

Bacterial Communities of Sediments in a Seawater-Freshwater Transition Zone During the Wet and Dry Seasons in Pearl River Estuary

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Abstract

Seawater intrusion has a detrimental effect on agriculture, industry and human health. One question of particular interest is how the microbial community responds to and reflects seawater intrusion with seasonal variation. The current study explored the seasonal changes in bacterial community composition and interaction in the vicinity of Pearl River Estuary in January (dry season) and September (wet seasons). Results indicated that the salinity (expressed as electrical conductivity value) of sediment samples obtained in dry season was higher than that in wet season. The salt stress induced a declined alpha diversity but resulted in a loosely connected and unstable biotic interaction network in the bacterial communities. Random forest prediction and redundancy analysis of bacterial community indicated that salinity substantially affected the bacterial communities. Multiple lines of evidence, including the enrichment of bacterial taxa in the high-salinity location, microbe-microbe interactions, environment-microbe interactions, and machine learning approach, demonstrated that the families *Moraxellaceae* and *Planococcaceae* were the keystone taxa and were resistant to salt stress, which suggested that both of them can be used as potential biological indicators of monitoring and controlling seawater intrusion in coastal zone areas.

1. Introduction

Saltwater intrusion has become a prominent environmental concern all around the world [1]. Salt accumulation has poses exacerbated threats to local agricultural/industrial production and coastal community health around the world [2, 3]. In Netherlands, 20 well fields were closed and 9 had to lean upon artificial replenishment owing to the salinization between 1880 and 1992 [4]. In Vietnamese Mekong Delta, rice yield loss was predicted about 2.5–4.05 tons ha⁻¹ per year due to the saltwater intrusion [5]. Moreover, saltwater intrusion caused the salinization of groundwater and agricultural soil, which resulted in increasing the risk of hypertension and cardiovascular diseases of human beings because of the high salt intake [6–8]. Currently, many countries around the world are suffering from ill effects of seawater intrusion, including France [9], America [10], Australia [11], China [12], Italy [13], Tunisia [14], the United Arab Emirates [15], Bangladesh [16]. Hence, cost-efficient strategies for monitoring and controlling seawater intrusion should be implemented to prevent freshwater quality deterioration [7].

The saltwater-freshwater transition zone has always been one of the intensive study areas in current estuarine research due to its important role in connecting terrestrial and Marine ecosystems [17]. However, majority of past studies have concentrated on the monitoring of water quality changes, the hydrodynamics of groundwater in the saltwater-invaded area, and the approaches used for the assessment and management of seawater intrusion, while only a few have addressed the response of microbial community to saltwater intrusion [18, 19]. The dominant bacteria contain many haloresistant and halotolerant microorganisms [20, 21]. These microorganisms can be used as potential indicators of saltwater intrusion [21]. Noticeably, some salt-tolerant enzymes extracted from these halophilic microorganisms performed well in a seawater system and thus have the potential to be applied as biocatalysts, which is beneficial to reduce demand for freshwater in large-scale industrial processes [22].

Due to seasonal changes, saltwater intrusion is influenced by drought frequency and duration [23]. Prolonged drought increases intrusion of saltwater into freshwater systems [24] and elevates the maximum salinity of high tides or streams during low flows [25]. A number of researches found that seasonal fluctuations showed prominent effects on microbial community at varied habitats [26]. Microbial community structure in marine ecosystems shifted significantly with seasonal alternations in Western English Channel [27]. The microbial community in coastal water samples collected in spring was the most diverse among all seasons, indicating the seasonal fluctuation alters the microbial community structure [28]. One recent study [29] pointed out that the microbial community composition in estuary reservoir exhibited significant seasonal variations, that is, *Synechococcus* was the dominant taxa in warm season, while in cold season, *Actinobacteria* and *Bacteroidetes* had higher abundances and exhibited co-occurrence patterns with salt ions. Consequently, it will be pivotal to know the seawater intrusion impacts in order to delineate the microbial spatiotemporal distributions in seawater-freshwater transition zone.

In China, the phenomenon of saltwater intrusion in the Pearl River Estuary has become more serious than ever before due to the exacerbated climate change and anthropogenic activities [30–32]. This estuary, therefore, provides excellent natural labs to investigate the effects of saltwater intrusion on microbial communities. Thus, in this study, the sediment samples were collected in January and September 2020 for physicochemical parameter analysis, genetic 16S rRNA amplicon sequencing, and multivariable statistics analysis. The aims are to i) investigate the effects of seasonal variations of seawater intrusion on the bacterial community, particularly in the biodiversity, composition, and biotic interactions, and ii) identify suitable microbial taxa as indicators for seasonal seawater intrusion variation.

2. Materials And Methods

2.1 Sample site

This study was done in Pearl River Estuary, Guangdong Province, China. There are three sampled sites. The nearest monitoring location (ZH, 113°24'18.01", 22°0'26.31") away from the sea is located at the beach. The other two sampling sites are JM (113°5'50.48", 22°38'48.17") and FS (112°54'17.82", 23°21'56.46"), which are about 85 km and 150 km away from the ZH site. The sediment samples were collected in January and September 2020, and a total of 30 samples (5 samples per site and time) were collected. The samples were cryopreserved with dry ice during the transportation and stored at -20°C.

2.2 Sample geochemical parameters

Samples were freeze-dried and ground with 200-mesh sieve. The electrical conductivity (EC) and pH were measured in suspension (dry sediment/water, 1:5). Sulfate and nitrate concentrations were detected with ion chromatography (DIONEX, Sunnyvale, USA) after slurry was centrifugated at 4000 *g* for 10 min at room temperature. Sediment samples were analyzed for total organic carbon (TOC) with a MACRO elemental analyzer (Elementar, Hanau, Germany).

