

Soil Microbial Community Variation With Time and Soil Depth in Eurasian Steppe (Inner Mongolia, China)

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Abstract

Background

Soil microorganisms play an indispensable role in the material and energy cycle of grassland ecosystem, and were affected by many environmental factors, such as time and space changes. However, there are few studies on the temporal and spatial transformation of soil microbial community in typical degraded steppe. We analyzed the community structure and diversity of soil bacteria and fungi and the effects of environmental factors on the community structure in Xilingol degraded steppe.

Results

The abundance and diversity of bacteria and fungi were significantly affected by depth. Bacteria and fungi diversity of 10 cm was higher than that of 20 cm and 30 cm. The abundance of Acidobacteria, Proteobacteria, Actinomycetes, Ascomycetes and Basidiomycetes varies significantly with depth. What's more, soil pH increased significantly with depth increasing, while SOM, AN, VWC and ST decreased significantly with increasing depth. In addition, Depth, TOC and AN had significant impact on the bacterial and fungi communities ($p < 0.05$).

Conclusions

Spatial heterogeneity (depth) is more important than temporal (month) in predicting changes in microbial community composition and soil properties. And the abundance of Acidobacteria, Proteobacteria, Actinomycetes, Ascomycetes and Basidiomycetes varies significantly with depth. We speculate that SOM and VWC account for the abundance variations of Acidobacteria and Proteobacteria, and pH cause the abundance changes of Actinomycetes, Ascomycetes and Basidiomycota.

1. Introduction

The grassland covering approximately 25% of the earth's terrestrial area, and plays an important role in the global material cycle and energy exchange (Foley et al. 2011.). Inner Mongolia grassland is the typical arid and semi-arid steppe which is located in the eastern of the Eurasian steppe, and is the representative of Eurasian steppe in terms of climate, terrain, soil properties, and vegetation composition. Meanwhile, Inner Mongolia grassland also has the unique history of land use and management policy (Wu et al. 2015). In grassland ecosystem, soil is the important place for material and energy exchange, and has a strong influence on the diversity of microorganisms as the medium for microorganisms' survival. Microorganisms in soil stay in an indispensable position during the process of organics decomposing and the regulation of C (Bardgett et al. 2008), N (Bahram et al. 2018), P (Handa et al. 2014), S (Kowalchuk et al. 2001). Soil fungal communities are highly sensitive to soil water content, nitrate, and organic matter content (Wang et al. 2018), while bacterial diversity and community differences are strongly correlated to soil pH (Griffiths et al. 2011). Microbes have the general symbiotic relationship with soil and plants. They

help plants to absorb nutrients (such as C, N, P and other) that restrict the growth of plants (Der Heijden et al. 2008), and ingest nutrients from plant secretions and litters (Zhalnina et al. 2018).

Due to the nonuniform distribution of effective nutrients and plant roots in soil, soil microbial communities have different biogeographic distributions (Eilers et al. 2012). Soil microorganisms respond significantly differently to environmental factors (soil physical & chemical properties and plant community changes) at different depths (0-10 cm and 10-20 cm). Also, from the research of a multi-scale spatial assessment of soil bacterial community across the UK, a significant correlation between bacterial community and spatial distance was found out (Griffiths et al. 2011). While, in wetlands (Wang et al. 2010) and fallow farmlands (Ko et al. 2017), the size, activity, and diversity of microbes will decrease when soil depth increases. But the researches on grassland soil microorganisms mostly focused on the fixed depth of the surface layer (0-10/20 cm) (Leff et al. 2015; Na et al. 2019), and a few studies on the depths of the surface layer.

Soil microorganisms will be different when plant phenology changed, which is influenced by the climate and ecosystem. In temperate forest soils, the relative abundance of Actinomycetes will increase obviously in winter, but the relative abundance of Acinetobacter and Proteobacteria will decrease (Santalahti et al. 2016). It is widely concerned in recent years about the changes of soil microbial communities during the vegetation growing season. In crops root microbial communities have been varied throughout the whole life cycle (Shi et al. 2015; Zhang et al. 2018), and they tend to deviate gradually from the soil microbiome and enrich microbial specific groups. Oppositely, the microbial community in desert steppe has remained relatively stable over the course of the year. However, in Eurasian grassland, the soil temperature and water content are very variable between seasons, which would lead to the instability of plant litter and secretions, and affects the soil carbon input ultimately (Bardgett et al. 2005). Also soil organic carbon is the most important factor in driving the spatial distribution of microbial communities. When roots grow in summer, the effectiveness of soil carbon sources will also increase, while the effectiveness of carbon sources drop when roots activities stop in autumn. As a result, the rapidly growing microbial populations (which prefer to use direct carbon sources) have to slowed down their growth rate, which ultimately affect the structure of microbial population (Barboza et al. 2018).

Our research was conducted on the representative area of Eurasian Steppes in Inner Mongolia, China. Contrastively analyzed were performed on soil bacterial and fungal community structure, diversity, and the impact of environmental factors on the community structure. Those analyses were to explore and answer the questions as follows: (1) what are the composition of soil bacterial and fungal communities in this area? (2) what or how did time(months) and soil depth affect the structure of soil bacterial and fungal communities? (3) How is the relationship between other environmental factors and soil bacterial / fungal communities?

