Decoupled diversity relationships among soil bacterial, fungal, and archaeal communities and coupled cross-taxon diversity relationships across temperate grasslands

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Manuscript for *Ecography or Science of total environment*?

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ABSTRACT

Soil microbes assemble in complex highly diverse polymicrobial communities, and their diversity patterns and associated drivers have been studied extensively. However, the microbial diversity relationships across-domains and across-taxa among and within domains remain poorly explored. Here we assess diversity relationships among bacterial, fungal, and archaeal communities and among their counterpart phyla in temperate grassland soils by evaluating operational taxonomic unit (OTU) richness and Shannon-Wiener diversity along a precipitation gradient covering ~1500-km long transect in the Inner Mongolian grassland. We find decoupled diversity relationships among bacterial, fungal and archaeal communities. This is partly because their environmental drivers are domain-specific, with water dominantly yet negatively affecting soil bacterial diversity, energy positively affecting soil fungal diversity, and water and plant positively affecting soil archaeal diversity. However, many coupled diversity relationships were observed between some phyla cross microbial domains, which represent a tradeoff for the overall decoupled diversity relationships. Analyses of cross-taxon diversity relationships within domains showed a complex coupled relationships (either positive or negative), which were most complex in bacterial communities followed by fungal and archaeal communities. The coupled diversity relationships are likely caused by their common or divergent response to environmental factors, which results high likelihood for cooperation and/or antagonism in these phyla. Understanding diversity relationship underlying the structure and the composition of soil polymicrobial communities and cross-taxon assemblies is essential for understanding the synergistic and competitive interactions among microbial taxa involved in the process of litter decomposition, biogeochemical cycles and climate regulation.

KEYWORDS: Diversity relationship, bacteria, fungi, archaea, temperate grassland

INTRODUCTION

It is well known that in most terrestrial ecosystems, the belowground biota supports a great diversity of microbes. These microbes assemble complex, highly diverse polymicrobial communities (including bacteria, fungi, and archaea) which play an important role in decomposition processes, carbon and nitrogen cycles and climate regulation (de Boer et al. 2005, Gubry-Rangin et al. 2011). Therefore, exploring the diversity relationships between different microbial domains is essential to assess the effects of microbial diversity on ecosystem functioning. However, while the relationships between macro-organisms diversity have been relatively well studied (e.g. the relationships between animal and plant diversity; Castagneyrol and Jactel (2012)), the relationships between microbial diversity of different domains across large spatial scales remains poorly understood.

Mechanistic links between diversity of different groups of macroorganisms have been extensively investigated (Castagneyrol and Jactel 2012). For example, the diversity of arthropods, herbs, birds and mammals showed positive correlation with plant richness, which may reflect the interactions between these different groups (Castagneyrol and Jactel 2012). Similarly, a range of complex positive (commensalism, mutualism) (Tiunov and Scheu 2005) and negative (amensalism, parasitism, or predation) (Forsberg et al. 2014, Treton et al. 2004) interactions occur in soil microbes. For example, some members of bacteria stimulate the establishment of mycorrhizal fungi (Founoune et al. 2002), but others, such as actinomycetes can suppress litter decomposer fungi in degrading organic substrates (Jayasinghe and Parkinson 2008), or compete with fungi for simple plant-derived substrates (de Boer et al. 2005, Mille-Lindblom et al. 2006). Consequently, microbial taxa that associate with each other through positive and negative interactions are likely to show specific positive or negative diversity relationships. Several studies have found negative relationships between diversity patterns of bacteria and fungi with contrasting diversity responses to precipitation and soil pH at the global scale (Bahram et al. 2018). Others have shown positive diversity relationship between bacteria and fungi across a gradient of heterogeneous land uses (George et al. 2019). Finally, previous research has also found that ammonia-oxidizing bacteria (AOB) frequently thrive in neutral, and/or alkaline soils, whereas ammonia-oxidizing archaea (AOA) thrive in acidic soil (Zhang et al. 2012), leading to negative relationships between the diversity of AOB and AOA. These

studies consistently suggest that the relationships between the diversity of different microbial domains in soil are associated with their response to common or contrasting environmental factors (Wardle 2006). However, the scope of these analyses spans over large ecological gradients and the diversity relationships between microbes may be easily susceptible to environmental factors. Thus, whether microbial diversity relationships are coupled in moisture- and N-limited grasslands remained to be explored.

Soil microbial diversity is influenced by a wide array of variables, including contemporary climate (Maestre et al. 2015, Wang et al. 2015, Zhou et al. 2016), plants (Porazinska et al. 2018, Prober et al. 2015), edaphic properties (e.g. soil pH and nutrients) (Bates et al. 2011, Delgado-Baquerizo et al. 2016, Fierer and Jackson 2006, Griffiths et al. 2011) and human disturbance (REF). Contemporary climate, including water (precipitation or aridity) and energy (characterized by temperature) availability are shown to have considerable effects on soil microbial diversity (Maestre et al. 2015, Wang et al. 2015, Zhou et al. 2016), by restricting microbial access to soil nutrients or moisture (Engelhardt et al. 2018, Sorensen et al. 2013) and/or accelerating metabolic rates and biochemical processes in microbes (Zhou et al. 2016). Plants could also significantly influence soil microbial diversity and their relationship can be directly mediated by plant labile carbon inputs and/or rhizo-deposition (Garbeva et al. 2004). In addition, edaphic properties (e.g., soil pH) are broadly considered as an important factor driving soil bacterial (Fierer and Jackson 2006, Griffiths et al. 2011), archaeal (Gubry-Rangin et al. 2011, Tripathi et al. 2015) and fungal diversity (Tedersoo et al. 2014). Human disturbance could also shape soil microbial diversity (Moora et al. 2014) due to its effect on plant composition and soil structure (Duhour et al. 2009, Partel et al. 2017). Although effects of environmental factors on soil microbial diversity have been widely studied, whether such effects have consistent footprint on diversity of soil bacterial, fungal and archaeal communities remains unknown.

Here, we explored the relationships between the diversity of soil microbial domains along a temperature grassland transect in Inner Mongolia, China. This transect is located at the eastern part of the Eurasian steppe and spans from arid to mesic ecosystems along a precipitation gradient from Northeast China to the west, covering a

broad variation in climate, vegetation types, soil properties and human disturbance. Along this transect, soil samples were collected in meadow steppe, typical steppe and desert steppe. Using amplicon-based sequencing of 16S rRNA and ITS genes, we explored the relationships between the diversity of soil bacteria, fungi and archaea and between these three different phyla. Then we evaluated the relative importance of climatic (water and energy), plant, edaphic and human disturbance variables on microbial diversity. With these analyses, we aim to test the following three hypotheses: (1) Diversity patterns between soil microbial domains are decoupled in moisture- and N-limited grasslands, which might partly be caused by their respective domain-specific environmental responses. (2) Relationships between diversity of different phyla across microbial domains are complex and represent a tradeoff for the overall decoupled diversity patterns. (3) Relationships between diversity of different phyla within microbial domains are most complex in bacterial communities, followed by fungal and archaeal communities.

