterial genera present in four different samples.




## Figure 7

Overall diversity of the samples. Principal coordinate analysis of four samples were showing the diversity (using an unweighted unifrac distance metric)

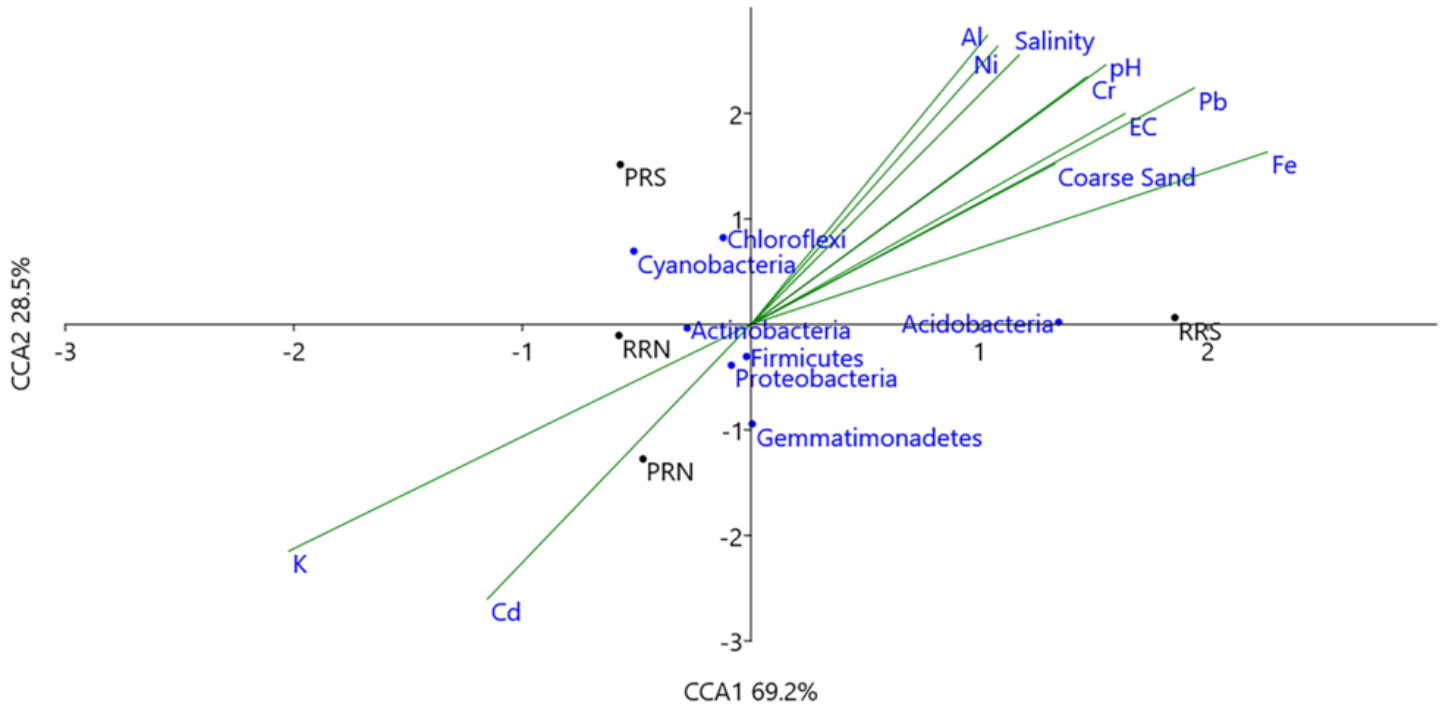



**Figure 8**

CCA plot based on the relative abundance of phylum in response to environmental factors

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SIIslametalSciRep.pdf