2. Materials And Methods

2.1 Study site and soil sampling

The experiment site is located in Erlitu Ranch of Zhengxiangbai Banner, XilinGol, Inner Mongolia, China (42°9' 14" N, 115°14' 39" E). Soil samples were collected every month from June to October in 2018 (represented by May, Jun, Jul, Aug, Sep). Each sample of 0-10 cm, 10-20 cm, and 20-30 cm depth (represented by 10, 20, and 30) was collected in the shape of "S" with a soil drill of 8cm in diameter. A total of six points were selected for soil collecting, and mixed into composite samples. After removing the animal / plant residues and other impurities like gravels by sieving (2 mm). Each composite sample was then divided into 3 subsamples as repeats for further DNA extraction (store in -80°C) and other soil properties analysis (store in -20°C).

2.2 Soil property measurements

Air-dried soil was used to measure soil properties at 0-10 cm, 10-20 cm and 20-30 cm depths, such as pH (soil-water ratio 1:5) and soil organic matter (SOM, potassium dichromate external heating method) (Bao, 2000). While other properties of soil like NH_4^+ and NO_3^- concentrations (reagent kit method) were measured using fresh soil. Soil temperature (ST) and volume water content (VWC) were observed automatically by in suit CS650 30 cm soil moisture and temperature sensor (Campbell Scientific Inc., Logo, UT, USA, <http://www.campbellsci.com>).

2.3 DNA extraction, PCR amplification and sequencing

The total DNA of soil microorganisms was extracted from 0.5 g soil samples using DNeasy PowerSoil Kit (Qiagen Benelux B.V., Venlo, The Netherlands), and each soil sample has three parallels. DNA concentration and quality were assessed by ultramicro nucleic acid quantifier, and DNA integrity was checked using 1% agarose gel electrophoresis.

Primers used in PCR were universal primers of bacterial 16S rRNA V3-V4 hypervariable region and fungal ITS1 region, which were based on Hiseq sequencing platform (Illumina Int., San Diego, CA, USA). Bacterial primer sequences are 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'); and fungal primer sequences are ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3'). The hot-start PCR reaction mixtures contained genomic DNA 40-60 ng, PCR buffer 15 μL , dNTP (10 mmol / L) 1 μL , upstream primer (10 μM) 1.5 μL , downstream primer (10 μM) 1.5 μL , Q5 high-fidelity DNA polymerase 0.2 μL , High GC Enhancer 10 μL , and ddH₂O to a final 50 μL . After mixing homogeneously the reaction system as above, the PCR program was carried out according to the following program. Pre-denaturation at 95 °C for 5 min, 15 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. PCR products were detected by electrophoresis with agarose 1% gels, followed by high-throughput sequencing and analysis, which were based on the Illumina HiSeq 2500 platform (Illumina Int., San Diego, CA, USA).

FLASH v1.2.7 (Magoč and Salzberg. 2011), Trimmomatic v0.33 (Bolger et al. 2014), and UCHIME v4.2 (Edgar et al. 2011) were used to control the quality of data and the effect of splicing to obtain Effective Tags. QIIME v1.8.0 (Caporaso et al. 2010) was used to cluster the obtained Effective Tags at a similarity level of 97%, and obtain OTU (Operational Taxonomic Units). The representative sequences of OTU are compared with the microbial reference database to obtain the classification information for each species corresponding to each OTU, and then the composition of each sample community was counted at each level (phylum, class, order, family, genus, species). And the taxonomic annotation was taken for OTU basing on Silva (for bacteria) and UNITE (for fungal) taxonomy database.

2.4 Statistical analysis

R (Mass Package) was used to carry out a general linear mixed model (GLMM). With month and depth as fixed factors and sampling point as random factors, the variation rules of soil physical and chemical properties, microbial abundance, uniformity and diversity with month and depth were analyzed. At the same time, we conducted multivariate analysis of variance with SPSS software to explore the effects of month, depth and their interaction on soil and microorganisms and their significance.

Alpha diversity was analyzed after the taxonomic annotation of OTU using Mothur (Schloss et al. 2009),

$$(H' = - \sum_{i=1}^S P_i \ln P_i,$$

and the Shannon-Wiener Index

S is the total number of species, P_i is the proportion of individuals of this species to the total number of individuals) was used to measure species. Beta diversity analysis was used to compare the similarity of species diversity among different groups. Among them, Principal coordinates analysis (PCoA) used dimensionality reduction thinking to observe the differences in microbial community composition between groups (Anderson and Willis. 2003). And analysis of similarities (Anosim) can test the significance of difference of beta diversity between samples of different groups. All these were analyzed and drew by the vegan package of R language (Anderson and Walsh. 2013; Team RC. 2014).

Another analysis used in this study is significance analysis of differences between groups. The LEfSe (Linear Discriminant Analysis (LDA) effect size) component of LEfSe software was used to analyzing the differences for the composition and abundance of soil bacterial and fungal communities under different treatments (Segata. 2011). And drawing on the LefSe website (<http://huttenhower.org/galaxy/>). LDA score of 2.0 indicates a significant difference, and a score of 4.0 indicates a very significant difference. In addition, structural equation modeling (SEM) was used to assess the potential correlation between various environmental factors and soil bacterial and fungal community structure. This model aimed to explore the direct and indirect effects of each treatment on soil microorganisms when multiple environmental factors are considered simultaneously. And the relationship between bacterial fungi and environmental factors was analyzed using software IBM SPSS Amos 24.0 (Chicago, IL: Amos Development Corporation) (Lefcheck. 2016).

3. Results

3.1 Variation of soil physicochemical properties

Our results showed that soil pH and NH_4^+ increased significantly with month, while VWC reached the maximum in August and September, and ST reached the maximum in July ($p < 0.05$, Table S1). Soil pH and NO_3^- increased significantly with soil depth, while the other soil properties decreased significantly with soil depth ($p < 0.05$, Table S1). Moreover, month had significant effects on soil characteristics (except SOM) and bacterial abundance and evenness, but had insignificant effects on bacterial diversity and fungal abundance, evenness and diversity. In addition, all indicators are significantly affected by depth (Table S2). The results of multivariate analysis of variance showed that the soil physical and chemical indexes (except NO_3^-) were also significantly affected by the interaction of month and depth ($p < 0.05$, Table 1).