2.3 DNA extraction and Illumina MiSeq sequencing

DNA was extracted using Qiagen DNeasy Powersoil kit (Dresden, Germany). The 16S rRNA gene (V4-V5 region) was amplified with 515F/806R primer pair (Caporaso et al., 2010). Amplification products were quantified using a microplate fluorescence reader FLX800T (BioTek Instruments, Inc. Winooski, Vermont) and pooled at equimolar ratios for paired-end sequencing on Illumina MiSeq platform at Personal Biotechnology (Shanghai, China). Then, raw reads were subjected to quality checking, trimming, and chimera elimination using QIIME2 [33], and then the clean sequences were clustered by DADA2 [34]. Representative amplicon sequence variants (ASVs) were assigned against the Nucleotide Sequence Database. The obtained raw data have been committed to the database of NCBI (accession no. PRJNA731343).

2.4 Statistical analysis

The alpha diversity estimation and Bray-Curtis distance matrices-based principal coordinate analysis (PCoA) of microbial communities were analyzed using QIIME2. Redundancy analysis (RDA) was analyzed using the OmicStudio (<https://www.omicstudio.cn/tool>). Statistical analysis of data was carried out with Wilcoxon rank sum test or Student's *t*-test in SPSS v.20. Correlations between physicochemical parameters and Simpson index were analyzed using random forest (RF) algorithm [35]. The R package ggplot2 was used to visualize the RF model based on the partial dependence plots.

To gain a better understanding of potential interactions of “microbe-microbe” and “environment-microbe”, co-occurrence network analysis was constructed with Gephi [36, 37]. A connected link denotes a high and significant Spearman's correlation ($|r| > 0.6$, $p < 0.05$). Link thickness corresponds to the absolute value of Spearman's correlation coefficient, and node size corresponds to the number of connections (i.e., degree).

3. Result

3.1 Physicochemical parameters

The physicochemical parameters of samples in three locations collected in January and September were measured according standard methodology (Fig. 1). For carbonic analysis, compared to the January samples, the September samples had significantly lower EC and TOC in three locations except FS. The nitrate concentration was significantly higher in January samples than in September samples in FS. pH values fluctuated markedly, in both FS and JM, in January (slightly acidic) and September (slightly alkaline), while in ZH, the pH values were fairly consistent in the two sampling points (ranging between 6.73 and 6.82). In three locations, the sulfate concentration was significantly higher in January than in September.

3.2 Bacterial community diversity

The bacterial community composition in the three collection sites was revealed by 16S rRNA amplicon sequencing. In ZH, the alpha diversity (including ACE, Fisher, Chao1, Shannon, Simpson, and Observed species) was higher in September than in January (Fig. 2A), demonstrating both evenness and richness were higher in September than in January. The effects of the physicochemical parameters on the Simpson index was evaluated using RF predictions (Fig. 2B). EC was the main factor, which adversely affected the alpha diversity of bacterial communities. Based on the PCoA and PERMANOVA analysis, the bacterial community compositions in different locations in January and September could be clearly separated (Fig. 2C, $p < 0.001$). ZH had a high EC and bacterial community with great changes and thus we mainly focused on the bacterial community in ZH in the subsequent section.

3.3 Composition of bacterial community

The above results showed important insights into how diversity of bacterial community responds to physicochemical parameters at general community level. Subsequently, the response of individual taxa at different levels to physicochemical parameters was further investigated. At phylum level, the abundance of *Proteobacteria* ranked first among all samples, accounting for 27.0–62.7% of the whole bacterial community, followed by *Acidobacteria*, *Chloroflexi*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Planctomycetes*, *Nitrospirae*, and *Gemmatimonadetes* (Fig. 3A). Specifically in ZH, *Proteobacteria* and *Firmicutes* were significantly enriched in January, while *Acidobacteria*, *Chloroflexi*, *Bacteroidetes*, *Planctomycetes*, and *Gemmatimonadetes* were significantly enriched in September (Fig. S1). Further analysis at family level were performed and revealed enrichment of distinct bacterial taxa in January and September (Fig. 3B). Some bacteria, including *Moraxellaceae*, *Planococcaceae*, *Anaerolineaceae*, *Syntrophaceae*, *Gaiellaceae*, *Micrococcaceae*, were significantly enriched in January samples, while *Thermoanaerobaculaceae*, *Gemmatimonadaceae*, *Saprospiraceae* were enriched in September samples in ZH (Fig. S2).

3.4 Interaction networks and distinct keystone taxa

The microbe-microbe interactions (biotic interaction) were predicted (three in January and three in September) using the interactive platform Gephi (Fig. 4A). The biotic interaction in January showed 19–51 nodes and 36–178 strong and significant connections (links). However, in September, the biotic interactions were more complicated, containing 40–56 nodes and 121–243 links (Fig. 4B). The results demonstrated that the potential interactions were weaker in January than in September, especially in ZH. Additionally, the ratio of positive link number to negative link number in each network was calculated. Expect for January samples in ZH, the ratios from other samples remained ~68%. However, in January samples in ZH, the proportion of positive link was much higher than that of negative link as a result of the reduced proportion of negative links. The keystone taxa were identified according to the standard of the nodes with low betweenness centralities and high degrees [38, 39]. In January samples in ZH, a total of sixteen ASVs were defined as keystone taxa (Table S1), which were further assigned to *Moraxellaceae* (eight ASVs) and *Planococcaceae* (eight ASVs) at family level.