MATERIALS AND METHODS

Study area

Our study area spans a ~1500 km transect from arid to mesic grasslands in Inner Mongolia (107.929° E ~ 119.970° E, 39.154° N~49.618° N) with varied climatic, edaphic, vegetation and human disturbance conditions (Figure 1 and Supplementary Dataset). This transect is composed of several vegetation types (desert steppe, typical steppe and meadow steppe) with increasing mean annual precipitation (MAP:165.0 ~ 411.5 mm) and decreasing mean annual temperature (MAT: 6.4 ~ -2.3 ºC) from southwest towards northeast. The desert steppe is arid and low in plant species richness, dominated by perennial drought-adapted species including *Stipa klemenzii* and *Stipa breviflora*, etc (Ma et al. 2010). The typical steppe has the highest coverage in Inner Mongolia with intermediate levels of net primary productivity (NPP) and plant species richness, dominated by *Stipa grandis*, *Stipa krylovii*, and *Artemisia frigida*, etc (Bai et al. 2008). The meadow steppe has the highest NPP and plant species richness, dominated by *Stipa baicalensis* and *Leymus chinensis*, etc (Bai et al. 2008). Soil types along this transect include Calcisols, Kastanozems and Calcic Chernozem from southwest towards northeast (Bai et al. 2008).

Soil and community sampling

Soil and grassland community samples were collected from 32 sites along the transect in August 2015. At each site, five subplots (1 m × 1 m) were set at the four corners and the middle of a larger plot (10 m × 10 m) and the aboveground communities of these subplots were sampled. The aboveground biomass (AGB) of each species was harvested by clipping the entire aboveground part, dried at 75°C to a constant weight and weighed separately for each subplot. NPP of each site was estimated using data from the Numerical Terradynamic Simulation Group (NTSG) with a spatial resolution of 1 × 1 km (http://www.ntsg.umt.edu/project/modis/default.php). Three subplots along the diagonal were selected for each large plot, and within each subplot, three soil cores were taken by excavating soils from predetermined depths to a total of 100 cm using a 50-mm diameter soil auger (Wang et al. 2017). Soils from the same depth and subplot were thoroughly mixed as a composite sample of a subplot and then were divided into two portions. One portion was kept in an ice box and stored at -80^{o}C immediately after transporting to the laboratory for DNA analysis while the other portion was air-dried for physicochemical analyses. In this study, only the topsoil (0-10 cm) samples were used and three subplot replicates were thoroughly mixed to constitute a representative sample for each site. All soils were sieved through a 2-mm mesh with visible roots removed before laboratory analysis.

Soil physicochemical analysis

Total carbon (TC) and total nitrogen (TN) concentrations of soil samples were measured by combustion using an elemental analyzer (Vario EL Ⅲ, Elementar, Hanau, Germany) as previously described (Dai et al. 2018). Soil organic carbon (OC) was calculated as the total carbon minus inorganic carbon, which was analyzed volumetrically by reaction with hydrochloric acid as previously described (Dai et al. 2018). Total phosphorus (Soliveres et al.) was extracted using perchloric acid-sulfuric acid ($HClO_4$-H_2SO_4) digestion and measured by colorimetric method with molybdenum blue (Cai et al. 2017). Soil pH was measured using a ratio of soil:water at 1:2.5 (*w*:*v*). Soil texture was examined by laser diffraction using Malvern Mastersizer 2000 (Malvern Instruments Ltd., UK) after removal of organic matter and calcium carbonates (Dai et al. 2018). Dithionite-extractable manganese (Mn_d) and aluminum (Al_d) were extracted from using the citrate-bicarbonate-dithionite (CBD) method

(Georgiadis et al. 2017, Spielvogel et al. 2008) and subsequently were determined on an inductively coupled plasma-atomic emission spectrometer (ICP-AES, ICAP6300, Thermo Scientific, USA).

DNA extractions and high-throughput amplicon sequencing

Total DNA from soil samples was extracted using the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacture's protocol.

For the soil bacterial community, we used the barcoding primer set 515F/806R (515F, 5'-GTGCCAGCMGCCGCGGTAA-3'; 806R, 5'-GGACTACHVGGGTWTCTAAT-3') targeting the V4 region of bacterial 16S rRNA gene (Leff et al. 2015). For the soil fungal community, we used the barcoding primer set ITS5-1737F/ ITS2-2043R (ITS5-1737F, 5'-GGAAGTAAAAGTCGTAACAAGG-3'; ITS2-2043R, 5'-GCTGCGTTCTTCATCGATGC-3') targeting the fungal ITS2 (Internal Transcribed Spacer) gene (Lu et al. 2013). For the archaeal community, we used the barcoding primer set 1106F/1378R (1106F, 5'-TTWAGTCAGGCAACGAGC-3'; 1378R, 5'-TGTGCAAGGAGCAGGGAC-3') targeting archaeal 16S rRNA gene (Zhang et al. 2016). All the barcodes were unique to every soil sample.

PCR reactions were performed in 30 μL reaction systems after mixing 15μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers labelled with specific barcodes, and about 10 ng template DNA. Thermal cycling was repeated following the procedure: initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, elongation at 72 °C for 30 s, and with a final step of 72 °C for 5 min. Sequencing library was generated using Illumina TruSeq DNA PCR-Free Library Preparation Kit (Illumina, USA). The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. The library was sequenced on an Illumina HiSeq2500 platform and paired-end reads were generated in fasta format with forward and reverse reads filtered into separated files.

Processing of sequence data

Raw DNA sequences generated from the Illumina HiSe2500 platform were

processed on the Galaxy pipeline in Metagenomics for Environmental Microbiology (http://mem.rcees.ac.cn:8080/root/index) (Feng et al. 2017) at the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The raw DNA sequences assigned to samples were cleaned by removing the barcodes and primer sequences and merged by FLASH (version 1.0.0) (Magoc and Salzberg 2011). The overlap length ranged from 30 bp to approximately 90% of read pairs (bp). The maximum allowed ratio of the number of mismatches to overlap length was set as 0.25, with the Phred Offset representing the quality values of bases set as 33 and the standard deviation as 10% of the average fragment length. After merging the pair-end reads, the sequences were filtered with the Btrim program using threshold of average quality score > 20 and a 5-bp window size (Kong 2011). As a result, we obtained a total of 3,531,946, 2,045,712 and 4,086,723 high-quality sequences for bacteria, fungi and archaea, and corresponding OUTs were clustered using the UPARSE pipeline (Edgar 2013) at 97% sequence similarity per sample.

Taxonomy annotation was conducted using Greengene database (gg_13_8_otus) for 16S rRNA OTUs of bacteria and archaea (McDonald et al. 2012), and Unite database for ITS2 OTUs of fungi (Abarenkov et al. 2010) with minimal 50% confidence score. However, because bacterial 505F/806R primer set can classify a handful of archaea due to primers's non-specificity, we removed the OTUs that were annotated to archaea in the following analysis. OTUs annotated to bacteria generated with the archaeal 1106F/1378R primer set were also removed. To make the data comparable among different sampling sites, we standardized the OUT table across all samples to 32,889, 10,207 and 50,347 sequences (the smallest number of sequences for each group across all samples) for bacteria, fungi and archaea per sample, respectively. We then grouped these sequences into 23,458, 6,318 and 3,152 OTUs at 97% sequence similarity per sample. All the following analyses were based on the standardized data. According to the rarefaction results (Figure S1), curves of bacterial, fungal and archaeal communities reached an asymptote, suggesting the sequencing depths were appropriate to survey most soil bacteria, fungi and archaea.

To build a phylogenetic tree sequences were aligned in MAFFT (Katoh and Standley 2013) and the resulting alignments were analyzed under maximum likelihood (ML) using ExaML (Kozlov et al. 2015) for bacteria and RAxML (Stamatakis 2014) for fungi and archaea. Both ExaML and RAxML were obtained from https://cme.h-

its.org/exelixis/software.html.

Climate data

To evaluate the effect of climate on soil microbial diversity, we used variables representing water availability and environmental energy. The variables representing water availability included mean annual precipitation (MAP, mm), precipitation of driest quarter (MPDQ, mm), and aridity index (AI). AI is calculated as the ratio of MAP to potential evapotranspiration (PET). The variables representing environmental energy included mean annual temperature (MAT, °C) and mean temperature of warmest quarter (MTWQ, °C). These variables have been shown to be the dominant factors of species diversity in both above- and belowground communities in the Inner Mongolian steppe in previous studies (Wang et al. 2015).

