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论文题目 Title of Paper: Vertical and temporal variations of soil bacterial and archaeal communities in maize-soybean rotation agroecosystem

Vertical and temporal variations of soil bacterial and archaeal communities in maize-soybean rotation agroecosystem

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Abstract

Soil microbes are an essential component of terrestrial ecosystems and drive many biogeochemical processes throughout the soil profile. However, the knowledge on soil microbial community in sub-surface soil remains insufficient. In the present study, I investigated the vertical and temporal patterns of soil bacterial and archaeal communities in maize-soybean rotation agroecosystem at a depth of millions of sequences per sample. Our results revealed different vertical bacterial and archaeal richness patterns: bacterial richness was lowest in the deep soil and peaked in the surface or middle soil layers. In contrast, archaeal richness did not differ among soil layers. PERMANOVA analysis indicated that both bacterial and archaeal community composition was significantly impacted by soil depth but unaffected by sampling time. Notably, the proportion of rare bacteria gradually decreased along with the soil profile. The rare bacterial community composition was the most important soil nutrient fertility index indicator, as determined by random forest analysis. The soil prokaryotic co-occurrence network in the surface and middle soil layers was more connected and harbored fewer negative links than the deep soil layer. Overall, our results highlighted that soil depth is a more important determinant than temporal variation in shaping prokaryotic soil community and interspecific interactions and revealed a potential role of rare taxa in soil biogeochemical function.

Keywords

Soil microbiome, high-throughput sequencing, keystone species

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Inspiration

Environmental degradation has been a buzzword since the start of high school biology. My mind has been stocked with facts regarding eutrophication and marine pollution, with chemical fertilizer use deemed the greatest culprit. Surface run-off into aquatic bodies contaminates the water with excess nutrients, leading to algae blooms which ultimately make the water anoxic.

With increasing attention towards environmental protection and conservation, schemes have been proposed to curtail the damage, one of which details the utilization of microorganisms to manage soil fertility in place of chemical substances. I could not contain my curiosity on how bacteria and fungi could provide nourishment and wondered what the prokaryotic soil community comprised of - the idea of a biofertilizer intrigued me. Initially, when I perceived 'Environmental Sciences,' I thought of the ecosystem, flora, and fauna, but never the life beneath our feet. My gravely and defectively narrow definition of the discipline caused me to overlook a potential enhancer for agriculture. Perhaps, visibility is a trap; the soil boundary has acted as a façade for the mysteries of nature.

Following the steady emergence of soil microbiomics, it has been recognized that the key to developing and advancing the application of this microbial tool is to expand the existing realm of knowledge on the diversity and richness of soil species. High-throughput sequencing combined with bioinformatics is an essential technique to

identify keystone species and abundant species that can be targeted for widespread use. Some of the world's largest food and agriculture companies have already invested in biological solutions as the future of agriculture (Batista & Singh, 2021). This proactive attitude affirms the biofertilizer market as one with great potential.

Overall, studying soil microbiomes can shed light on how to manipulate soil composition to increase soil fertility, improve crop yield, and anticipate the response of soil microbiomes to ongoing climate change. This aims to contribute my efforts to the investigation into microbial resources for agricultural use.

Introduction

Soil microbes drive many biogeochemical processes throughout the soil profile and are an essential component of terrestrial ecosystems (Cao et al., 2012; Caporaso et al., 2011; Fierer, 2017; Jiao et al., 2018). A large body of studies have explored their local, regional, or continental patterns, yet, the understanding of soil microbial diversity and community composition remains limited to the surface soil layer (top ~20 cm) when deep soil (> 20 cm) is also crucial for crop growth in agroecosystems (Cao et al., 2012; Caporaso et al., 2011; Crowther et al., 2019; Fierer, 2017; Jiao et al., 2018). Crop roots generally extend below 20 cm, even to depths as deep as 150 cm (Fierer, 2017). Given that the deeper soil harbors more than two-thirds of the total soil organic carbon and nearly equal amounts of phosphorus (Gy & Jackson, 2000), determining the distribution of soil microbes in deeper soil layers would contribute to the prediction of their roles in

carbon sequestration and nutrient cycling.

The vertical soil profile is highly heterogeneous, with drastic differences in soil texture, nutrients availability, and organic carbon levels among different soil depths (Jiao et al., 2018; Kim et al., 2016). Previous surveys of vertical microbial distributions revealed that soil depth could be a key determinant in shaping microbial diversity and community assembly in alpine meadows, forests, and agroecosystems (Eilers et al., 2012; Hao et al., 2020; Xu et al., 2021). However, existing surveys of vertical microbial distributions mainly employed one-time sampling, which only captures a specific status of soil microbes with temporal variations neglected. Soil microbial communities could be highly dynamic and show temporal patterns (Kivlin & Hawkes, 2020; Yang et al., 2019; Yang, Yang, et al., 2020). In previous meta-analyses, soil microbial biomass and community composition were temporally variable across the globe (Shade et al., 2013; Wardle, 1998). Therefore, incorporating temporal variations could be crucial to understanding vertical distributions of soil microbes.

Although bacteria and archaea are abundant in soil, they occupy different environmental niches and are sometimes filtered by different soil variables (Wei et al., 2020). Soil pH was identified as the primary ecological filter for bacterial communities, while soil C/N ratio or salinity were the primary ecological filters for archaeal communities (Auguet et al., 2010; Bates et al., 2011). These differences lead to contrasting vertical distribution patterns between archaea and bacteria, corroborated by

previous reports. Bacteria diversity generally decreased towards deeper soil depth, while archaeal diversity increased along with soil depth in the desert and alpine ecosystems (Wang et al., 2021; Xu et al., 2021). However, others reported that bacterial and archaeal richness negatively correlated with soil depth in paddy soils (Yuan et al., 2020). Together, the varying conclusions of these studies emphasize the need for further comparisons between the vertical distribution patterns of archaeal and bacterial communities.