The environment-microbe interactions in January samples in ZH further demonstrated that EC was the main factor, which influenced 16 individual ASVs (Spearman $> |0.6|$ and $p < 0.05$) (Fig. 5). The predominant influence of EC in ZH was also supported by RDA analysis (Fig. 6), and by the bacterial diversity result in Sect. 3.2. Other physicochemical parameters, including sulfate, TOC, and nitrate, also had great influences on the community composition. Importantly, all identified sixteen keystone taxa were also detected in this environment-microbe network, and all of them exhibited positive correlations with EC (Tables S2, S3).

RF analysis indicated that EC was the main factor influencing the relative abundances of *Moraxellaceae* and *Planococcaceae* (Fig. 7). Indeed, *Moraxellaceae* and *Planococcaceae* had high relative abundances within a certain range of EC value (e.g., $EC > 1650 \text{ us cm}^{-1}$), indicating that *Moraxellaceae* and *Planococcaceae* could be halotolerant and/or halophilic bacteria.

4. Discussion

Seawater intrusion is a universal environmental issue, esp. under the current scenario of exacerbated climate change and human activities. However, to date, studies focused on the response of the microbial community to saltwater intrusion was still scarce, though the application of microorganisms to monitor and control seawater intrusion is being widely considered as an effective and economical strategy. In this study, we selected three sampled places in Pearl River Estuary suffering from varying degrees of seawater intrusion, and collected the sediment in January and September 2020, respectively. Aims of this research were to investigate the influences of saltwater intrusion on bacterial communities and detect some potential microbial indicators for seawater intrusion.

4.1 Diversity and composition of bacterial community

In JM and ZH, the January samples showed higher EC values than September samples (Fig. 1), which suggested that seawater intrusion was aggravated during dry season (January). Owing to heavy rainfall during monsoon season (represented by September samples), the recharge rates were considerably high in most coastal aquifers with a high groundwater table, which raises the freshwater level in coastal aquifers and restricts intrusion of seawater [7, 40]. Environmental stresses including salt stress are considered as major drivers of microbial community diversity [41, 42]. In this study, bacterial community alpha diversity was lower in January than in September in ZH, suggesting an inverse relationship between salinity and diversity of bacterial community (Fig. 2). Similarly, the alpha diversity was lower in seawater than in irrigation water and groundwater [43]. RF analysis further demonstrated that EC exerted adverse effects on alpha diversity (Fig. 2B). The effects of salinity on microbial community diversity is mainly due to the alternation of plasma membrane, especially the solute contents which plays a vital role in water fluxes regulation [44]. An increase in salinity would lead to water efflux and dehydration, while a decrease in salinity cells would induce water influx of high solute contents, cell swelling and eventually resulted in cell lysis [45].

4.2 Microbial interaction networks

Network analysis has been increasingly used as a critical tool for exploring microbial community symbiosis patterns in ecosystem studies [46, 47]. In this study, salt stress suppressed the potential interactions within microbial communities, especially in ZH (Fig. 4). This can be interpreted with the deleterious effects of high salinity on microbial growth due to ionic, osmotic and oxidative stresses [48]. Salt stress made the nodes dispersed and loosely linked in the bacterial interaction networks [49]. When a microbial community possessing a large percentage of members linked together through positive correlation, it tends to be unstable. This is because in such a community, members are likely to react uniformly to circumstantial fluctuations, giving rise to a positive feedback and resonance. On the contrary, negative links are likely to stabilize the resonance of such communities and thus contribute to the stability of the network [50]. Therefore, the study suggested that salt stress suppressed network potential interactions and affected the network stability.

4.3 *Moraxellaceae* and *Planococcaceae* as the keystone taxa

Generally, keystone taxa are considered part of the core microbial communities [51, 52]. These keystone taxa significantly influence material transformation, energy metabolism, and/or nutrient cycling in response to geochemical and biological factors because they can choose different environment-dependent survival strategies to develop the whole microbial communities in their favor [53–55], and the absence of these keystone taxa can potentially cause tremendous shifts in the composition and function of the microbial communities [56, 57]. Usually, the keystone taxa were identified according to the criteria of high degree nodes and low betweenness [38, 39]. In current study, *Moraxellaceae* and *Planococcaceae* were identified as the keystone bacteria based on multiple pieces of evidence, and they had positive correlations with EC (Figs. 6,7). It suggested that the identified keystone bacteria may be resistant to salinity. In fact, some members of *Moraxellaceae* and *Planococcaceae* have been isolated from various hypersaline habitats [58–61].

In this study, *Psychrobacter* and *Acinetobacter* belonging to *Moraxellaceae* family were detected. *Psychrobacter* could survive in extremely cold habitats, such as in ice, soil, sediments in Antarctic, as well as in deep sea environments [58]. This finding is consistent with our result that *Psychrobacter* was enriched in January when low temperature condition existed. Besides, some strains of *Psychrobacter* have been isolated and/or detected in variety of hypersaline surroundings [59, 62–64]. *Psychrobacter* sp. ANT206 was isolated from Antarctic sea-ice, which not only had high catalytic efficiency at low temperatures but also displayed remarkable salt tolerance [59]. *Psychrobacter* was identified as the predominant bacterium at high salinity ($15 \text{ g L}^{-1} \text{ NaCl}$) [65]. The another genus *Acinetobacter* belonging to *Moraxellaceae* were also reported to tolerant to salt stress [66–68].

Some genus belonging to the family *Planococcaceae* have been isolated in variety of hypersaline surroundings [60, 61, 69]. For example, the xylanase from *Planococcus* sp. SL4 which was obtained from the sediment in soda lake Dabusu was found to be salt-tolerant, showing great prospect of basic research and industrial applications [60]. Similarly, *Planomicrobium* and *Planococcus halotolerans* could grow

optimally under conditions with high salinity [61, 70]. Therefore, these results further suggested that keystone *Planococcaceae* identified in this study could be resistant to salinity.