3.2 Variation in bacterial and fungal community richness

The bacterial communities of all soil samples were mainly Actinomycetes, Proteobacteria, Acidobacteria, Chloroflexi, Verrucobacteria, and Bacillus (95%). The fungal community was dominated by Basidiomycota, Ascomycota and Mortierella (85%) (Table S3). According to GLMM analysis results, bacterial abundance, diversity, and fungal abundance, evenness, and diversity all decreased significantly with the increase of depth, while bacterial evenness and fungal diversity increased significantly with month (Table S1). Our results showed that the changes in the abundance of phyla of bacteria and fungi were significantly affected by depth and month (Table 2). The abundance of Actinobacteri, Verrucomicrobia, Gmatimonadetes, Ascomycota, Basidiomycota and Mortierellomycota increased significantly with the increase of depth. While the abundance of Proteobacteria, Acidobacteria and Chloroflexi decreased significantly with the increase of depth (Table S3). In addition, the abundance of Ascomycota increased significantly with the increase of month, while the abundance of Verrucomicrobia, Gmatimonadetes, Basidiomycota and Mortierellomycota decreased significantly with the increase of month (Table S4).

3.3 Bate diversity of bacterial and fungal community

According to PCoA results, the bacterial and fungal community compositions of 10 cm were clearly different from that of 20 cm and 30 cm soil layers (Figure 1A-B). As shown in Figure 1C-D, the bacterial community composition in August was quite different from other months, and the community composition in August and September was more specially. Moreover, according to ANOSIM 's analysis, soil fungi were not well grouped by depth and month, and soil bacteria were not well grouped by month either (Figure 1B-D, Supplementary Figure 1).

3.4 Taxonomic composition

With the increase of soil depth (without considering the month factor), the abundance of Actinomycetes, Thermoleophilia, MB_A2_108, and some other bacteria were significantly increased, while the abundance of Acidobacteria, Proteobacteria, Rhizobiales, and Alphaproteobacteria were decreased (Figure 2A). Similarly, the abundance of Ascomycetes, Basidiomycetes, Agaricomycetes and some other fungi increased significantly with the increase of soil depth, while the abundance of Dothideomycetes, Hypocreales, Pleosporales and other fungi was significantly reduced (Figure 2B).

If soil depth wasn't considered when the abundance of soil bacteria and fungi in different months were analyzed contrastively, the month did not have a significant effect on bacteria's abundance (Figure 2C). As compared with bacteria, the abundance of soil fungi was impacted more greatly by month. As shown in Figure 2D, the abundances of Mortierellomycetes and Phaeosphaeriaceae (belonging to Ascomycota) increased significantly in September; the abundances of Cantharellus and Ceratobasidiaceae (all belonging to Basidiomycetes) were highest in May; the abundance of Hypocreales (belonging to Ascomycota) increased in June, and the abundance of Fusarium (also belonging to Ascomycota) increased in August.

3.5 Factors driving bacterial and fungal communities composition

Through Pearson correlation analysis ($p < 0.05$), we found that month and depth were significantly correlated with bacteria and fungi respectively (Figure 3). TOC and VWC were significantly related to the bacterial community, while TOC and ST were significantly related to fungal aggregation ($p < 0.05$, Figure 3). We build a SEM model, which was based on the correlation analysis (Figure 4). The result showed that month, depth, pH, TOC and AN had significant impact on the bacterial communities ($p < 0.05$, Figure 4A). As shown in Figure 4B, TOC and AN had directly positive effects on fungi, soil depth and ST influenced fungi negatively ($R^2=0.35$). The month weakened positive effect of AN on fungal communities and enhanced the negative effect of ST on fungal communities by its negative effect on AN and ST (Figure 4B).

4. Discussion

This study took typical degraded grassland in Inner Mongolia as the basis for multiple sampling at fixed point. Month and depth were used to represent time and space. The variation of pH, VWC and ST with were analyzed to understand the soil physicochemistry and nutrients characteristics varied as time and space changed. Meanwhile, the next generation sequencing technology was used to investigate the changes of microbial diversity and the driving factors of microbial community structure transformation with temporal and spatial variation. Our results provide strong evidence that spatial heterogeneity (depth) is more important than temporal (month) in predicting changes in microbial α -diversity and β -diversity. Variation in microbial community composition was driven by changing environmental factors in their habitat. Considering that soil microbial community composition was related to community function

(Fierer et al. 2012), temporal and spatial changes mainly interfered with the ecological function of soil microbial community, rather than the stability of grassland soil ecosystem.

4.1 Vertical spatial variation of microbial communities and soil properties

Our results indicate that all measured soil physicochemical indices are significantly affected by depth. Soil pH increased significantly with depth increasing, while ST, VWC, SOM and AN decreased significantly with increasing depth. This may be because the top soil layer (0-10 cm) was seriously influenced by external environment conditions. In particular, in Inner Mongolia, grasslands had been influenced by human activities like grazing and mowing for very long time, which resulted in the decline of the productivity and diversity of grassland vegetation (Xun et al. 2018). Furthermore, human activities also resulted in the increased bare area, the aggravated erosion and coarseness of surface soil, and reduced nutrient content (Fierer et al. 2009). Relatively, the environment of the deeper layer (20-30cm) is stable. However, as increasing of soil depth, the distribution of plant roots decreased, and the plant litter and secretions decreased. It may lead to a lower soil nutrient content than the surface layer (Truong and Marschner. 2018). As soil bulk density increases, porosity and oxygen content decrease, which is not conducive to the survival of microorganisms and inhibits the activities of enzymes involved in decomposition (Bagheri et al. 2013; Holt. 1997). And leads to the decrease of soil carbon and nitrogen availability (Wang et al. 2014). According to our results, most of the soil physical and chemical indexes was significantly affected by the month, but each index varies without rule between months, which requires further study and discussion.