Microbial communities typically display a skewed species abundance distribution, with relatively few abundant species co-existing with many rare species (Jousset et al., 2017). Previous studies have indicated that abundant and rare species may possess different functional traits (Jiao & Lu, 2020). Although key roles of abundant microbial taxa in soil function have been well understood, relatively less attention has been paid to rare taxa (Chen et al., 2020). Rare taxa are important reservoirs of genetic diversity, as they provide functional redundancy and impact ecosystem stability (Jousset et al., 2017). In addition, rare taxa may be functionally dissimilar to the abundant members (Liang et al., 2020), offering complementary functions or unique metabolic pathways to support the overall community functioning. Despite their importance, rare species in soil are often neglected due to the low sequencing coverages. Therefore, deep sequencing with millions of reads would provide higher resolution for the rare species detection and a more comprehensive depiction of the overall soil microbial community along with the soil profile.

The present study investigated the vertical and temporal patterns of soil bacterial and archaeal communities in maize-soybean rotation agroecosystem at a scale of millions of sequences per sample. The aims were to determine the following: (1) Which factor from soil depth and temporal fluctuations is the key determinant in shaping bacterial and archaeal communities; (2) Whether there exist differences in the vertical distribution pattern between soil bacteria and archaea; (3) Whether rare taxa occupy a larger proportion in the surface soil layer than in the deep soil layer.

Materials and methods

Field description

The field experiment was carried out at the Chinese Academy of Agricultural Science, located in the North China Plain (39.97 N, 116.33 E), an important food-producing region in China. This region has a warm temperate monsoon climate, with an average annual temperature and annual precipitation of 11–13 °C and 500–700 mm (80% from July to August). The soil is classified as fluvo-aquic soil (Chinese soil taxonomy) with a loamy texture. The field was fertilized before wheat planting (September 10) under the wheat-soybean rotation system; other agricultural practices, including weeding and tillage, were identical to those done by the local agricultural management.

Soil sampling and physiochemical analysis

Soil sampling was conducted on October 18 (wheat season) and December 18 (wheat

season) of 2019 and April 17 (wheat season), and August 17 of 2020 (soybean season). In brief, six plots (1 × 1 m) were randomly selected from within an area of 20 × 20 m in our study site. For each plot, five soil cores were collected between two plant individuals from a 100 cm long vertical profile that corresponded to depths of 0–20 cm (surface soil layer), 20–50 cm (middle soil layer), and 50–100 cm (deep soil layer). Therefore, each soil sample was a mixture of five soil cores (3.8 cm diameter) for a given soil layer. Seventy-two soil samples (6 plots × 4 sampling times × 3 soil depth) were taken from the four sites. The freshly collected soil samples were treated separately: one part was sieved (< 2 mm) and stored at −80 °C for DNA extraction; the remaining was sieved (< 0.25 mm and < 1 mm) to determine the soil physiochemical parameters, stored at 4 °C or room temperature (Yang, Guan, et al., 2020). Soil physiochemical characteristics including soil total carbon (TC), total nitrogen (TN), dissolved organic carbon (DOC), dissolved organic nitrogen (DOC), nitrate (NO_3^--N) and ammonium (NH_4^+-N), moisture, pH, clay, silt, and sand content were determined.

Miseq sequencing and bioinformatics

The soil DNA was extracted using a PowerSoil DNA Isolation Kit (MO BIO Laboratories, USA). The V4 region of 16s rDNA was amplified using primer pairs 515F/806R (Caporaso et al., 2012). Primer 515F contained a 12 bp barcode unique to each sample for Miseq sequencing detection. Following Caporaso et al., all PCR reactions were carried out in triplicate in a 25 μl system (Caporaso et al., 2011; Yang, Guan, et al., 2020). The PCR products were then pooled, purified, and sequenced on an

Illumina MiSeq platform at Majorbio Biotech Co., Ltd. (Shanghai, China). The raw sequence data have been deposited on the NCBI SRA (Accession No. SRP271579).

The raw sequences were trimmed to the shortest sequence length using QIIME Pipeline Version 1.8.0 (Caporaso, 2010). Subsequently, the sequences were dereplicated, with all singletons discarded. Sequences were then error-filtered and grouped into amplicon sequence variants (ASVs) using the Deblur software (Amir et al., 2017), with ASVs containing less than two reads removed. Further, potential chimeras were discarded using the Vsearch software. The number of sequences per sample was rarefied to 1,000,000 using the "vegan" package in R. The ASVs were blasted against the silva 16s database to assign their taxonomic annotation. Then, the ASV abundance tables were rarefied at 845,591 for bacteria and 10,413 for archaea to ensure even sampling depth within each prokaryote group.

Data analysis

ASVs with relative abundance below 0.01% were defined as "rare" taxa, while those with relative abundances above 0.1% were defined as "abundant" taxa. Soil fertility index is a synthetic variable calculated from the sum of z-score transformation of TN, TC, DON, DOC, NH_4^+-N, and NO_3^--N. Alpha-diversity indices of bacteria and archaea, such as richness and Pielou evenness index, were calculated using the "vegan" package in R (Oksanen et al., 2013). Two-way analyses of variance (ANOVAs) were then performed to examine the effects of soil depth, sampling time, and their interaction on

alpha-diversity indices and the proportion of rare ASVs. All data were tested for normality and homogeneity of variance before two-way ANOVAs. A Tukey's HSD posthoc test determined paired comparisons among the treatments at a 5% significance level. Random forest analysis (Breiman, 2013) was used to examine the soil physiochemical drivers of bacterial and archaeal richness and evenness using the "randomForest" package (Liaw & Wiener, 2002). The "rfPermute" package (Archer, 2016) was then utilized to estimate the significance of important metrics for a random forest model by permuting the response variable.