4.4 Bacterial community application in seawater intrusion

Diverse microorganisms have previously been found in the sediment [71, 72]. Microorganisms are highly sensitive to pollutants, and this character makes them frequently applied as potential ecological indicators to diagnose environmental pollutions, such as organic contaminations [73] and heavy metal(loid) contaminations [74–76]. However, there is still a paucity on the application of microbial analysis in the study of seawater intrusion. Since seawater is regarded as a pollutant in the context of seawater intrusion, it seems logical and significant to adopt microbes as biological indicators to evaluate the seawater intrusion.

In this study, it is apparently showed that seawater intrusion changed the diversity, composition, and microbe-microbe interactions of microbial communities. The keystone taxa were recognized in the place with high salinity. The results of this study can provide a reference for exploring new microbial indicators of seawater intrusion. For example, seasonal variations in microbial interactions could be applied to characterize the degree of seawater intrusion [7]. Order *Oceanospirillales* and family *Alteromonadaceae* have already been suggested as indicators of seawater intrusion [43]. But more detailed microbial analyses with the variation of seawater intrusion need to be conducted, which will pave the way for rational seawater intrusion monitoring and management.

5. Conclusion

In this study, the Pearl River Estuary was selected to investigate the microbial adaption and response to seawater intrusion. Compared to the September (wet seasons) samples, the January (dry season) samples had higher salinity. In ZH, salt stress significantly affected alpha diversity and composition of bacterial communities. The nodes in microbe-microbe networks were loosely connected in the high salinity sediment, while they were densely interconnected in the low salinity samples. Besides, salt stress reduced the stability of the microbial community. In this study, based on multiple lines of evidence, members of *Moraxellaceae* and *Planococcaceae* were regarded as the keystone taxa and were resistant to salinity. Therefore, the microbial family *Moraxellaceae* and *Planococcaceae* may be used as biological indicators of seawater intrusion.

Declarations

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Compliance with ethical standards

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

Data availability All data related to this publication are made available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no conflict of interest.

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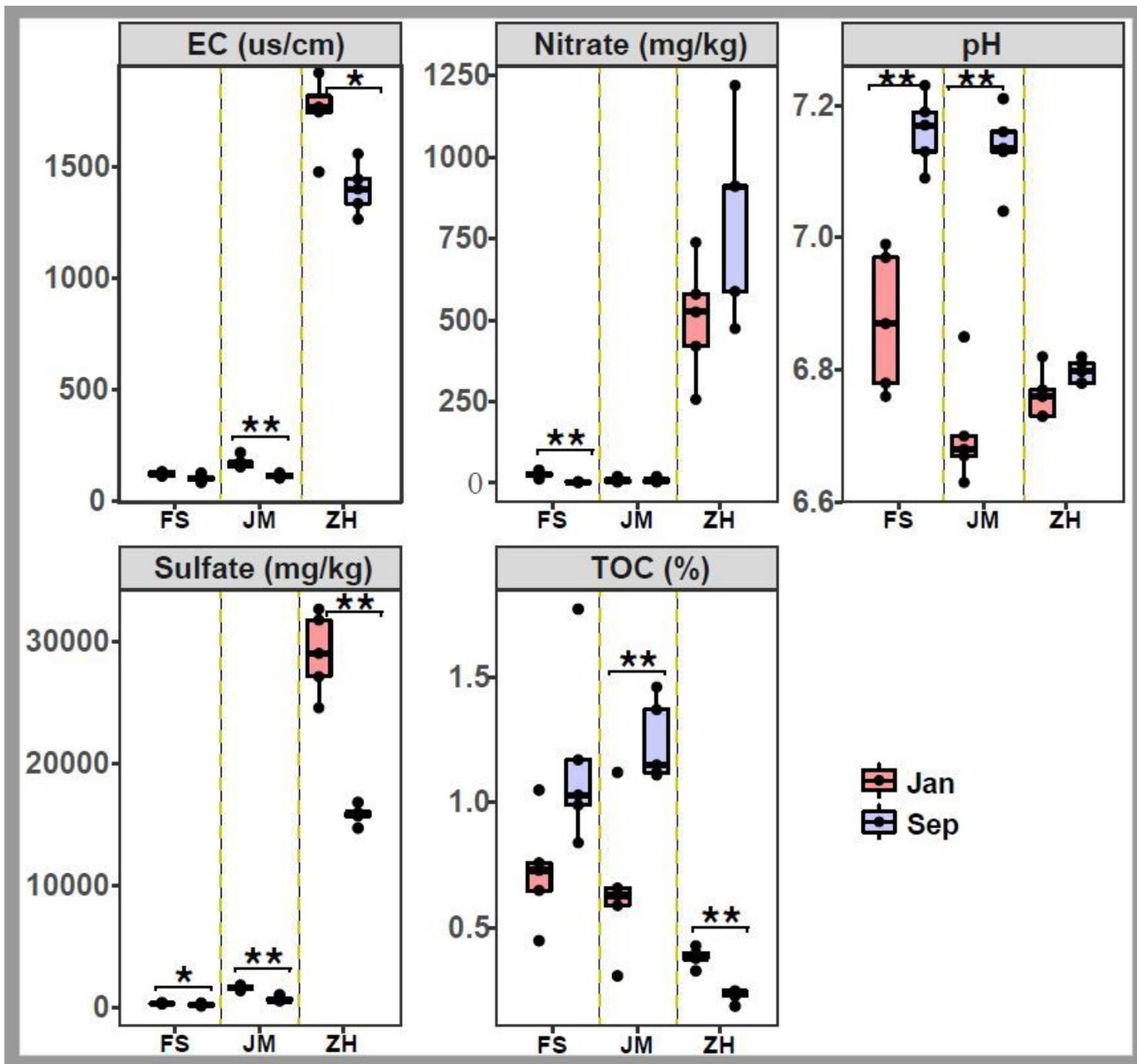
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Figures



Physicochemical parameters of sediment samples. * and ** indicate significant differences at $p < 0.05$ and $p < 0.01$, respectively.