The data of MAP, MPDQ, MAT and MTWQ with a spatial resolution of 30 arc seconds were obtained from the WorldClim website (http://worldclim.org/version2) (Hijmans et al. 2005). The PET data with a spatial resolution of 30 arc seconds were obtained from the CGIAR-CSI Global PET database (www.cgiar-csi.org/data/global-aridity-and-pet-database) (Trabucco and Zomer 2009).

Human disturbance

To evaluate the effect of human disturbance on soil microbial diversity, we used the number of villages (NV) and total road length (TRL) within a radius of 10 km from the site to represent the levels of human disturbance. The data of village locations and road distributions in China were obtained from Geographic Data Sharing Infrastructure (College of Urban and Environmental Science, Peking University, http://geodata.pku.edu.cn). In our analyses, roads included highways, national roads, and provincial roads.

Statistical analysis

We used the R (R version 3.4.3) 'vegan' package (Lozupone and Knight 2007) to estimate soil microbial diversity based on OTU richness and Shannon-Wiener diversity. Relationships between microbial OTU richness and Shannon-Wiener diversity were evaluated by linear regression.

To explore the determinants of soil microbial diversity, we analyzed the Pearson correlations of microbial diversity with 19 environmental variables, including MAP, MPDQ, aridity index, MAT, MTWQ, plant aboveground biomass, NPP, soil total nitrogen, soil total carbon, soil organic carbon, soil total phosphorus, soil pH, soil extractable Mn, soil extractable Al, soil silt, soil sand, number of villages and total road length (Table S1 and Supplementary Dataset). Relationships among all the environmental variables was evaluated by Pearson correlation (Table S2) with the R package 'Hmisc' (Hollander and Wolfe 1973).

To avoid collinearity between environmental variables in the following multiple regression analysis, we classified all variables into eight groups based on their ecological significance as: 1) water (including MAP, MPDQ and aridity index); 2) energy (including MAT and MTWQ); 3) plant (including aboveground biomass and NPP); 4) soil fertility (including total nitrogen, total carbon, organic carbon and total phosphorus); 5) soil pH; 6) soil metal concentration (including Mn and Al); 7) soil texture (including silt and sand); 8) human disturbance (including number of villages and total road length). Principal component analysis (PCA) was conducted for each group with more than one variable, and the first principal component (PC 1) was extracted to represent each variable group. These components explained 81.5% - 99.9% of the variations in the original variables (Table S3). PCA's feasibility was evaluated using the Kaise-Meyer-Olkin (KMO) test and the Bartlett test of sphericity (BS) (Table S3). Results from both rests indicate that use is PCA appropriate for our data (Dziuban and Schirkey 1974).

To compare the relative importance of different environmental variables on microbial diversity, we conducted hierarchical partitioning using the R package 'hier.par' (Heikkinen et al. 2004). The relative importance referred to their independent effects in the total variations. Multiple linear regression models were used to evaluate the significance of environmental variables (Table S4). All the statistical analysis were performed in the R computing environment (version 3.4.3) (R 2016).

Accession number(s)

All sequence data have been deposited in the public database of the National Center for Biotechnology Information (NCBI) under BioProject accession number PRJNA557316.

RESULTS

Taxa distribution of soil microbes along the transect

In the sampled soil bacterial communities we detected forty-five phyla. The eleven most dominant phyla contributed 98.7% - 99.6% of the bacterial OTU abundance, including *Actinobacteria* (28.1% - 55.6%), *Proteobacteria* (12.8% - 29.8%), *Acidobacteria* (5.8% - 24.5%), *Gemmatimonadetes* (2.4% - 9.7%), *Firmicutes* (1.4% - 12.0%), *Chloroflexi* (2.2% - 5.4%), *Bacteroidetes* (0.6% - 2.8%), *Planctomycetes* (1.0% - 6.2%), *Nitrospirae* (0.7% - 1.8%), *Verrucomicrobia* (0.6% - 20.5%), *Armatimonadetes* (0.2% - 0.8%, Figure 2a). Moreover, these eleven phyla were also OTU-rich phyla, and contributed 96.0% - 97.8% of the bacterial OTU richness (Figure 2d). The fungal communities included five phyla and contributed 83.6% - 98.9% of the fungal OTU abundance, including *Ascomycota* (19.8% - 80.4%), *Basidiomycota* (4.1% - 49.2%), *Zygomycota* (2.3% - 30.0%), *Chytridiomycota* (0.1% - 1.4%), *Glomeromycota* (0% - 1.0%, Figure 2b). Meanwhile, these phyla contributed 80.5% - 90.1% of the fungal OTU richness (Figure 2e). In soil archaeal communities, three phyla were detected and contributed 5.2% - 42.1% of the archaeal OTU abundance, including *Crenarchaeota* (4.7% - 41.9%), candidate division *Parvarchaeota* (0.1% - 13.9%), *Euryarchaeota* (0% – 0.3%; Figure 2c). These phyla only contributed 21.0% - 26.9% of archaeal OTU richness (Figure 2f), suggesting that rare and unclassified archaeal taxa deserved to be further studied.

The OTU richness and the Shannon-Wiener diversity in soil bacterial, fungal and archaeal communities where highest in bacteria lower in fungi and lowest in archaea in the temperature grassland (Figure 3). Bacterial OTU richness was on average 4037 ~ 4913 per sample across the transect, peaked in typical grassland in Xilingol League region and the eastern part of Hulunbeir League region, and was low in desert steppe and meadow steppe (Figure 3a). Fungal OTU richness was on average 554 ~ 1032 per sample and was irregularly scattered along the transect (Figure 3b). Archaeal OTU richness was on average 153 ~ 262 per sample and was high in meadow and typical steppe (Figure 3c). The patterns of Shannon-Wiener diversity of bacteria (Figure 3d),

fungi (Figure 3e) and archaea (Figure 3f) were similar to those of their OTU richness.

Relationships between diversity of different microbial domains and taxa

There was no significant correlation ($p > 0.05$, Figure 4) among overall bacterial, archaeal and fungal OTU richness or Shannon-Wiener diversity. However, complex positive or negative correlations were found between the diversity of different phyla among and within microbial domains evaluated by OTU richness and Shannon-Wiener diversity (Figure 5a). Specifically, more positive than negative correlations ($p < 0.05$) were found between the OTU richness of bacterial and fungal phyla, as well as between their Shannon-Wiener diversity. Positive correlations occurred between three oligotrophic bacterial phyla, four copiotrophic bacterial phyla and a few fungal phyla, while negative correlations occurred especially between a few oligotrophic bacterial phyla and the majority of fungal phyla evaluated by OTU richness (Figure 5a). In contrast, positive correlations occurred between three oligotrophic, one copiotrophic bacterial phyla and the majority of fungal phyla, while negative correlations occurred between two oligotrophic bacterial phyla, one copiotrophic bacterial phyla and two small fungal phyla evaluated by Shannon-Wiener diversity (Figure 5a).