The effects of soil depth, sampling time, and their interaction on soil bacterial and archaeal community compositions were evaluated using a permutational multivariate analysis of variance (PERMANOVA) with 999 permutations in the "vegan" package (Oksanen et al., 2013). The bacterial and archaeal community compositions were subsequently ordinated using principal coordinates analysis (PCoA) based on the Bray-Curtis dissimilarity matrices in the "vegan" package (Yang, Guan, et al., 2020). A ternary plot was used to demonstrate bacterial and archaeal ASVs distribution across the vertical soil profile.

Soil prokaryotic co-occurrence networks for the surface, middle, and deep soil layers were built. Each network was based upon 24 prokaryotic soil communities. ASVs with relative abundances > 0.5% and occurred in > 50% communities were included in the networks to focus solely on abundant ASVs. Spearman's correlation coefficients were

calculated between ASVs using the "Psych" package (Revelle, 2015). P-values for multiple tests were calculated using the false discovery rate (FDR), as described by Benjamini and Hochberg (Benjamini & Hochberg, 1995). The correlations with a Spearman's coefficient < 0.8 and a P-value > 0.001 were eliminated (Widder et al., 2014). Subsequently, calculations were done for the average degree, connectedness, number of positive and negative correlations, and modularity in the networks through the "igraph" packages. Then, the topological roles of each node were evaluated by the threshold values of Zi and Pi in each network, as proposed by Guimera and Amaral (Guimerà & Nunes Amaral, 2005). All analyses above were carried out in R (v.3.6.2).

Results

Sequencing data analysis and prokaryotic diversity

A total of 185,093,268 reads were obtained after quality control and chimera checks, assigned as 225,608 ASVs. The read numbers were normalized to 1,000,000 for each sample, resulting in a normalized dataset containing 163,862 prokaryotic ASVs. Then, the read numbers of bacteria and archaea were normalized to 845,591 and 10,413 after species annotation, resulting in two normalized datasets containing 162,802 bacterial ASVs and 213 archaeal ASVs. Among these ASVs, 7382 ASVs (4.5%) occurred in at least half of all samples.

The bacterial and archaeal diversities were assessed using the richness and Pielou evenness indices. One of the most interesting findings was that the vertical pattern of

soil bacterial and soil archaeal diversity differed. Two-way ANOVA analysis indicated that both bacterial richness and evenness were significantly affected by soil depth (Table 1). Generally, bacterial richness and evenness were lowest in the deep soil layer and peaked in the surface or middle soil layer (Fig. 1). In contrast, soil depth unaffected archaeal richness, while its evenness showed a decreasing trend along with the soil profile (Fig. 1).

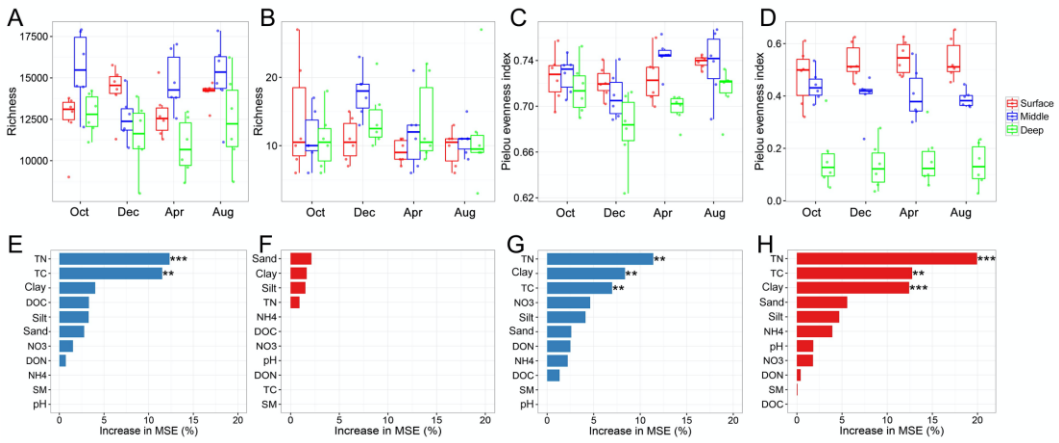

Figure 1. Bacterial richness (**A**), archaeal richness (**B**), bacterial Pielou evenness index (**C**), and archaeal evenness index (**D**) among soil layers in October, December, April, and October; random forest mean predictor importance of soil variables on bacterial richness (**E**), archaeal richness (**F**), bacterial Pielou evenness index (**G**), and archaeal evenness index (**H**). Significance level: $P < 0.05$, *; $P < 0.01$, **; $P < 0.01$, ***. Abbreviations: TC, total carbon; TN, total nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; NO_3^-, nitrate; NH_4^+, ammonium; SM, soil moisture.

I did not find an apparent temporal variation of archaeal richness or evenness (Table 1). Although bacterial richness did not show temporal variation (Table 1), its evenness index was significantly higher in October than in April (Fig. 1). Random forest analysis indicated that TN, TC, and sand content were the key determinants for bacterial and archaeal diversity (except for archaeal richness).