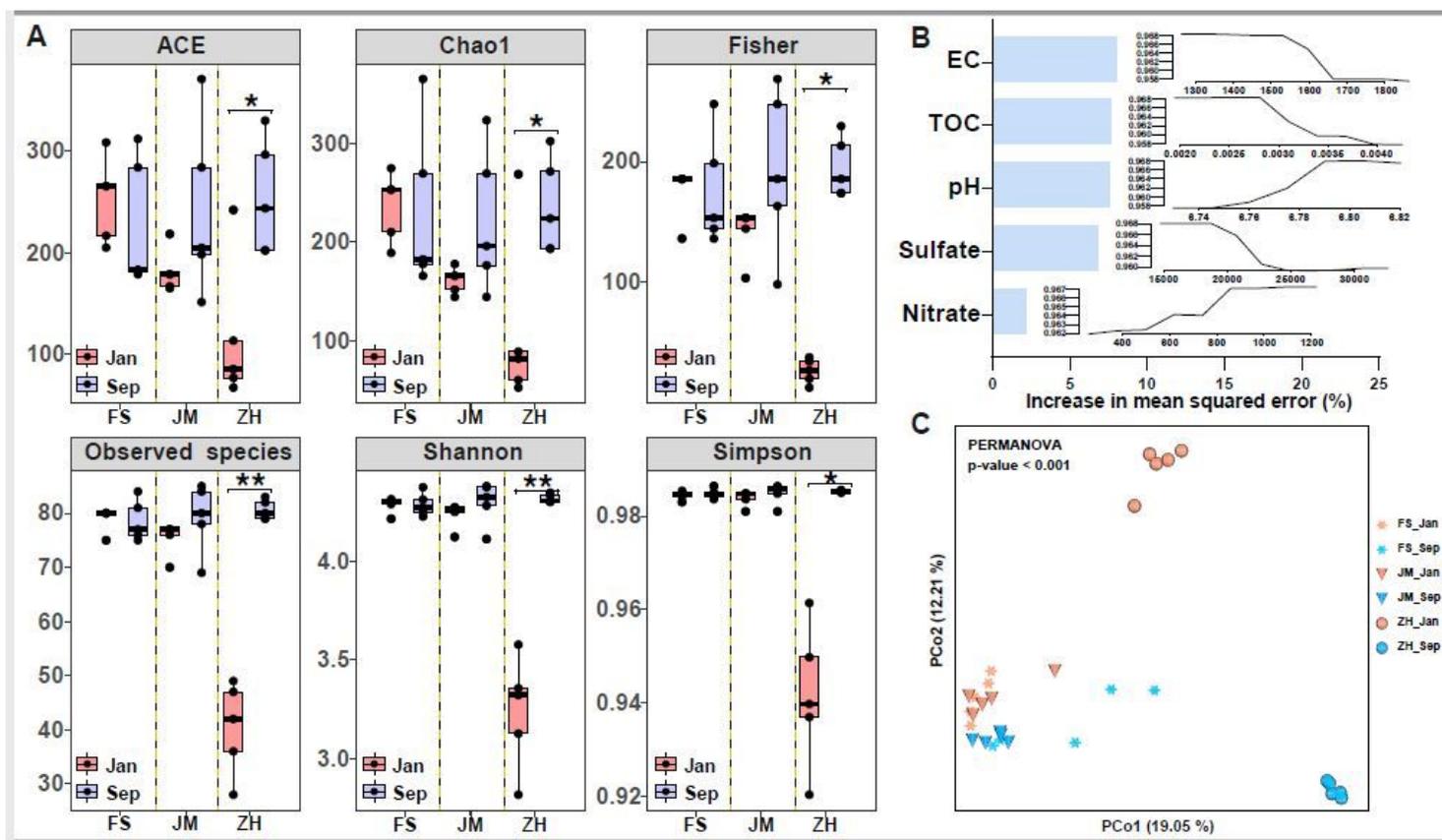


Figure 2

The diversity indices (A); random forest prediction of the relationships between physicochemical parameters and the diversity indices in ZH (B); the PCoA analysis based on the Bray-Curtis distance (C). * and ** indicate significant differences at $p < 0.05$ and $p < 0.01$, respectively. The insert in the RF model represents the partial dependence plots revealing the dependencies of the diversity indices on the physicochemical parameters.