More negative than positive correlations were detected between the diversity of bacterial and archaeal phyla, especially when evaluated by Shannon-Wiener diversity. Negative correlations occurred between four bacterial (*Chloroflexi*, *Armatimonadetes*, *Verrucomicrobia*, *Proteobacteria*) and two archaeal phyla (*Parvarchaeota* and rare, unclassified archaea), and positive correlations occurred between two copiotrophic, two oligotrophic bacterial phyla and most archaeal phyla (Figure 5a). While only one negative and one positive correlation occurred between two oligotrophic bacterial (*Nitrospirae*, *Verrucomicrobia*) and one archaeal phyla (rare, unclassified archaea). Likewise, between fungal and archaeal phyla, two correlations between the Shannon-Wiener diversity of different phyla were positive, involving two fungal phyla (*Ascomycota*, *Chytridiomycota*) and two archaeal phyla (*Crenarchaeota*, *Parvarchaeota*) ($p < 0.05$, Figure 5a), and one correlation was negative, involving Zygomycota and rare, unclassified archaea. There were no significant correlations between OTU richness of fungal and archaeal phyla.

Diversity relationships among phyla within each domain were most complex in bacterial communities (Figure 5a), with twenty-one positive and thirteen negative

correlations ($p < 0.05$) based on OTU richness, and fifteen positive and five negative correlations ($p < 0.05$) based on Shannon-Wiener diversity. Positive correlations were mostly observed within oligotrophic and within copiotrophic phyla, with a few occurring between oligotrophic and copiotrophic phyla (Figure 5a). In contrast, negative correlations were mostly observed between oligotrophic and copiotrophic phyla, with a few occurring within oligotrophic phyla (Figure 5a). In comparison with bacteria, diversity relationships within fungal and within archaeal domains were mostly positive ($p < 0.05$) based on either OTU richness or Shannon-Wiener diversity (Figure 5a).

To explore which phyla have similar biogeographic patterns, we conducted hierarchical clustering analysis through correlation coefficients. The dominant bacterial, fungal and archaeal phyla were clustered into four groups based on either OTU richness or Shannon-Wiener diversity. All microbial phyla within each clustered group had highly positive correlations and thus similar biogeographic patterns (Figure 5b and c). Between clustered groups evaluated by OTU richness, the correlations between group 1 and group 2, between group 1 and group 3, between group 1 and group 4, between group 2 and group 4 were mostly negative, while the correlations between group 2 and group 3, between group 3 and group 4 were mostly positive. Between clustered groups evaluated by Shannon-Wiener diversity, the correlations between group 1 and group 2, between group 1 and group 4, between group 3 and 4 were mostly positive, while the correlations between group 1 and 3, between group 2 and 3, between group 2 and 4 were mostly negative.

Environment – diversity relationships of soil bacteria, fungi and archaea

Using hierarchical partitioning and multiple linear regression models (Figure 6a and Table S4), we found that the eight environmental variables explained 36.4% and 51.9% of the variation in bacterial OTU richness and Shannon-Wiener diversity, respectively. Among them, water had the highest independent and negative effect, explaining 9.7% and 17.3% of the variation in bacterial OTU richness and Shannon-Wiener diversity, respectively ($p < 0.05$) (Figure 6a, Figure S2, Table S4). The diversity of major bacterial phyla was dominated by water ($R^2 = 0.83$ for Shannon-Wiener diversity) and energy ($R^2 = 0.77$ for OTU richness) as revealed by linear fitting of

environmental factors to the Canonical Correspondence Analysis (CCA), followed by plant, soil pH and soil fertility (Table 1 and Figure S3).

For soil fungi, the eight environmental variables explained 41.9% and 53.8% of the OTU richness and Shannon-Wiener diversity, respectively, as revealed by hierarchical partitioning and multiple linear regression models (Figure 6a and Table S4). Among them, energy had the largest independent effect, explaining 12.7% and 22.3% of the variation in fungal OTU richness and Shannon-wiener diversity (Figure 6b and Table S4). CCA analysis revealed that water (R^2 = 0.46) and soil metal concentration (R^2 = 0.46) explained the highest proportion of the variation in OTU richness of the dominant fungal phyla, while plant (R^2 = 0.45) explained the highest proportion of the variation in Shannon-Wiener diversity of the counterpart (Table 1 and Figure S3).

For soil archaea, the eight environmental variables explained 38.9% and 65.4% of the OTU richness and Shannon-Wiener diversity, respectively, as revealed by hierarchical partitioning and multiple linear regression models (Figure 6c and Table S4). Water had the highest independent and positive effect on soil archaeal OTU richness, explaining 12.5% of its variation (Figure 6c), while plant had the highest independent and positive effect (25.1%) on archaeal Shannon-Wiener diversity (Figure 6c and Table S4). In addition, energy and water also had significant independent effect on archaeal Shannon-wiener diversity, explaining 13.4% and 9.3% of its variation (Figure 6c). CCA analysis showed that soil pH (R^2 = 0.21) and human disturbance (R^2 = 0.21) explained the highest proportion of the variation in OTU richness of the dominant archaeal phyla, while energy (R^2 = 0.32) explained the highest proportion of the variation in their Shannon-Wiener diversity (Table 1 and Figure S3).

DISCUSSION

While the variation in soil microbial diversity and its drivers have been widely studied, the relationships between microbial diversity of different domains and phyla remain poorly known. Here, using amplicon-based sequencing of 16S rRNA and ITS genes, we find that the diversity of soil bacteria, fungi and archaea is decoupled, which is distinct from previously documented positive correlations between the diversity of plants and animals at large spatial scales (Scherber et al. 2010). The underlying reasons for this pattern might be that the diversity of the three microbial domains is driven by different predominant factors; water for soil bacteria, energy for soil fungi, and water

and plant for soil archaea. Specially, the diversity of major phyla within bacterial, fungal and archaeal communities are driven by different environmental factors. However, by comparison to the overall decoupled diversity relationships among bacterial, fungal and archaeal communities, a complex mixture of positive or negative correlations between the diversity of dominant bacterial, fungal and archaeal phyla both within and across domains emerges. This aroused a tradeoff against some phyla leading to the decoupled diversity relationships among soil bacteria, fungi and archaea.

Decoupled diversity patterns between soil microbial domains

Our study reveals decoupled patterns in the overall bacterial, fungal and archaeal diversity (Figure 4), corroborating our first hypothesis. A speculation for the general lack of cross-domain concordance in diversity patterns is that variations in soil bacterial, fungal and archaeal diversity are dominated by different environmental variables (Johnson et al. 2017, Ma et al. 2017). This hypothesis is verified by our finding that environmental drivers of soil microbial diversity are domain-specific, with water dominating bacterial diversity, energy dominating fungal diversity, and water and plant dominating archaeal diversity (Figure 6). The negative effect of water (Table S4) on soil bacterial diversity is in contrast to previous findings that water (and/or aridity index) is strong yet positive driver of soil bacterial community diversity in arid and semi-arid temperate grassland ecosystems (Maestre et al. 2015, Wang et al. 2015), but consistent with the findings of Fierer et al. (2013) in tallgrass prairie soils in the United States (Figure S4). These differences between different studies suggest that there might be an optimum water adaptation (ca. aridity index ~ 0.5, Figure S3) where soil bacterial diversity peaks. For example, some studies have found that optimum water potential for soil microorganisms is relatively dry (-0.3 MPa) due to the limited diffusion of oxygen by high water potentials (Lennon et al. 2012). Among all bacterial phyla, the oligotrophic and/or dry-adapted phyla may drive the negative relationships between overall bacterial diversity and water since most oligotrophic and/or dry-adapted phyla show negative correlations with water (see Table S5). Comparatively, water has weaker effect on soil fungal diversity than on bacterial diversity, likely due to fungi's ability to access water from distant micropores with their extensive hyphal network (de Boer et al. 2005). Similarly to bacteria, archaeal diversity is also affected by water, albeit in the opposite direction. The positive effects of water on archaeal diversity is consistent with

previous findings that soil moisture shapes archaeal community composition in the eastern Tibetan Plateau (Shi et al. 2016). The divergent effect of water on bacteria and archaea indicates that they thrive in different water conditions.