We found that both soil characteristics and microbial community structure were more significantly correlated with soil depth than with time variations. It was consistent with previous findings, which confirm the importance of spatial heterogeneity (Fierer et al. 2006; Lauber et al. 2013), and can vary even on the scale of meters or even centimeters (O'Brien et al. 2016). Some studies have found that microbial community structure and abundance respond to changes in environmental factors to the same degree (Bell et al. 2014; Na et al. 2019), but some studies have shown that community composition is more sensitive than community diversity (Fierer et al. 2006). However, in our study was no found that response differences in community composition and diversity. Instead, it was found that bacterial abundance and evenness were significantly affected by both soil depth and month, while the abundance, evenness and diversity of fungi were only significantly affected by depth. We speculate that the response difference between bacteria and fungi is due to their own factors. Because bacteria are more susceptible to local changes in soil properties (Sorensen et al. 2013), while the evolutionary life history of fungi enables them to form hyphae structures and highly resistant spores that are able to withstand sudden environmental changes (Sun et al. 2017). In addition, the individual size of fungi is usually larger than that of the bacterial members of the community, which results in transmission limitations severely (Young. 2006; Schmidt et al. 2014). It is also worth noting that the interaction between month and depth weakened the effect of depth on microbial community, and only had a significant effect on fungal diversity. This indicates that the results of single factor and multi-factor influence are quite different and unpredictable.

Therefore, future research on microbial ecology should set up as many control factors as possible to understand more really changes of microorganisms.

4.2 Driving factors of soil microbial community structure

Changes of soil properties had the potential impacts on variation of soil microorganisms in the vertical section. According to our results of LEfSe analysis, Acidobacteria and Proteobacteria were enriched in the top layer (0-10 cm), while Actinomycetes, Ascomycetes and Basidiomycetes had the high abundance in deeper layer (20-30 cm) (Figure 3). Simultaneously, VWC and SOM gradually reduced as depth increasing, in the opposite pH increased gradually (Table S1). Therefore, the variation of VWC and SOM explained here the abundance changes of Acidobacteria and Proteobacteria, and the variation of pH explained the abundance changes of Actinomycetes, Ascomycetes, and Basidiomycetes. Studies had shown that changes in soil fungal communities are significantly correlated with soil moisture and pH (Zheng et al. 2009), and pH is the main driving force for formation of soil microbial communities (Wang et al. 2014). On the other hand, different microbial communities have different utilization of nutrients. Compared with fungi and actinomycetes, bacteria use smaller organic matter molecules, while fungi and actinomycetes can decompose substrates with relatively large molecules by producing lignin degrading enzymes (Bonanomi et al. 2017; Bonanomi et al. 2017). This may also be the reason why the fungal diversity in the soil layer of 30cm was greater than that of 10cm in the analysis of α diversity.

We also found that the temperature is highest in July and August, and the soil moisture content is highest in June and July. Soil pH is alkaline, NO_3^- , NH_4^+ content is limited. The contents of NH_4^+ and NO_3^- are the minimum in August, and the pH is the highest in August. According to the analysis of influencing factors, month has a direct and significant positive effect on bacteria, and promotes its significant positive effect on bacteria by indirectly affecting pH and VWC. However, from the perspective of taxonomic composition, this effect did not significantly affect the species abundance of the community. Indicating that the composition of soil bacterial community was relatively stable during the whole plant growing season. However, month has no direct effect on fungi, but indirectly affects fungi through AN and ST. This indirect effect causes significant changes in species abundance of fungal community. In other words, AN and ST affected the changes of Ascomycetes, Basidiomycetes and Mortierellomycetes. However, some studies have shown that in the arid and semi-arid grassland ecosystem in the eastern part of Inner Mongolia, soil microbial biomass (C_{mic} , N_{mic}), soil TOC, TN, NH_4^+ all increase with the increase of precipitation, while pH value decreases with the increase of precipitation (Yao et al. 2017)

Nevertheless, 65-70% of the variation in microbial composition was not explained by month and depth, or environmental variables in our study. The possible reason is the existence of other unmeasured environmental factors that vary in space and time (Bahram et al. 2015), including biotic interactions such as competition, mutualism, and predation between microbial taxa (Zhou et al. 2017) and ecological processes such as dormancy and persistence traits of microbial communities and their members (Averill et al. 2019).

5. Conclusion

The article analyzed the spatiotemporal variation and driving factors of soil microbial community structure in typical degraded steppe. The results show that both month and soil depth had significantly effect on microbial community structure and soil properties, but depth has a more significant effect. The abundance and diversity of bacteria and fungi were significantly affected by depth. The abundance of Acidobacteria, Proteobacteria, Actinomycetes, Ascomycetes and Basidiomycetes varies significantly with depth. What's more, soil pH increased significantly with depth increasing, while SOM, AN, VWC and ST decreased significantly with increasing depth. Therefore, we speculate that SOM and VWC account for the abundance variations of Acidobacteria and Proteobacteria, and pH cause the abundance changes of Actinomycetes, Ascomycetes and Basidiomycota. In addition, this study only analyzed the microbial changes in each month of the plant growing season in a year, which may underestimate the real microbial time changes. Therefore, more and longer time points should be included in the design of future similar studies, including those on microbial biogeography. In conclusion, spatial and temporal studies of soil microbial ecology provide a more comprehensive basis for understanding the key factors that regulate biodiversity in soil ecosystems.