Table 1. Two-way ANOVA examining the effects of soil depth and sampling time on bacterial and archaeal richness (S) and Pielou evenness (J) indices.

			Soil depth (D)			Sampling time (T)			D × T	
			F	P		F	P		F	P
Bacteria	S		11.97	< 0.001		1.70	0.18		2.11	0.07
	J		13.60	< 0.001		5.50	0.002		1.28	0.28
Archaea	S		1.10	0.34		1.60	0.20		1.27	0.28
	J		121.34	< 0.001		0.13	0.94		0.63	0.71

Soil prokaryotic community composition

PERMANOVA analysis indicated that both bacterial and archaeal community composition were significantly impacted by soil depth (Bacteria: $r2 = 0.38$, $P < 0.001$; Archaea: $r2 = 0.70$, $P < 0.001$), but unaffected by sampling time and their interaction (all $P > 0.05$). This pattern was further evidenced by PCoA ordination based on Bray-Curtis dissimilarity, which indicates that the bacterial and archaeal community composition was separated by soil depth. I then tested for the temporal variations of bacterial and archaeal community composition in each soil depth. I observed that the archaeal soil community did not show any temporal variations in any soil depth (all $P > 0.05$, Fig. 2). In contrast, bacterial community composition exhibited strong temporal variation in the middle soil layer (PERMANOVA: $r2 = 0.13$, $P = 0.006$, Fig. 2)).

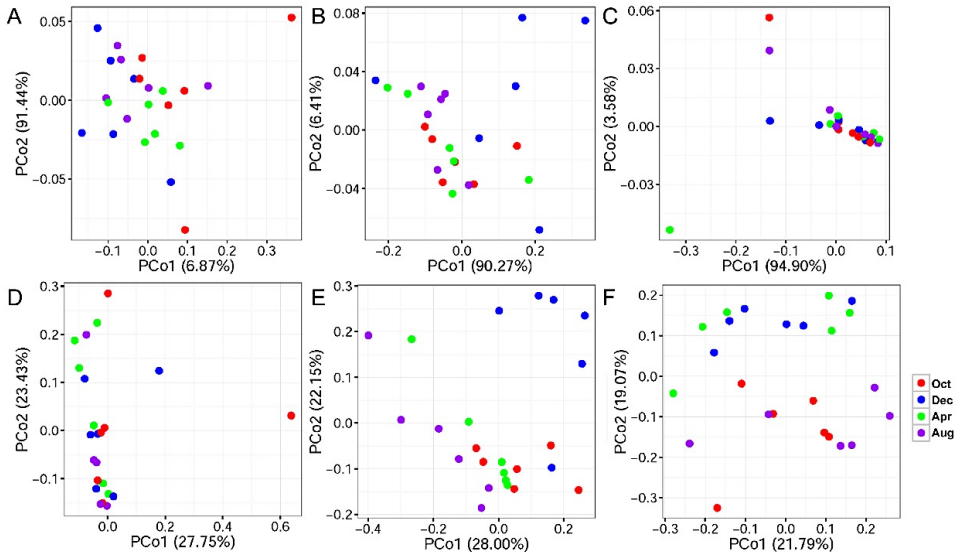

Figure 2. Principal coordinate analysis (PCoA) of soil archaeal community in surface (**A**), middle (**B**), and deep (**C**) soil layer among seasons; principal coordinate analysis (PCoA) of soil bacterial community in surface (**D**), middle (**E**), and deep (**F**) soil layer among seasons.

At the phylum level, the bacterial community was mainly dominated by Actinobacteria, Proteobacteria, Firmicutes, Chloroflexi, and Acidobacteria, while the archaeal community was mainly dominated by Crenarchaeota in our study site, accounting for 69.19% of the total prokaryotic sequences (Fig. 3).

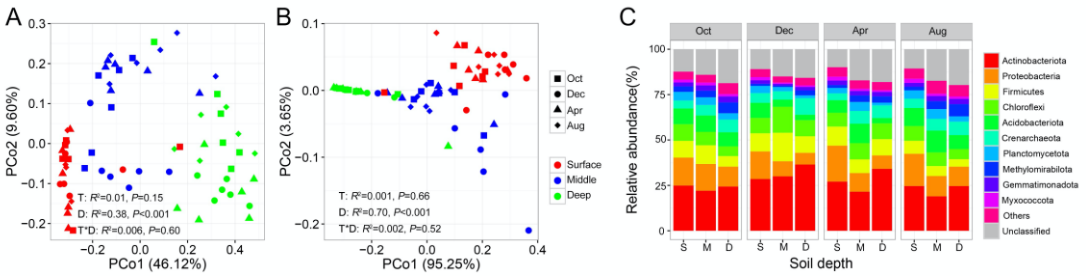

Figure 3. Principal coordinate analysis (PCoA) of soil bacterial community (**A**) and archaeal community (**B**) along soil profile in October, December, April, and August; relative abundance of prokaryotic phyla shifts along soil profile in October, December, April, and August (**C**). Abbreviations: S, surface soil layer; M, middle soil layer; D, deep soil layer.

Next, I investigated the changes in relative abundance patterns of prokaryotic phyla associated with soil profile. The relative abundance of nearly all phyla was significantly different among soil layers in all sampling time, except for Gemmatimonadetes and Chloroflexi. I observed that Proteobacteria, Firmicutes, Acidobacteria, Planctomycete, and Myxococcota, especially, decreased along with soil profile, while Actinobacteria and Crenarchaeota exhibited the opposite trend (Fig. 3, Table 2). Most strikingly, the proportion of unclassified ASVs showed an increasing trend along with the soil profile (Fig. 3).