Compared with the strongest effect of water on soil bacterial diversity, energy characterized by temperature, had the strongest effect on soil fungal diversity. This is in accordance to previous findings that temperature mediates fungal diversity in forest soils (Zhou et al. 2016). However, energy showed weak effect on bacterial diversity and archaeal diversity, indicating that fungi are more sensitive to energy because they have larger genomes (37.7-225.3 Mbp) than bacteria (1000 to 9000 Kbp) and archaea (Konstantinidis and Tiedje 2004, Tavares et al. 2014, Trevors 1996). The large genome makes fungi metabolically versatile and they need more energy to motivate metabolic processes and biogeochemical cycles (Zhou et al. 2016). In contrast to the dominant effects of climate on bacterial and fungal diversity, plant shows the strongest effect only on archaeal diversity. The higher importance of plant for soil archaeal diversity, rather than bacteria and fungi, indicates that archaea are more sensitive to labile carbon inputs provided by plant than bacteria and fungi. This finding is consistent with previous results showing that an increased atmospheric CO_2 can only significantly increase the soil archaeal diversity rather than the soil bacterial and fungal diversity (Lee et al. 2015). Overall, the differentiated environmental determinants of soil bacteria, fungi and archaea diversity may have led to optimum ecological niche separation and thus decoupling of their cross-domain diversity relationships (Watanabe et al. 2012).

Relationships between diversity of different phyla across microbial domains

Although no concordant patterns in diversity were found between the three microbial domains, significant positive and negative relationships were detected between different phyla across microbial domains (Figure 5), such as between bacterial and fungal phyla (de Boer et al. 2005, Mille-Lindblom et al. 2006), between bacterial and archaeal phyla (Zhang et al. 2012), and between fungal and archaeal phyla (Figure 5). The diversity of different phyla across microbial domains have opposite relationships (either positive or negative), which might represent a tradeoff against each other and thus contribute another reason for the decoupled diversity relationships among overall soil bacteria, fungi and archaea (Soliveres et al. 2016), corroborating our second hypothesis.

The positive and negative correlations indicate that these phyla show similar and contrasting responses to corresponding environmental variables (Table S5) (Castagneyrol and Jactel 2012). Between dominant bacterial and fungal phyla, there are more positive than negative diversity relationships (Figure 5), suggesting that many bacterial and fungal phyla share common ecological niche (Table S5). This is consistent with previous studies revealing that some bacterial and fungal phyla show consistent positive relationship with either soil pH (Griffiths et al. 2011, Tedersoo et al. 2014) or aridity (Maestre et al. 2015) or soil metal concentration (Ma et al. 2017) or productivity (George et al. 2019). Phyla sharing common ecological niche thus have higher likelihood to form strong functional interactions by occupying different functional niches, especially in decomposition processes, such as their cooperation in decomposing the recalcitrant organic matter (e.g., lignin) (de Boer et al. 2005). Negative correlations, albeit less common, were also detected, mostly between the diversity of some oligotrophic bacterial phyla and the majority of fungal phyla, which might be due to their divergent response to water and soil fertility (Table S5). For example, most oligotrophic bacterial phyla (such as *Gemmatimonadetes*, *Chloroflexi*, and *Nitrospirae*) are arid- and low-nutrient adapted (DeBruyn et al. 2011, Fierer et al. 2012, Yao et al. 2014), whereas members of some fungal phyla (e.g. *Basidiomycota*, *Zygomycota*, *Chytridiomycota*) are saprotrophs and thrive in high water and nutrient rich soils (Tedersoo et al. 2014).

In contrast, between dominant bacterial and archaeal phyla, there are more negative than positive diversity relationships (Figure 5), suggesting more bacterial and archaeal phyla have divergent ecological niches and the likelihood that these phyla coexist is low. This results reaffirms the divergent response of some bacterial and archaeal phyla to either soil pH (Zhang et al. 2012) or productivity (George et al. 2019). Few positive relationships were also detected, such as between diversity of bacterial *Planctomycetes* and archaeal *Euryarchaeota*, which is caused by their common and consistently negative response to human disturbance (Table S5). That is because the two taxa predominantly function as anammox bacteria (Humbert et al. 2010) and methanogenic archaea (Leff et al. 2015) and they both need anoxic conditions to function. Human disturbance could disrupt anoxic conditions by affecting plant composition and soil structure (Duhour et al. 2009, Partel et al. 2017) and thus yields negative effect on diversity of bacterial *Planctomycetes* and archaeal *Euryarchaeota*.

553 Only a few diversity relationships were found between diversity of fungal and archaeal
554 phyla, suggesting that most phyla of these two domains respond very differently to
555 environmental factors.
556 Although several positive and/or negative diversity relationships were found
557 among several taxa across different domains, further experimental studies are needed
558 to understand their functional interactions, such as cooperation and/or antagonism
559 (Jayasinghe and Parkinson 2008, Zhang et al. 2018).
560
561 **Relationships between diversity of different phyla within microbial domains**
562 We found complex positive or negative diversity relationships between different
563 phyla within each of the three microbial domains (Figure 5). The most complex
564 relationships were among phyla within the bacterial domain, followed by the fungal
565 domain and the archaeal domain, which may reflect the fact that bacteria have the
566 highest diversity in soil, followed by fungi and archaea (Fierer et al. 2007b),
567 corroborating our third hypothesis. The diversity relationships within oligotrophic
568 and/or arid-adapted bacterial phyla and within copiotrophic phyla were mostly positive
569 (Figure 5). These positive relationships are largely due to their consistent responses to
570 the majority of the environmental variables examined in this study (Table S5). For
571 example, most oligotrophic and/or arid-adapted phyla are adapted well to limited water
572 and nutritional availability, while copiotrophic taxa are adapted ample range of water
573 and nutritional availability (Fierer et al. 2007a). Most diversity relationships between
574 oligotrophic and/or arid-adapted phyla and copiotrophic phyla were negative. These
575 negative diversity relationships are largely due to niche partitioning evidentiated by
576 their divergent responses to environmental variables (Baran et al. 2015), and due to
577 competition for soil nutrients between oligotrophic and copiotrophic taxa (Fierer et al.
578 2007a). Therefore, these complex positive or negative diversity relationships between
579 different bacterial phyla indicate the functional interactions (a mixture of collaboration
580 or competition) between different phyla, and promote the formation of a well-connected
581 network within soil bacterial communities (Banerjee et al. 2016).
582 In contrast to the complex positive and negative relationships between bacterial
583 phyla, only significant positive relationships were detected between diversity of fungal
584 phyla. These relationships are mainly due to their consistent responses to water, energy,
585 and soil fertility (Figure S3, Table S5). These results suggest that the dominant fungal

phyla may share similar ecological niche. This is partly consistent with previous findings that most groups of fungi thrive in regions with similar environment (such as tropical forests) (Tedersoo et al. 2014). Similarly, only significant positive relationships were detected between diversity of archaeal phyla especially the rare, unclassified archaeal and other archaeal phyla, which stresses the importance of uncultured archaeal clades (Soliveres et al. 2016) and indicates that the remaining undescribed archaeal phyla have unique environmental preferences and/or niche specialization (Gubry-Rangin et al. 2011).

CONCLUSION

Our study demonstrates decoupled relationships between the diversity of the bacterial, fungal and archaeal domains in soil, highlighting that dominant drivers of their diversity are domain-specific. In contrast, significant positive or negative diversity relationships between different phyla were observed both within and across the three microbial domains, which indicates that: 1) similar or divergent ecological niche selection of different phyla, and 2) synergistic and/or antagonistic interactions between different phyla. Our findings provide important evidence for understanding collaboration or competition between different phyla in polymicrobial communities. However, network analysis should be further employed to explore relationships between diversity and ecosystem functioning (Feng et al. 2017).