Declarations

Data Availability Statement

Sequence data have been deposited in the NCBI (<https://www.ncbi.nlm.nih.gov/sra>) under the accession numbers PRJNA664840.

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Author Contributions

Hongbin Zhao: The conception of the study (lead); writing - final draft & review & editing (equal). Wenling Zheng: Conceptualization (equal); data analysis (lead); writing – original draft (lead). Shengwei Zhang: The conception of the study (lead); funding acquisition (lead). Wenlong Gao: Methodology (supporting). Yueyue Fan: Writing - review & editing (supporting).

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Conflict of Interests

The authors declare that there are no conflict of interests.

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

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Tables

Table 1 Multivariate analysis of soil properties and microbial community α -diversity index (two-way ANOVA)

Variables	Month		Depth		Month* Depth	
	f value	p value	f value	p value	f value	p value
pH	3.184	0.018	5.841	0.000	2.186	0.038
SOM	1.598	0.184	4.608	0.013	3.651	0.001
NO ₃ ⁻	4.909	0.001	120.863	0.000	1.120	0.360
NH ₄ ⁺	44.690	0.000	53.510	0.000	7.236	0.000
VWC	88.411	0.000	5.098	0.008	14.011	0.000
ST	14102.554	0.000	21.398	0.000	188.361	0.000
Bacterial OTU richness	2.027	0.099	29.239	0.000	1.744	0.102
Bacterial ACE estimator	4.180	0.004	16.832	0.000	1.700	0.112
Bacterial Chao1 estimator	3.025	0.023	6.511	0.002	1.427	0.199
Bacterial Simpson index	1.239	0.302	3.590	0.032	1.232	0.293
Bacterial shannon index	0.831	0.509	67.406	0.000	1.162	0.333
fungus OTU richness	1.157	0.337	72.667	0.000	0.603	0.773
fungus ACE estimator	1.135	0.347	17.069	0.000	0.399	0.918
fungus Chao1 estimator	1.274	0.288	29.850	0.000	0.398	0.918
fungus Simpson index	0.258	0.904	22.016	0.000	1.173	0.327
fungus Shannon index	5.841	0.000	2.564	0.084	3.257	0.003

Note: f value is the value of F test, p value significance level, p < 0.05 means significance, p < 0.01 means extremely significant.

Table 2 GLMM results showing effects of month and depth on relative abundance of dominant bacterial and fungal phyla.

Bacterial phylum	Month (Fixed effect)			Depth (Fixed effect)		
	df	t _{1,4}	p value	df	t _{1,2}	p value
Actinobacteria	4	4.357	0.107	2	16.564	0.000
Proteobacteria	4	2.692	0.018	2	22.837	0.000
Acidobacteria	4	4.39	0.002	2	12.143	0.000
Chloroflexi	4	7.067	0.046	2	23.571	0.000
Verrucomicrobia	4	6.481	0.004	2	4.464	0.001
Gemmatimonadetes	4	11.206	0.000	2	11.987	0.000
Rokubacteria	4	5.419	0.020	2	7.972	0.000
Bacteroidetes	4	4.878	0.037	2	10.093	0.000
Planctomycetes	4	7.435	0.014	2	13.84	0.000
Firmicutes	4	6.376	0.000	2	7.032	0.000
Ascomycota	4	9.658	0.001	2	3.445	0.056
Unclassified	4	8.643	0.002	2	9.465	0.000
Basidiomycota	4	10.82	0.002	2	4.817	0.001
Mortierellomycota	4	7.009	0.001	2	4.848	0.006
Unassigned	4	7.889	0.097	2	5.094	0.008
Glomeromycota	4	0.391	0.987	2	0.386	0.127
Chytridiomycota	4	3.547	0.265	2	6.439	0.001
Aphelidiomycota	4	1.913	0.878	2	0.76	0.890
Olpidiomycota	4	2.239	0.463	2	0.255	0.964
Cercozoa	4	1.686	0.981	2	1.383	0.703

The effects of month and soil layer depth on relative abundances of bacterial phyla were determined by using GLMM with altered precipitation regime as the fixed factor and block as random factor. p < 0.05.