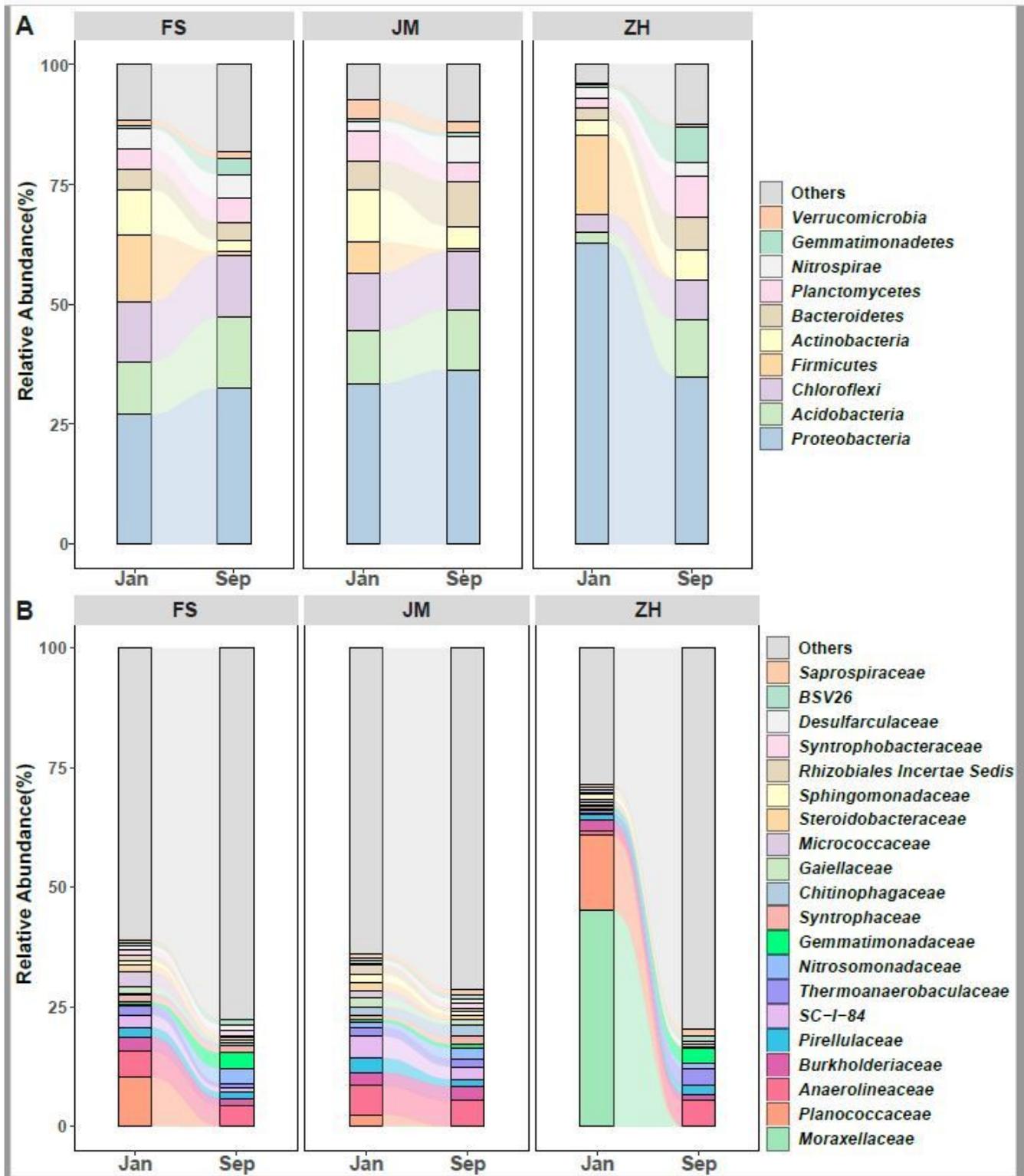


Figure 3

The relative abundances at the phylum (A) and genus (B) level based on the 16s rRNA amplicon sequencing.