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AUTHOR CONTRIBUTIONS

Z.W., X.F. and N.L. designed the research. W.M., H.H., Y.L. and B.H. collected soil samples. N.L. and X.Z. conducted microbial and chemical analyses with Y.D. contributing the analytical platform for sequencing data. D. D. built the ML tree of bacteria. N.L. and Z.W. performed data analyses. N.L., X.F. and Z.W. wrote the paper with inputs from all other coauthors.

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781

Figure 1. Sampling sites along a precipitation gradient in Inner Mongolian steppes. In total, 32 sites were sampled across desert, typical and meadow steppes from the southwest towards the northeast. MAP – denotes mean annual precipitation in mm.

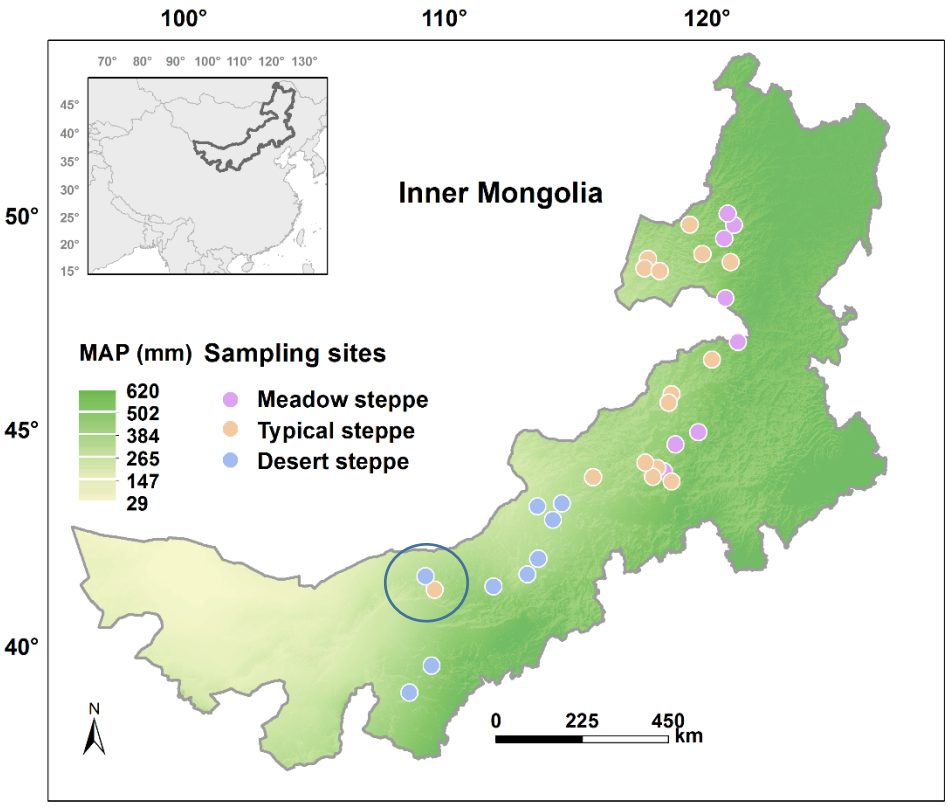

Figure 2. The relative abundance (a, b, c) and relative diversity (d, e, f) of the dominant phyla of soil bacteria (a, d), fungi (b, e) and archaea (c, f) in Inner Mongolian steppes. The relative abundance (or frequency) of a phylum within a community was estimated as the percentage of the sequences (or OUT richness) of this phylum in the total sequences (or OUT richness) of the community.

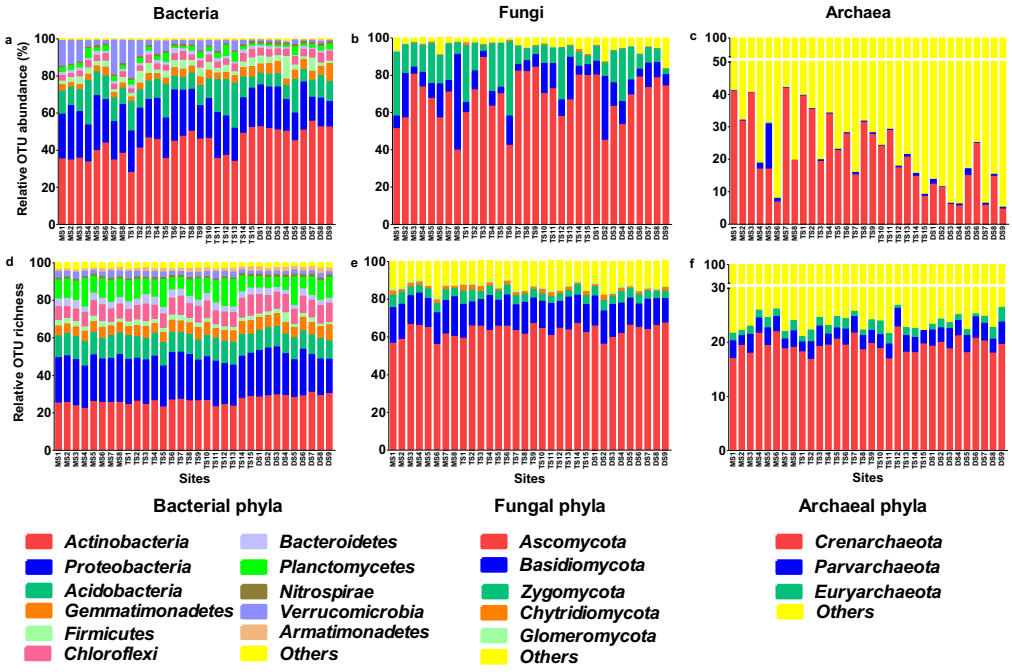

Figure 3. Biogeographic patterns in the OTU richness and Shannon-Wiener diversity of soil bacteria, fungi and archaea in Inner Mongolian steppes. The size of the dots represents the magnitude of OTU richness and Shannon-Wiener diversity of soil bacteria (a, d), fungi (b, e) and archaea (c, f). Land cover classification is from the Global Land Cover Characteristics Database v2.0 (https://lta.cr.usgs.gov/GLCC). MS- meadow steppe, TS - typical steppe, DS – Dry steppe (for definitions see text).