Figures

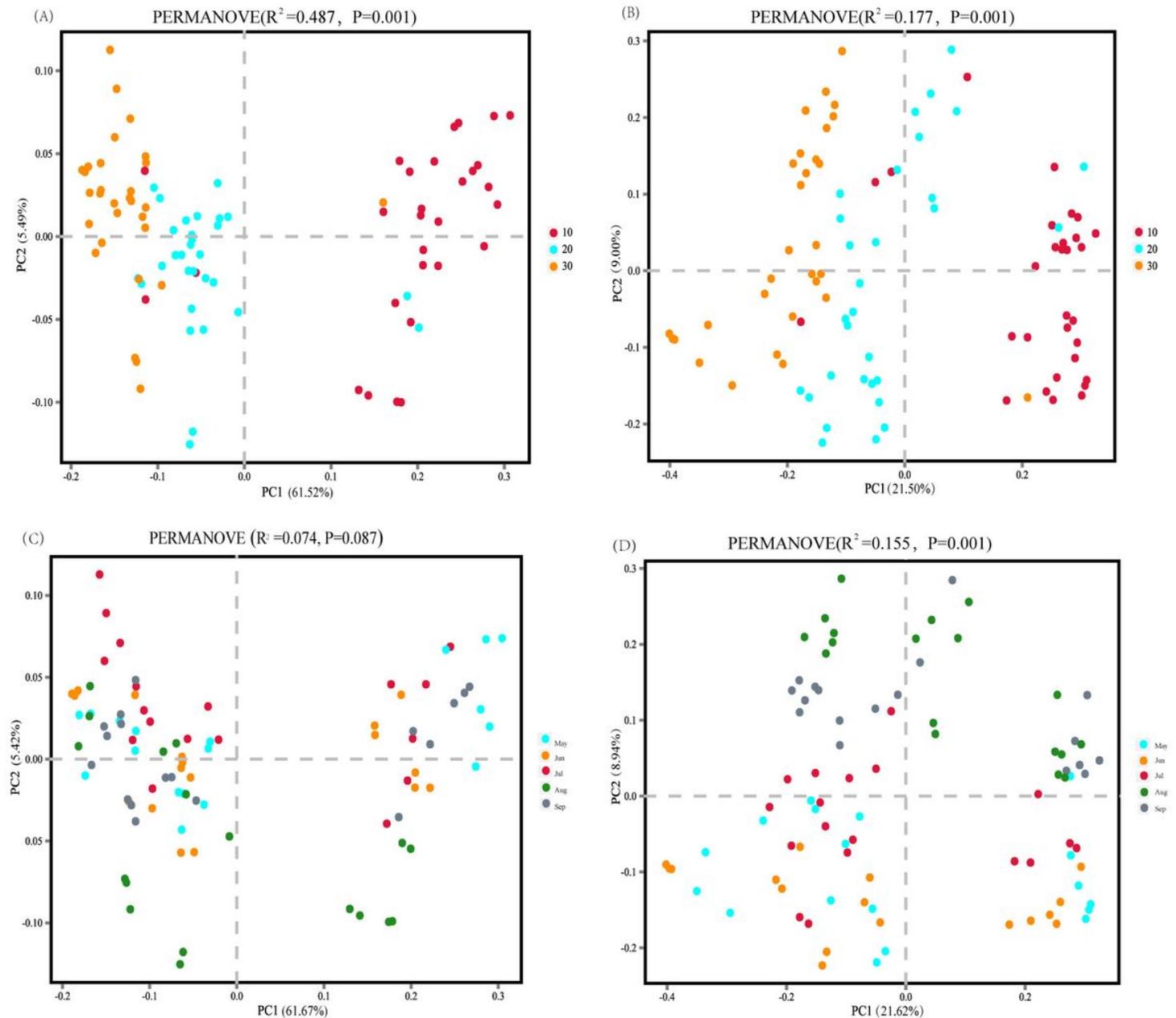


Figure 1

PCoA analysis of soil microbial community structure based on Bray-Curtis distance algorithm. (A) and (B) represent bacteria and fungi at different soil depths, and (C) and (D) represent bacteria and fungi in different month. The dots represent each sample; different colors represent different groupings. The horizontal and vertical coordinates are the two characteristic values that cause the largest difference between samples, and reflect the main degree of influence as a percentage. 10, 20, and 30 represent 10 cm, 20 cm, and 30 cm of soil depth, respectively. May, Jun, Jul, Aug, and Sep represent the soil sampled in May, June, July, August, and September, respectively. The PERMANOVA analysis is based on the Bray-Curtis distance algorithm, the result shows that depth had a significant effect on both bacterial

($R^2=0.487$, $p=0.001$) and fungal ($R^2= 0.177$, $p=0.001$) communities. Month had a significant effect on fungal communities ($R^2= 0.074$, $p=0.087$) and a little effect on bacterial communities ($R^2= 0.155$, $p=0.001$).