Table 2. Pearson correlations between soil variables and main soil prokaryotic phyla.

	depth	Soil moisture	pH	TN	TC	NH4	NO3	DOC	DON	clay	silt	sand
Actinobacteriota	.298*	.008	.221	-.297*	-.398**	.178	.175	-.069	.236*	.268*	.159	-.181
Proteobacteria	-.592**	.266*	-.265*	.478**	.296*	.282*	.274*	.240*	-.007	-.386**	-.193	.230
Firmicutes	-.263*	.092	.137	.206	.039	.319**	.423**	.193	.474**	-.221	-.131	.150
Chloroflexi	-.219	-.057	.160	.064	.053	.069	.168	-.076	.338**	-.088	-.074	.079
Acidobacteriota	-.334**	.053	-.093	.280*	.405**	-.285*	-.177	-.074	-.291*	-.229	-.138	.157
Crenarchaeota	.440**	-.193	.001	-.282*	-.148	-.231	-.212	-.228	-.146	.170	.081	-.098
Planctomycetota	-.357**	.041	-.081	.312**	.394**	-.247*	-.083	-.035	-.210	-.281*	-.198	.217
Methylomirabilota	.554**	-.219	-.087	-.365**	-.226	-.263*	-.444**	-.040	-.333**	.325**	.215	-.239*
Gemmatimonadota	.221	.145	-.109	-.044	-.039	.004	-.219	.205	-.296*	.113	.009	-.027
Myxococcota	-.503**	.194	-.248*	.411**	.305**	.230	.153	.155	-.126	-.384**	-.217	.250*

Abbreviations: TN, total nitrogen; TC, total carbon; NH4, ammonium; NO3, nitrate; DOC, dissolved organic carbon; DON, dissolved organic nitrogen.

The shifts in bacterial and archaeal communities were also reflected in the abundant ASVs, with 50%-72.49% of total abundant ASVs unevenly distributed along with the soil profile among sampling times.

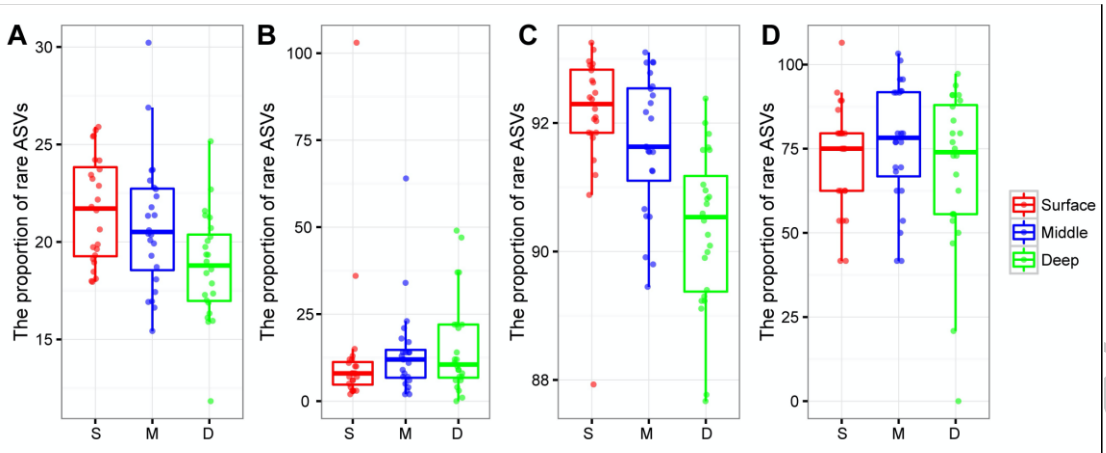

Figure 4. Box plots showing the proportion of rare bacterial richness (**A**) and archaeal richness (**B**), the proportion of rare bacterial abundance (**C**), and archaeal abundance (**D**) among soil layers. Abbreviations: S, surface soil layer; M, middle soil layer; D, deep soil layer.

ASVs enriched in the surface soil layer, mainly Solirubrobacterales and Propionibacterales, accounted for a more significant proportion than those in the middle and deep soil layers (Fig. 4). ASVs enriched in the middle and deep soil layer, primarily classified as Rokubacterales and Gaiellales, differed from the surface soil layer (Fig. 4). Meanwhile, abundant ASVs also exhibited temporal variations: less enriched ASVs (50%) in October than in other sampling times (71.5%-72.5%). Interestingly, the proportion of unclassified ASVs was less abundant in the surface soil than in middle and deep soil layers (Fig. 4).

Rare bacterial and archaeal ASVs

The proportion of rare bacterial richness and abundance was highest in the surface soil layer and showed a decreasing trend along with the soil profile (Fig. 5). On the other hand, the proportion of rare archaeal richness and abundance were not impacted by soil

depth (Fig. 5).