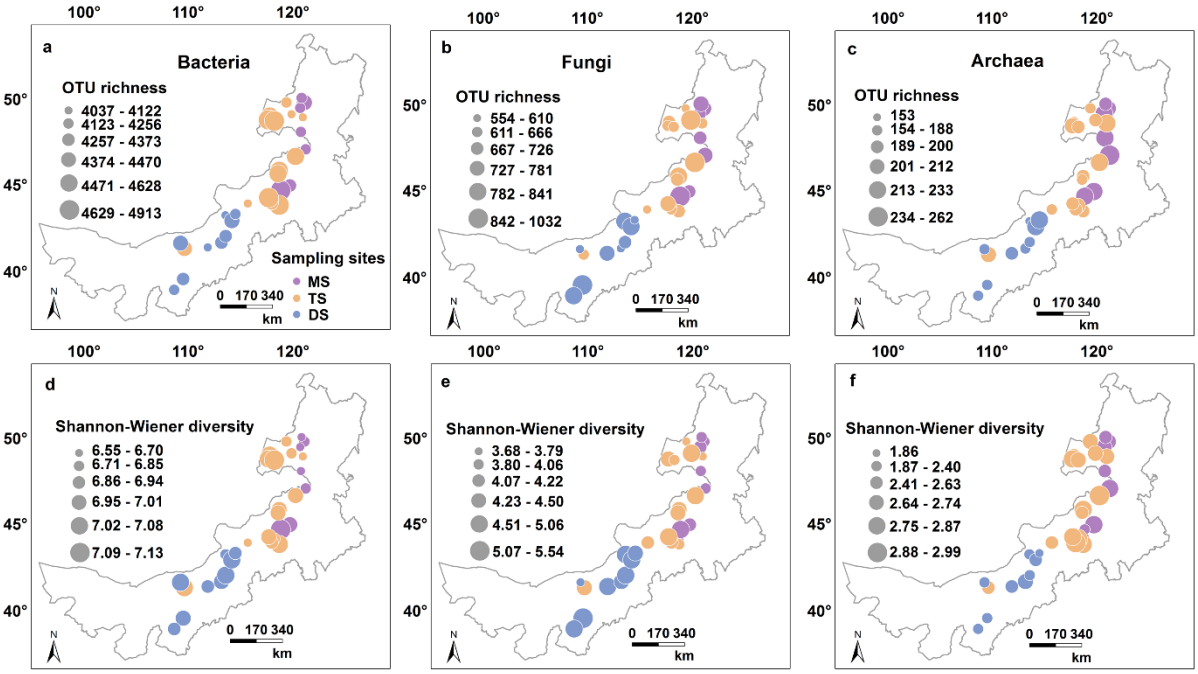

Figure 4. The relationships between the diversity of soil bacteria, fungi and archaea evaluated by OTU richness (a, b, c) and Shannon-Wiener diversity (d, e, f). MS- meadow steppe, TS - typical steppe, DS – Dry steppe (for definitions see text).

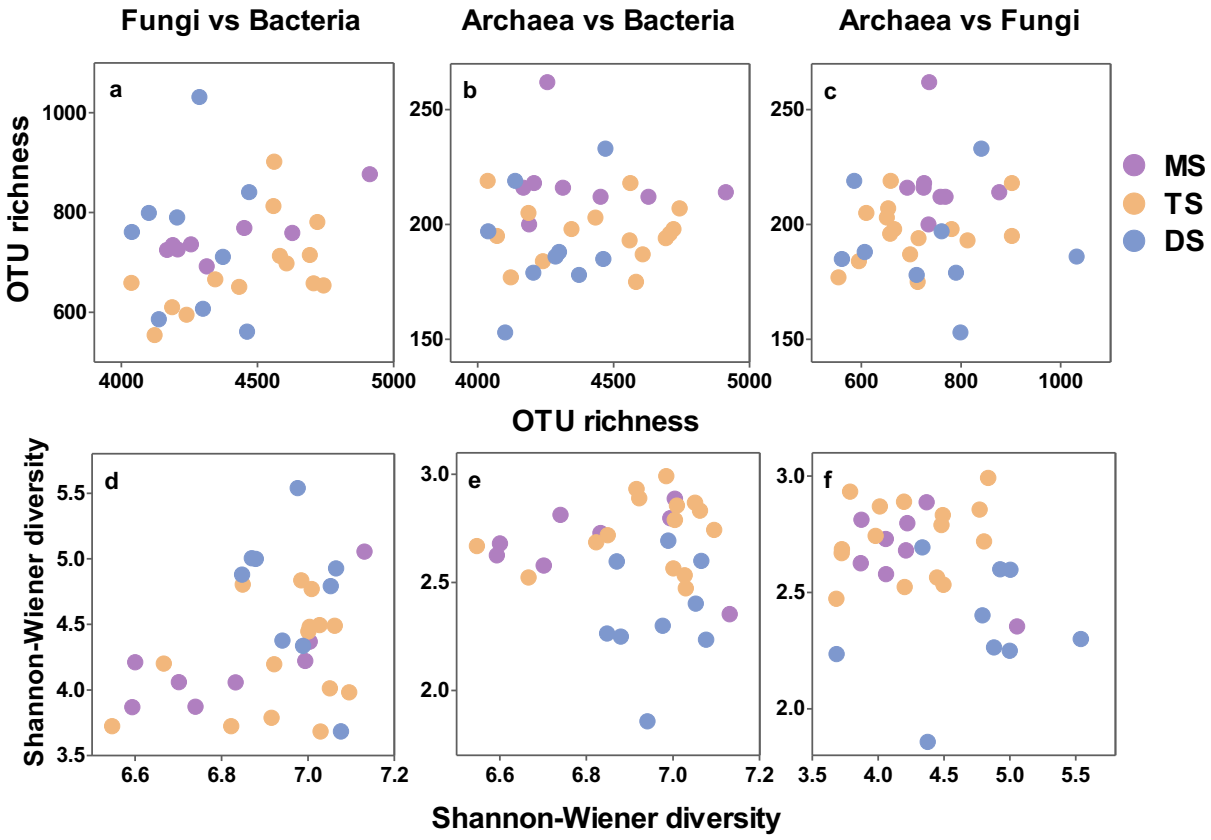

Figure 5. (a) Coefficients of Pearson correlations between diversity of different microbial phyla evaluated by OTU richness (lower triangle) and Shannon-Wiener diversity (upper triangular). The size of dots represents the magnitude of Pearson correlation coefficients, blue and red colors represent positive and negative correlations, respectively. Only significant correlations ($p < 0.05$) are shown. Taxa are labeled as: soil bacterial phyla (pink colors), soil fungal phyla (green colors), and soil archaeal phyla (dark blue colors). (b and c) Hieratical cluster analyses for microbial phyla based on correlation coefficients of OTU richness (b) and Shannon-Wiener diversity (c).