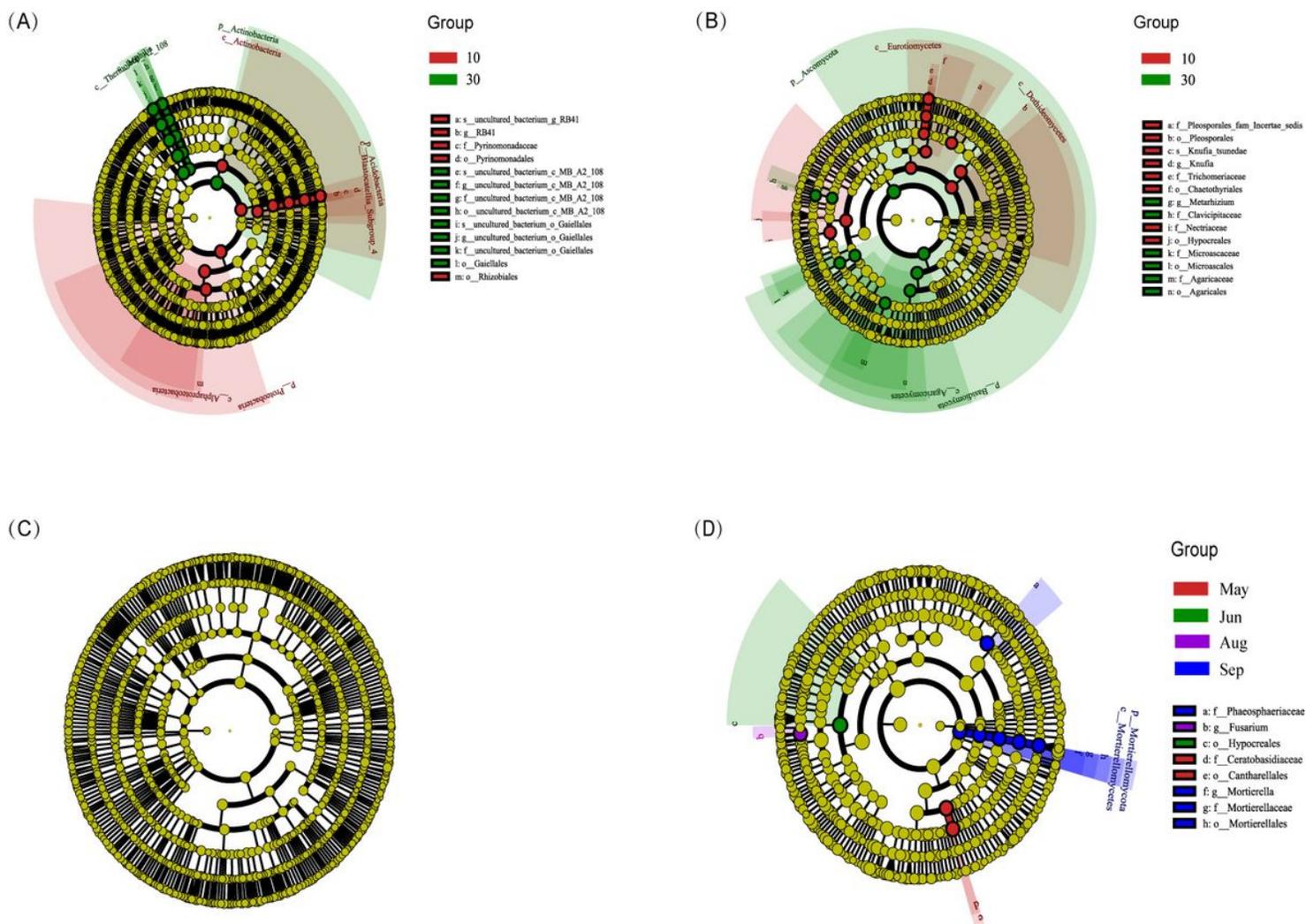


Figure 2

LefSe analysis of soil bacteria and fungi. (A) and (B) represent bacteria and fungi at different soil depths, and (C) and (D) represent bacteria and fungi in different months. The figure shows species with LDA Score greater than 4.0 (LDA Score greater than 4.0 indicates a significant difference), p-value less than 0.05, and different colors indicate species in different groups. 10 represents 10cm soil layer and 30 represents 30cm soil layer. May, Jun, Aug, and Sep respectively represent sampling in May, June, July, August, and September.