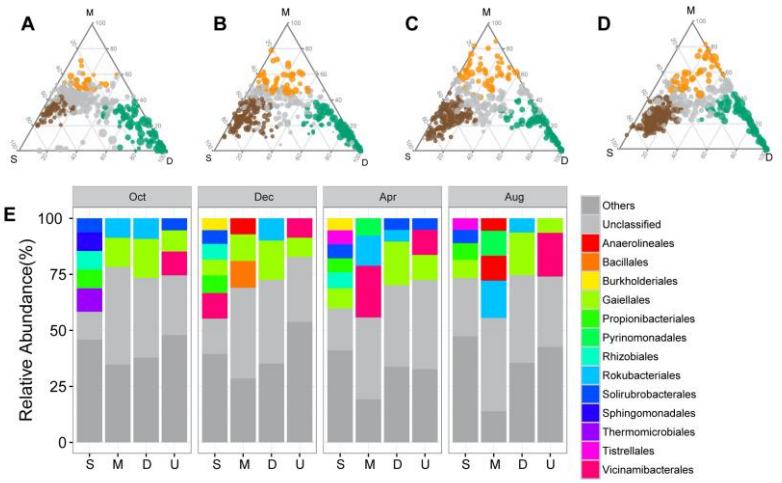

Figure 5. Ternary plots showing the distribution of enriched prokaryotic ASVs in surface (brown), middle (orange), and deep (green) soil layers in October (**A**), December (**B**), April (**C**), and August (**D**). The size of each circle is equivalent to its relative abundance. The orders of enriched ASVs are displayed in bar plots (**E**). Abbreviations: S, ASVs enriched in surface soil layer; M, ASVs enriched in middle soil layer; D, ASVs enriched in deep soil layer; U, unenriched ASVs.

Random forest analysis further indicated that the rare bacterial community was the key determinant of the soil fertility index (Fig. 6).

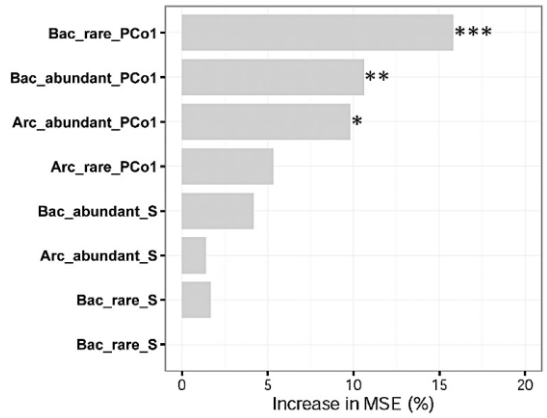

Figure 6. Random forest mean predictor importance of PCo1 of rare bacterial community (Bac_rare_PCo1), PCo1 of abundant bacterial community (Bac_abundant_PCo1), PCo1 of rare archaeal community (Arc_rare_PCo1), PCo1 of abundant archaeal community (Arc_abundant_PCo1), rare bacterial richness (Bac_rare_S), abundant bacterial richness (Bac_abundant_S), rare archaeal richness (Arc_rare_S), abundant archaeal richness (Arc_abundant_S) on soil fertility index. Significance level: $P < 0.05$, *; $P < 0.01$, **; $P < 0.01$, ***.

Moreover, the potential role of 43 archaeal and bacterial phylum on soil fertility was assessed. Among these phyla, Latescibacterota, Zixibacteria, Dadabacteria, Dependentiae, Firmicutes, Micrarchaeota, and Calditrichota were the main predictors of the soil fertility index (Fig. 7); these phyla, except Firmicutes, were relatively rare taxa, accounting for 0.00034%-0.31% of the total reads.

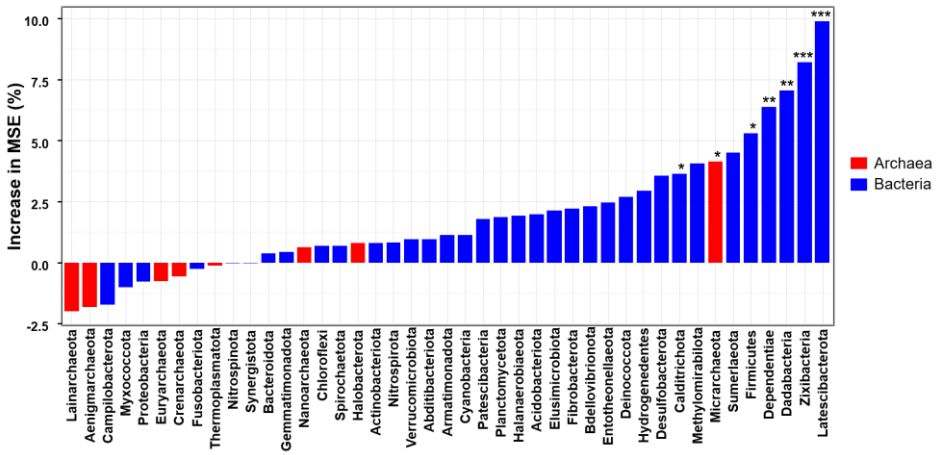

Figure 7. Random forest mean predictor importance of prokaryotic phyla on soil fertility index. Significance level: P < 0.05, *; P < 0.01, **; P < 0.01, ***.

Prokaryotic co-occurrence network

I constructed a soil prokaryotic co-occurrence network for each soil layer. The network size generally became larger along with the soil depth, with 214, 247, and 274 nodes in the surface, middle, and deep soil layer, respectively. Additionally, I observed that only a small proportion of nodes (54) were shared among these three networks. The network of the surface soil layer was more analogous with the network of the middle soil layer (153 nodes shared) but was quite dissimilar with that of the deep soil layer (65 nodes shared). The surface and middle soil layers' network was more connected than the deep soil layer (Fig. 8). This pattern was also affirmed by their topological characteristics (Table 3). Furthermore, the positive/negative links gradually decreased with the soil profile (Table 3).