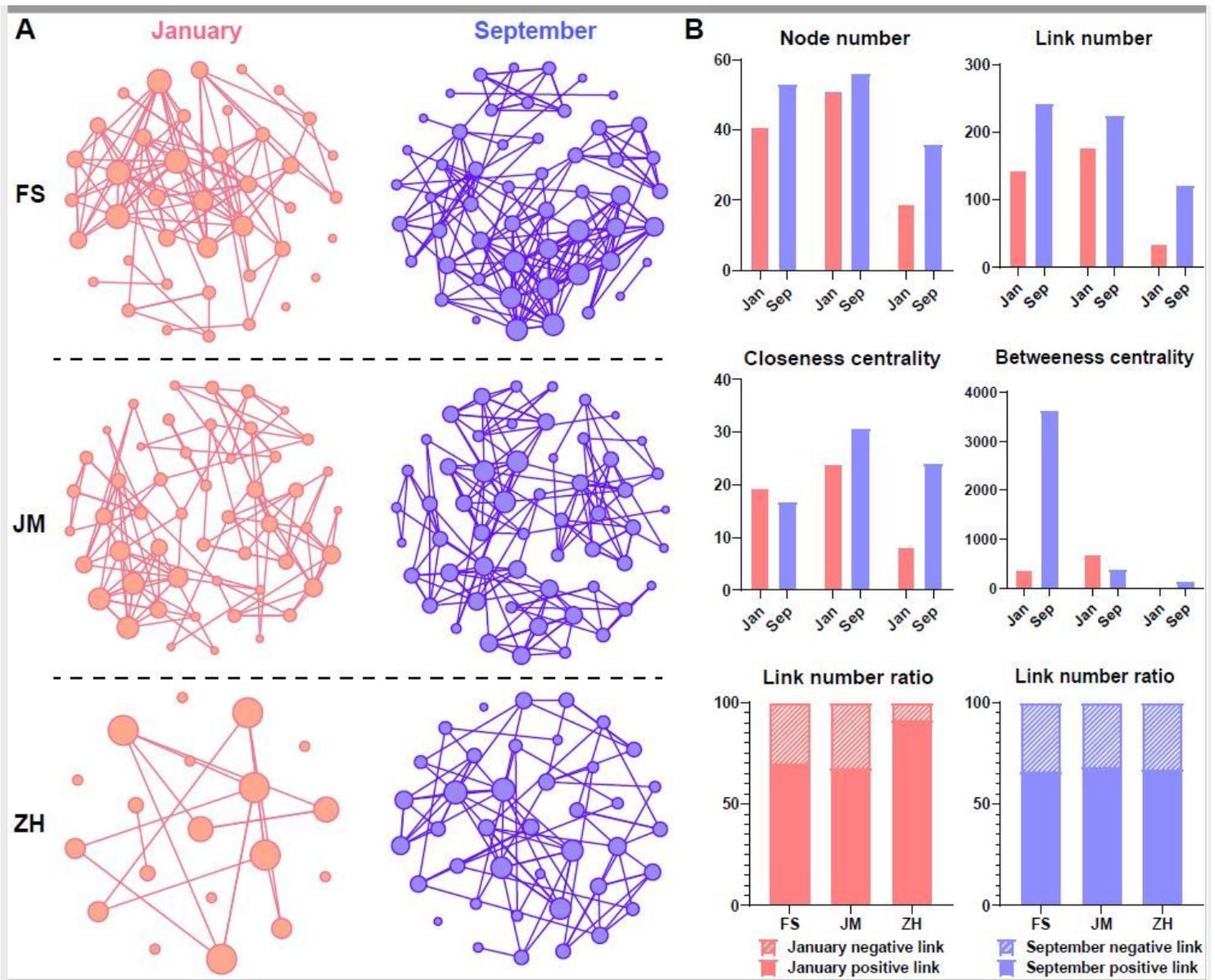


Figure 4

The co-occurrence network of the microbe-microbe at different sampled sites in January and September (A). The topological characterizations (B). The connection stands for the Spearman correlation with significance ($p < 0.05$) and high significance ($0.6 < |r| < 1$).

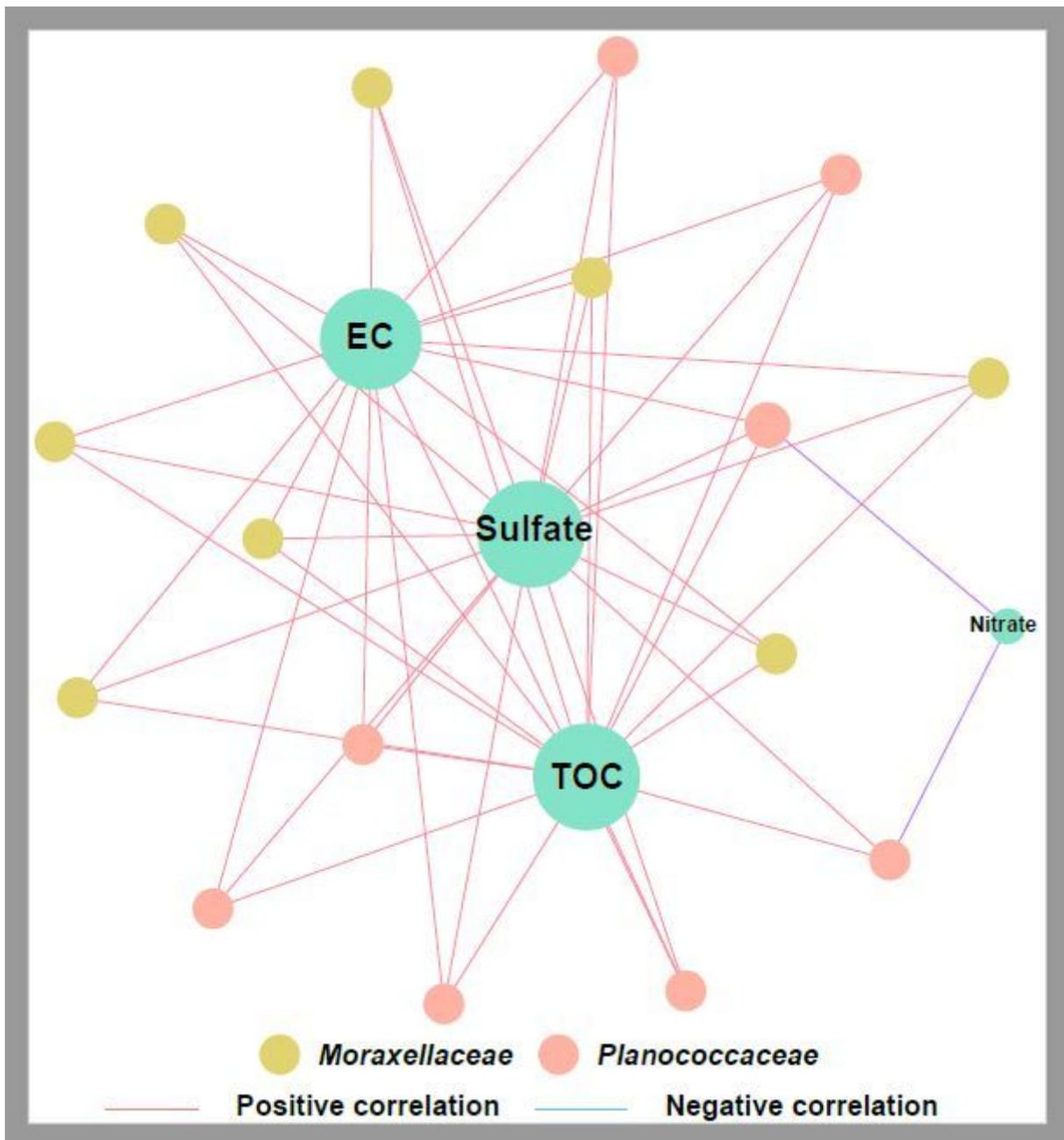


Figure 5

The co-occurrence network of the environment-microbe representing the correlations between the relative abundances of the bacterial taxa and the physicochemical parameters in ZH. The connection stands for the Spearman correlation with significance ($p < 0.05$) and high significance ($0.6 < |r| < 1$).