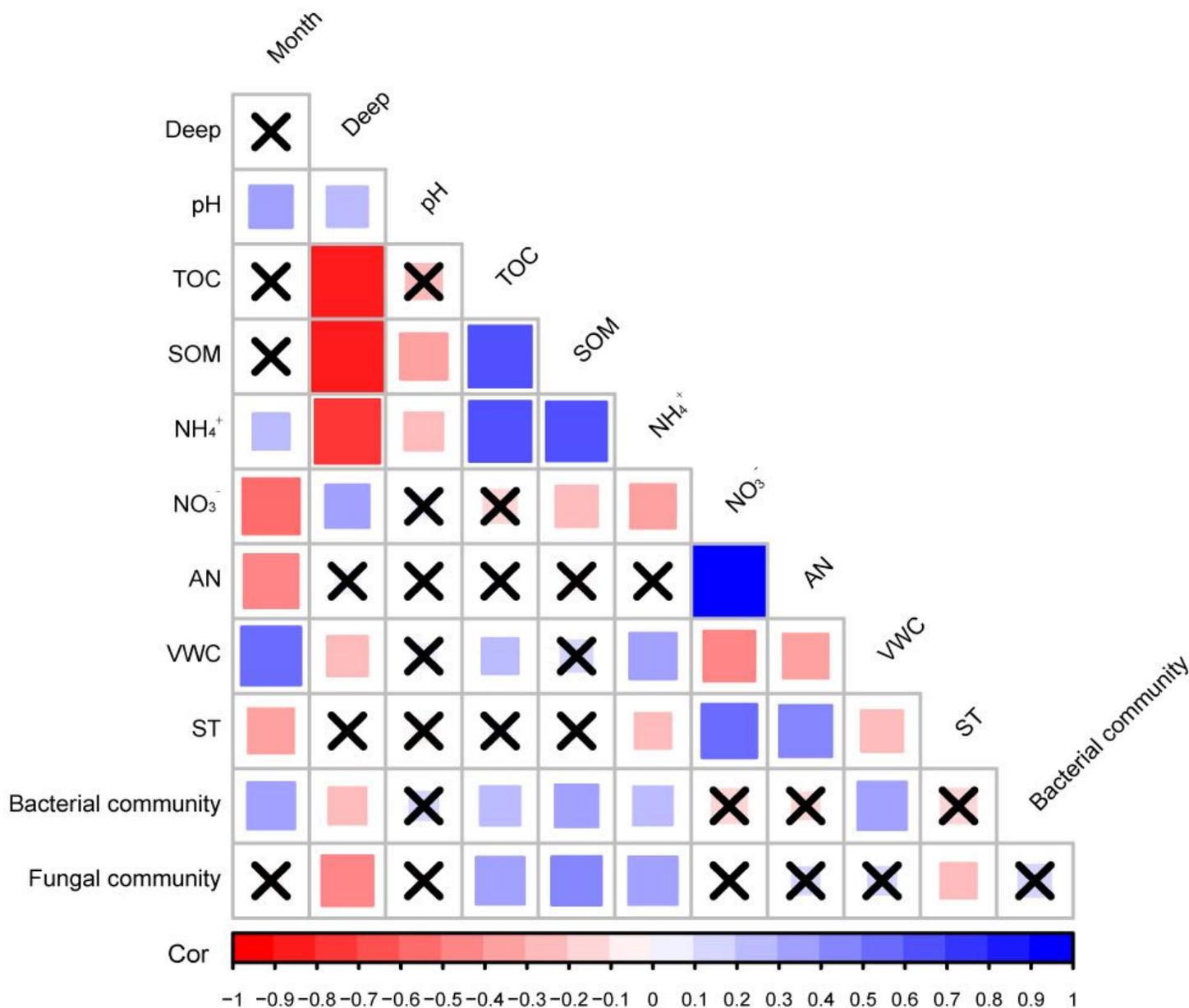


Figure 3

Pearson correlation analysis between month, depth and soil properties and bacterial and fungal community structures. TOC was short for total soil organic carbon; SOM was short for soil organic matter; AN was short for available ammonium (the sum of NO₃⁻ and NH₄⁺); VWC was short for volume water content of soil; ST was short for soil temperature. The bolding indicates significance at p < 0.05.

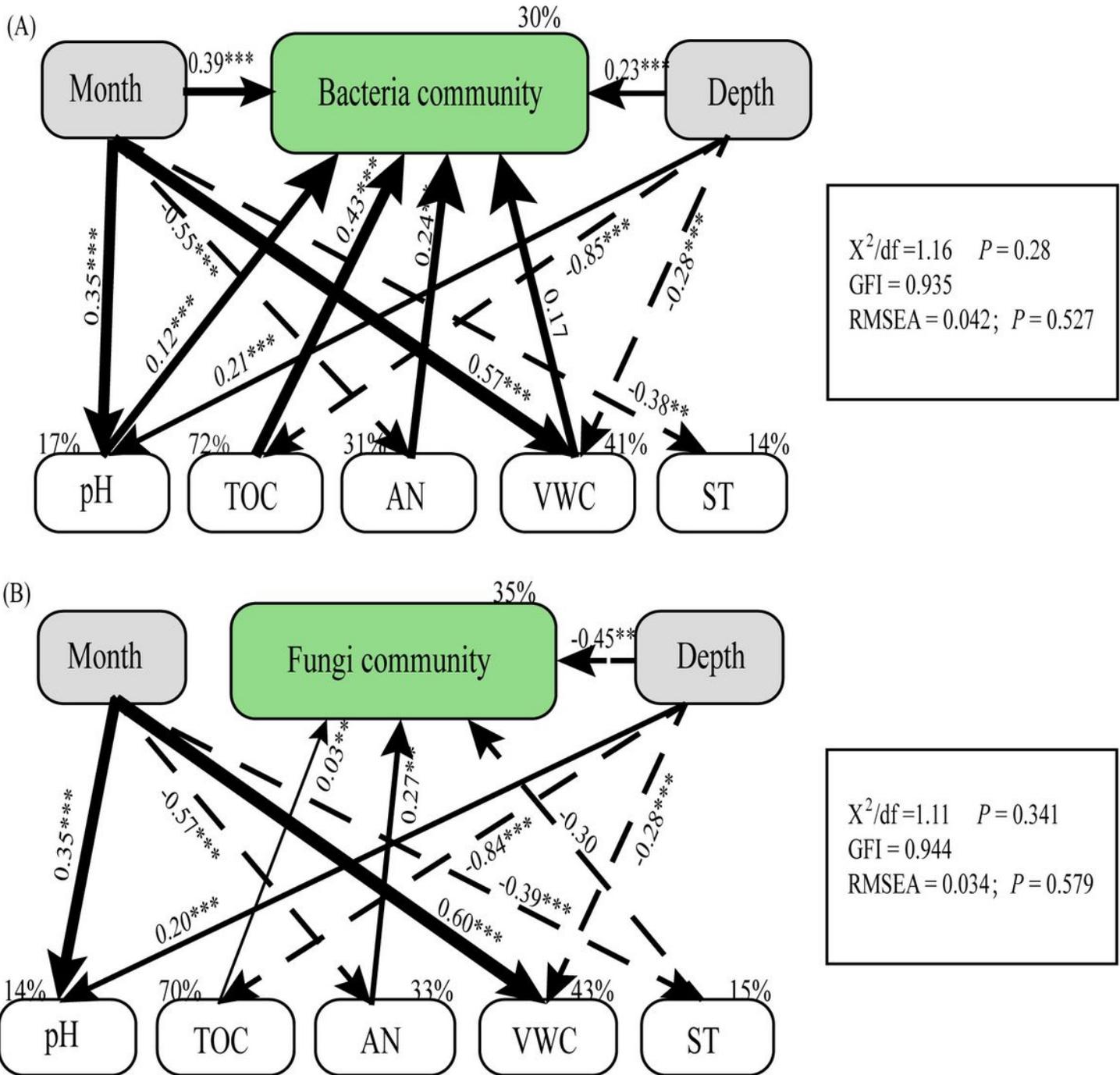


Figure 4

SEM analysis of bacteria and fungus s with environmental factors. (A) and (B) represent structural equation model analysis of bacteria and fungi. TOC, AN, VWC, ST represent as above; BC was short for bacterial community; FC was short for fungi community. Solid arrows indicate positive correlations, and dotted arrows indicate negative correlations. Values associated with arrows indicate standardized path coefficients (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Arrow width represents the size of the corresponding normalized path coefficient. Percentage (R²) associated with response variables represent the proportion of explained variation by other variables.

Supplementary Files

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