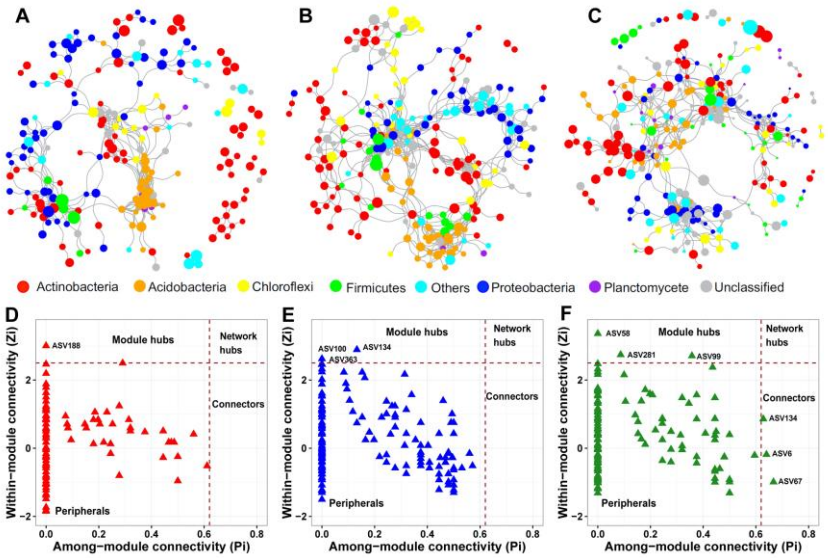

Figure 8. Prokaryotic co-occurrence networks in surface (A), middle (B), and deep (C) soil layers, the size of each node is proportional to its relative abundance. Pi-Zi plots showing the distribution of prokaryotic soil ASVs based on their topological roles in surface (D), middle (M), and deep (D) soil layers.

Table 3. Co-occurrence network topological parameters. Major topological properties of the soil prokaryotic networks in surface, middle and deep soil layers.

	Edges	Vertices	Connectance	Average degree	Average path length	Clustering coefficient	Modularity	Positive links	Negative links
Surface	684	217	0.029	6.30	5.32	0.69	0.65	675	9
Middle	807	247	0.027	6.53	5.15	0.44	0.63	681	126
Deep	719	274	0.019	5.25	5.39	0.45	0.74	580	139

From the plot of Zi (a value measuring within-module connectivity) and Pi (a value measuring among-module connectivity), the different roles of each node in the network were identified (Fig. 8). Notably, there were more keystone species observed in the deep soil layer (six) than in the surface (one) and middle (three) soil layers (Fig. 8). However, these keystone species were not shared among the three networks. The classification of these keystone species is listed in Table 4.

Table 4. The classification at each taxonomic level of the keystone species.

Soil layer	Keystone species	Classification
Surface	ASV188	d__Bacteria;k__norank_d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Tistrellales;f__Geminicoccaceae;g__norank_f__Geminicoccaceae
Middle	ASV134	d__Bacteria;k__norank_d__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Subgroup_9;f__norank_o__Subgroup_9;g__norank_f__norank_o__Subgroup_9
Middle	ASV100	d__Bacteria;k__norank_d__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Gaiellales;f__norank_o__Gaiellales;g__norank_f__norank_o__Gaiellales
Middle	ASV363	d__Bacteria;k__norank_d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micromonosporales;f__Micromonosporaceae;g__Micromonospora
Deep	ASV67	d__Bacteria;k__norank_d__Bacteria;p__Acidobacteriota;c__Blastocatellia;o__Pyrinomonadales;f__Pyrinomonadaceae;g__RB41
Deep	ASV58	d__Bacteria;k__norank_d__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Gaiellales;f__norank_o__Gaiellales;g__norank_f__norank_o__Gaiellales
Deep	ASV281	d__Bacteria;k__norank_d__Bacteria;p__Methylomirabilota;c__Methylomirabilia;o__Methylomirabilales;f__Methylomirabilaceae;g__wb1-A12
Deep	ASV99	d__Bacteria;k__norank_d__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Subgroup_17;f__norank_o__Subgroup_17;g__norank_f__norank_o__Subgroup_17
Deep	ASV6	d__Bacteria;k__norank_d__Bacteria
Deep	ASV134	d__Bacteria;k__norank_d__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Subgroup_9;f__norank_o__Subgroup_9;g__norank_f__norank_o__Subgroup_9

Discussion

Different vertical patterns between bacterial and archaeal diversity

Iobserved that the deep soil layer harbors the lowest bacterial diversity, which is consistent with studies from alpine meadow (Xu et al., 2021), soybean cropland (Hao et al., 2020), and deciduous birch forest (Mundra et al., 2021). This finding confirmed the environmental principle that more extreme environments are expected to be occupied by a less diverse microbe community (Gaston, 2000). In contrast to previous studies, Iobserved that bacterial richness and evenness in the middle soil layer were roughly equal to that in the surface. This observation may have the following two explanations. First, this is likely attributable to the soil carbon content, which did not differ between the surface and middle soil layer. Soil organic carbon content is the most important factor that has a notable influence on soil microorganisms in addition to pH (Fierer, 2017) and was observed to be the best predictor of bacterial diversity, as indicated by random forest analysis in this study. Second, on a depth-weighted basis, the microbial biomass in the middle soil layer may be not less than that in the surface soil layer, thus, containing an equal diversity with the surface soil layer.

For archaea, its richness was unaffected by soil depth and cannot be explained by any measured soil variable. Archaea are reported to be highly adaptable to environmental stress (Cao et al., 2012), so may survive in the nutrient-poor and oxygen-deficient deep soil layer.