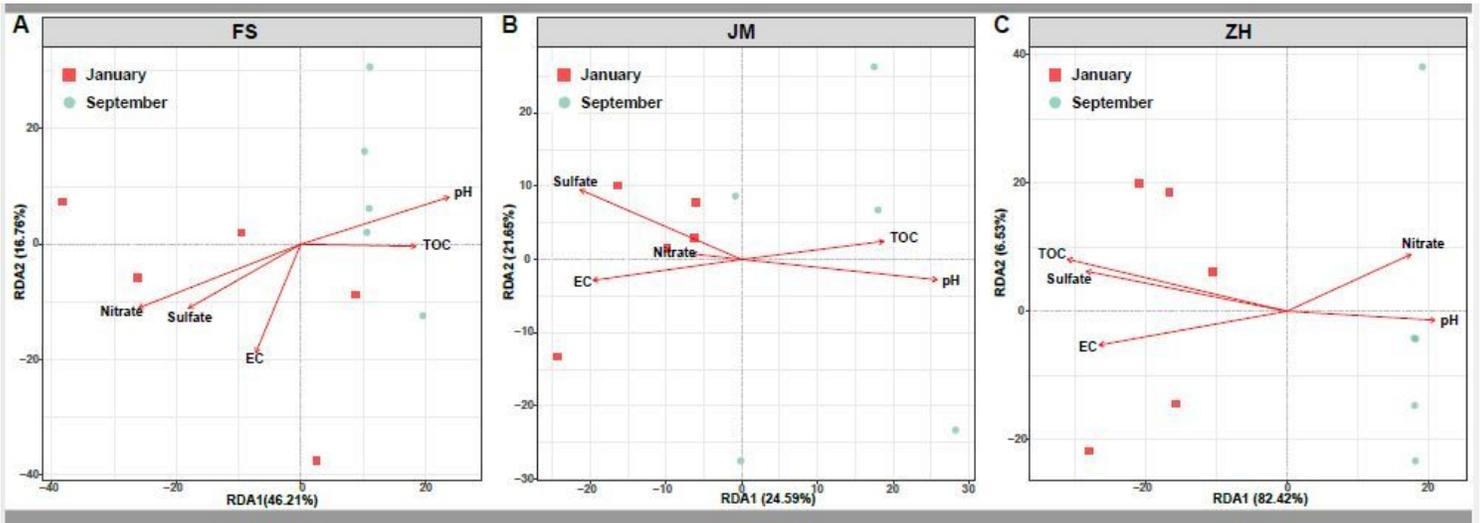


Figure 6

Redundancy analysis (RDA) demonstrating the impact of environmental factors on the bacterial communities in FS (A), JM (B), and ZH (C).

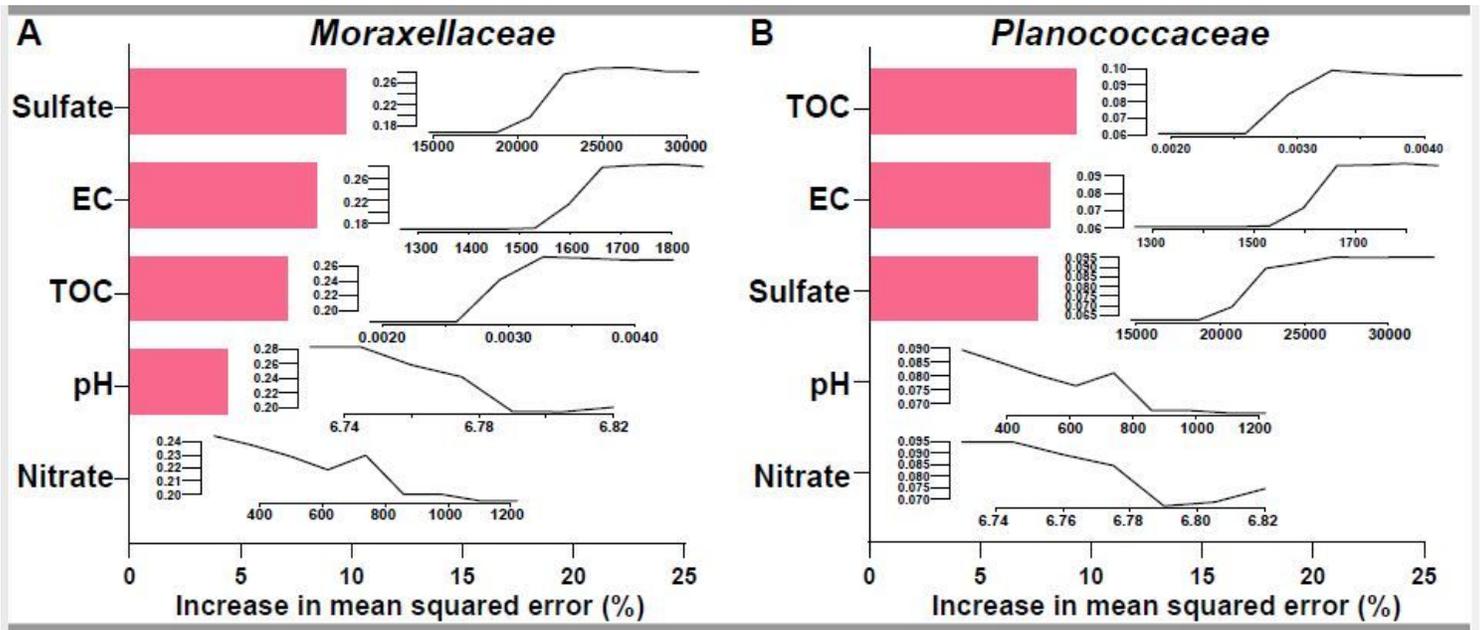


Figure 7

The random forest (RF) prediction of the relationships between the individual physicochemical parameters and the relative abundance of Moraxellaceae and Planococcaceae. The insert in the RF model represents the partial dependence plots revealing the dependencies of the relative abundance on the physicochemical parameters.

Supplementary Files

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