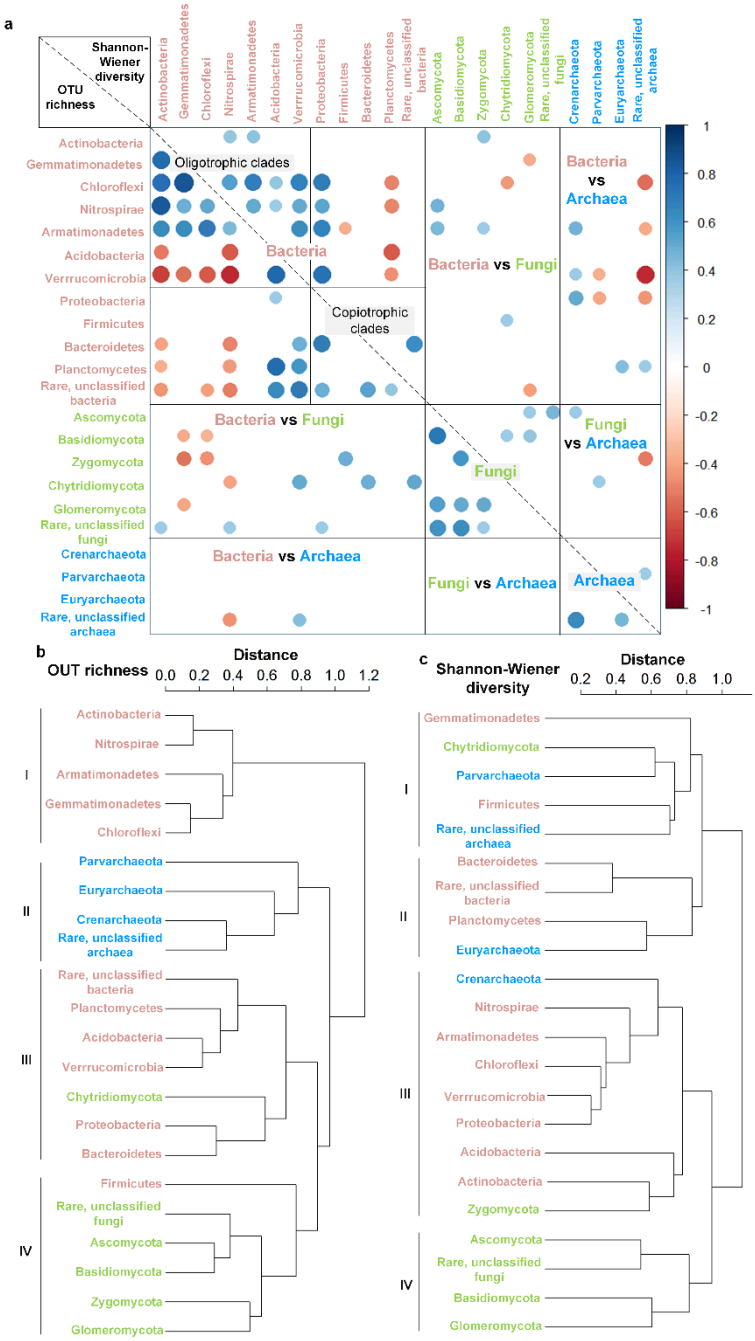

Figure 6. Relative importance of environmental variables on the geographic patterns in OTU richness and Shannon-Wiener diversity of soil bacteria, fungi and archaea evaluated by hierarchical partitioning. The relative importance of different environment variables was calculated as their independent effects using hierarchical partitioning (Table S4). The asterisks indicate significant independent effects ($p < 0.05$; n = 32).

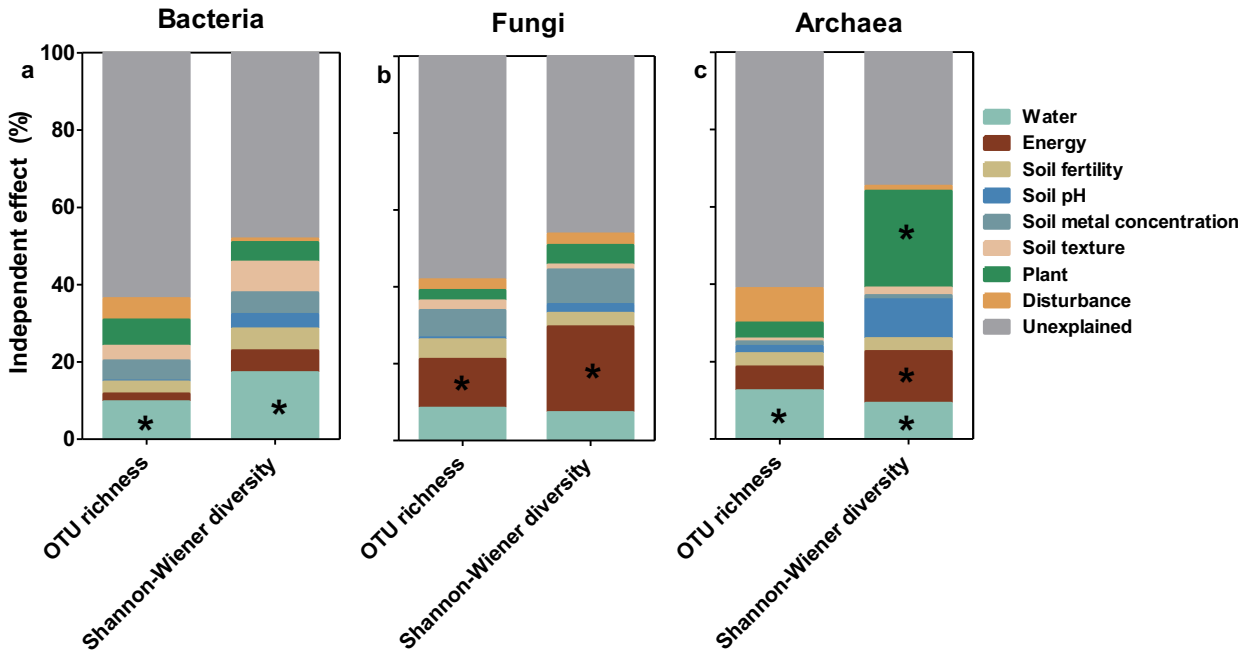

Table 1 Relationships amongst environmental factors and microbial community composition. CCA1 and CCA2 refer to the CCA (Canonical Correspondence Analysis) axes from Figure S4, and the numbers represent the association between each variable and the respective CCA axes. R^2 refers to the goodness of fit statistics and represents the contribution of environmental variables to microbial community composition.

Variables	**OUT richness of dominant bacterial phyla**				**OUT richness of dominant fungal phyla**				**OUT richness of dominant archaeal phyla**			
	CCA1	**CCA2**	R^2	***P* value**	**CCA1**	**CCA2**	R^2	***P* value**	**CCA1**	**CCA2**	R^2	***P* value**
Water	-0.97	-0.25	0.74	0.001	**0.00**	**1.00**	**0.46**	**0.001**	-0.74	0.68	0.14	0.120
Energy	**0.98**	**0.20**	**0.77**	**0.001**	-0.23	-0.97	0.33	0.007	0.86	-0.51	0.16	0.101
Soil fertility	-0.98	-0.22	0.44	0.001	0.34	0.94	0.35	0.001	-0.75	0.67	0.05	0.474
Soil pH	0.97	0.23	0.48	0.001	-0.11	-0.99	0.27	0.011	**0.80**	**-0.60**	**0.21**	**0.037**
Soil metal concentration	-1.00	-0.05	0.21	0.023	**0.79**	**0.61**	**0.46**	**0.001**	-0.99	0.11	0.03	0.584
Soil texture	1.00	0.05	0.10	0.233	-0.70	-0.72	0.27	0.013	0.74	-0.68	0.03	0.615
Plant	-0.97	-0.24	0.73	0.001	0.11	0.99	0.25	0.021	-0.79	0.62	0.12	0.159
Disturbance	0.96	-0.27	0.27	0.013	-0.70	-0.71	0.01	0.813	**0.99**	**-0.13**	**0.21**	**0.037**
Variables	**Shannon-Wiener diversity of dominant bacterial phyla**				**Shannon-Wiener diversity of dominant fungal phyla**				**Shannon-Wiener diversity of dominant archaeal phyla**			
	CCA1	**CCA2**	R^2	***P* value**	**CCA1**	**CCA2**	R^2	***P* value**	**CCA1**	**CCA2**	R^2	***P* value**
Water	**-1.00**	**0.06**	**0.83**	**0.001**	-0.40	-0.92	0.27	0.010	-0.92	-0.40	0.27	0.010
Energy	0.97	-0.23	0.80	0.001	0.25	0.97	0.34	0.004	**0.95**	**0.32**	**0.32**	**0.006**
Soil fertility	-1.00	0.09	0.56	0.001	-0.90	-0.43	0.34	0.001	-0.93	-0.36	0.14	0.089
Soil pH	0.99	-0.13	0.45	0.001	0.42	0.91	0.19	0.044	0.88	0.47	0.21	0.045
Soil metal concentration	-0.91	0.41	0.39	0.003	-0.98	-0.20	0.29	0.009	-0.98	-0.21	0.18	0.047
Soil texture	0.95	-0.31	0.17	0.075	0.96	-0.26	0.33	0.002	0.93	0.37	0.04	0.504
Plant	-0.99	0.13	0.72	0.001	**-0.30**	**-0.95**	**0.45**	0.001	-0.91	-0.41	0.25	0.017

Disturbance	0.73	-0.68	0.28	0.007	0.19	0.98	0.05	0.447	1.00	-0.06	0.26	0.012

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