Vertical and temporal patterns of Bacterial and archaeal community

Both bacterial and archaeal community composition exhibited distinct distribution patterns with the vertical soil profile, mainly due to the drastic differences in soil carbon and nitrogen content and soil texture (Eilers et al., 2012; Gu et al., 2017; Gy & Jackson, 2000). Ialso detected different ecological preferences for certain phyla. For instance, Actinobacteria and Crenarchaeota showed increasing abundance along with the vertical soil profile. This result corresponds with the conclusions of previous studies that archaea occupy a larger proportion in the deep soil layer due to their anaerobic characteristic. However, some bacterial phyla, including Proteobacteria, Firmicutes, and Acidobacteria, displayed a sharply decreasing trend in the deep soil layer. This trend may be owing to the copiotrophic-oligotrophic trade-off theory (Ramirez et al., 2012) and/or the deficiency in oxygen availability.

Only a slight temporal variation in bacterial and archaeal community composition was detected. In a meta-analysis, Shade et al. found that soil microbial communities were consistently less temporally variable than other ecosystems (Shade et al., 2013). The temporal variations of the soil microbial community were attributable to two factors:

the shift of soil nutrients and/or the plant growth stage (Yang, 2017). The soil samples were collected from two plant individuals in the present study, meaning microbial communities may be less impacted by plant physiology. The minimal impact may also be due to no apparent difference in soil environmental conditions among sampling times. In addition, soil contains many dormant microbes (Lennon & Jones, 2011), indiscriminately captured using the 16s rRNA sequencing approach (Shade et al., 2013). Based on the reasons mentioned above, the soil prokaryotic communities only fluctuated within a narrow range over the given time range.

Vertical pattern of rare ASVs and their potential function

Our deep sequencing enabled us to explore reliable rare taxa in soil. For the first time, the vertical patterns of rare bacterial and archaeal taxa were evaluated. Iobserved that the proportion of rare bacterial ASVs decreased along with the soil profile. The root system in the surface soil layer was more abundant than that in the middle or deep soil layers (Chaparro et al., 2014) and may provide more niches for rare species with specialized functions. Alternatively, it was proposed that rare species may occur from stochastic processes (Jousset et al., 2017), whereas deterministic processes would reduce rare taxa. Since soil nutrient availability generally decreased along with the vertical soil profile, stochastic processes would play a less predominant role in the deep soil layer.

Recently, the ecological importance of rare taxa in terrestrial and aquatic ecosystems

has been increasingly recognized (Chen et al., 2020; Jousset et al., 2017). Ialso found that rare taxa were key determinants of soil fertility, indicating that rare taxa had an over-proportional role in the soil biogeochemical cycling process. In contrast, the dominant bacterial and archaeal phyla (e.g., Proteobacteria, Actinobacteria, and Acidobacteria) showed little control over soil fertility. In long-term fertilized soils, Chen et al. (Chen et al., 2020) also observed that rare bacterial and fungal taxa (less than 3% of total reads) were the major drivers of ecosystem multifunctionality. Overall, our knowledge of the ecological role of the rare microbial taxa remains limited and is in need of more attention.

Vertical patterns of prokaryotic co-occurrence networks

Our results emphasized that the prokaryotic co-occurrence network was more complex in the surface and middle soil layers than in the deep soil layer, reflected by the higher connectedness and average degree. Likewise, Xu et al. observed a more complex and more extensive microbial network on the surface than in the deep soil layer of meadows and shrubland (Xu et al., 2021). Higher network complexity in the surface and middle soil layer might be explained, in part, by greater amounts of substrate and nutrient in the surface soil layer (Creamer et al., 2016), as microbial interactions feeding on these substrates would be largely strengthened. Many studies have found that soil microbial networks would be more connected after nutrient amendments (Yang et al., 2019; Yang, Yang, et al., 2020). Moreover, the microbial richness and biomass were extremely low in the deep soil layer (Eilers et al., 2012; Xu et al., 2021), reducing the opportunity for

different species to interact. It has been proposed that complex networks contribute to better environmental adaption and higher efficiency of resources transfer than simple networks (Morriën et al., 2017). In this sense, the complex prokaryotic networks in the surface and middle soil layers would lead to better biogeochemical functions and provide a stable soil environment for plant growth.

Iobserved a gradually increased proportion of negative links by assessing the correlations among microbial species, which indicates that microbial competition for the same resources increases with soil depth. The niche differentiation would be a possible reason for this result. The surface and middle soil layers were easily impacted by external disturbance; the roots within these layers also create heterogeneous environments (Morriën et al., 2017), leading to significant niche differentiation and reduction of competition. In contrast, the deep soil layer was deficient in roots and was less impacted by external disturbance, possessing a more homogeneous environment and weak niche differentiation (Faust & Raes, 2012). The nutrient-limited environment in the deep soil layer would also induce intense competition among species.

In conclusion, Ievaluated the effects of soil depth and sampling time on soil bacterial and archaeal community and co-occurrence networks in maize-soybean rotation agroecosystem at a depth of millions of sequences per sample. Our results showed that soil depth is more important than temporal variation in shaping prokaryotic community compositions and co-occurrence networks and revealed different vertical distribution

patterns of bacteria and archaea. Notably, our study also revealed the potential of rare microbial taxa in soil biogeochemical function.

Acknowledgment

The author would like to acknowledge the contribution of Yupeng Guan and Nana Jiang from the College of Resources and Environment, affiliated with the Northeast Agricultural University in Harbin, China, for their assistance in sample collection and processing. She would also like to recognize the contribution Ximei Zhang and her professor Wei Yang, from the Key Laboratory of Dryland Agriculture, Ministry of Agriculture, Institute of Environment and Sustainable Development in Agriculture, affiliated to the Chinese Academy of Agricultural Sciences in Beijing, China, for suggesting the research question and guiding the author through her entire research: from isolating DNA from soil samples to coding in Python, the author has gained immensely from this experience. Supervision was done without charge.

Supported by the National Natural Science Foundation of China (32071547).

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