Zhao et al. Annals of Microbiology (2021) 71:21
https://doi.org/10.1186/s13213-021-01633-9

Annals of Microbiology

ORIGINAL ARTICLE Open Access

Soil microbial community variation with time and soil depth in Eurasian Steppe (Inner Mongolia, China)

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Abstract

Purpose: Soil microorganisms play an indispensable role in the material and energy cycle of grassland ecosystems. The abundance of these organisms vary according to environmental factors, such as time of year and soil depth. There have been few studies on the transformation of soil microbial communities in degraded typical steppe according to these temporal and spatial changes. In this study, we analyze the community structure and diversity of soil bacteria and fungi, and the impact of these changing temporal and spatial factors upon the community structure.

Methods: From May to September 2018, we collected 90 soil samples from different depths (10, 20, and 30 cm) from the typical degraded steppe area of Xilingol. We carried out studies on soil physical and chemical properties and soil microbial diversity using high-throughput sequencing technology.

Results: We found that depth significantly affected abundance and diversity of bacteria and fungi. Bacteria and fungi diversity at 10 cm was higher than that at 20 cm and 30 cm. The abundance of Acidobacteria, Proteobacteria, Actinomycetes, Ascomycetes, and Basidiomycetes varies significantly with depth. In addition, soil pH increased significantly with increasing depth, while soil organic matter (SOM), available nitrogen (AN), volume water content of soil (VWC), and soil temperature (ST) decreased significantly with increasing depth. Finally, the depth, total organic carbon (TOC), and AN had a significant impact on the bacterial and fungal communities' abundance ($p < 0.05$).

Conclusions: Spatial heterogeneity (in soil depth) is more significant than the time of year (month) in predicting changes in microbial community composition and soil properties. SOM, VWC, and the abundance of Proteobacteria and Actinomycetes positively correlate with soil depth, while pH and the abundance of Acidobacteria, Ascomycetes, and Basidiomycetes negatively correlate with soil depth. We speculate that SOM and VWC account for the variations in the abundance of Acidobacteria and Proteobacteria, while pH causes variations in the abundance of Actinomycetes, Ascomycetes and Basidiomycota.

Keywords: Soil microorganism, 16S rRNA gene, ITS rRNA gene, Eurasian steppe

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Introduction

Grasslands cover approximately 25% of the earth's terrestrial area and play an essential role in the global material cycle and energy exchange (Foley et al. 2011). The grassland of Inner Mongolia is typical of arid and semi-arid steppe located in the eastern region of the Eurasian steppe and is representative of the Eurasian steppe in terms of climate, terrain, soil properties, and vegetation composition. Although it should be noted that Inner Mongolian grasslands possess a unique history of land use and management policy (Wu et al. 2015). The soil is an important place for material and energy exchange within the grassland ecosystem and has a strong influence on the diversity of microorganisms, as it is the medium for microbial growth and metabolic activities. Microorganisms in soil occupy an indispensable position in the process of organics decomposing and the regulation of carbon (C) (Bardgett et al. 2008), nitrogen (N) (Bahram et al. 2018), phosphorous (P) (Handa et al. 2014), and sulfur (S) (Kowalchuk and Stephen 2001). Soil bacterial community diversity is strongly correlated to soil pH (Griffiths et al. 2011) while fungal communities are highly influenced by soil water content, nitrate, and organic matter content (Wang et al. 2018). Microbes have a broad symbiotic relationship with soil and plants. They play a key role in the availability of nutrients and elements (such as C, N, P, and others) directly affecting the growth of plants (Der Heijden et al. 2008). In addition, they decompose plant secretions and litter through their metabolic uptake of nutrients (Zhalnina et al. 2018).

The biogeographic distribution of soil microbial communities varies due to the non-uniform distribution of nutrients and plant roots in the soil (Eilers et al. 2012). The response of soil microorganisms to environmental factors such as soil physical and chemical properties, and plant community also varies significantly at different soil depths (0–10 cm and 10–20 cm). A multi-scale spatial assessment of the soil bacterial communities across the UK also found a significant correlation between the bacterial community and spatial distance (Horizontal) (Griffiths et al. 2011). The abundance, activity, and diversity of microbes decrease when soil depth increases in wetlands and fallow farmlands (Wang et al. 2010; Ko et al. 2017). Despite this, the research on grassland soil microorganisms mostly focuses on the fixed depth of the surface soil layer (for example 0–10 cm or 0–20 cm, or 10–30 cm) (Leff et al. 2015; Na et al. 2019), and few studies have analyzed the differences between different depths of the topsoil.

Climate and ecosystems drive plant phenology, which in turn influences soil microorganism communities. In temperate forest soils, the relative abundance of Actinomycetes will significantly increase in winter, but the relative abundance of Acidobacteria and Proteobacteria will decrease (Kuffner et al. 2012). In recent years, there has been wide concern about changes in soil microbial communities during the growing season for vegetation. In crops, the taxonomic and phylogenetic composition of root microbial communities varies throughout the growth cycle (Shi et al. 2015; Zhang et al. 2018). There is a gradual change from the background soil microbiome as the plants cause the enrichment of specific microbial groups. In contrast, the microbial community in the desert steppe remains relatively stable over the year (Barboza et al. 2018). In Eurasian grassland, seasonal variations in the soil temperature and water content lead to changes in the abundance of plant litter and secretions, which directly affects the input of carbon to the soil (Bardgett et al. 2005). Soil organic carbon is the most critical factor in driving the spatial distribution of microbial communities. When roots grow in summer, the production of soil carbon sources will also increase, while the production of carbon sources drop when root activities stop in autumn (Griffiths et al. 2003). As a result, the rapidly growing microbial populations (which prefer to use direct carbon sources) have to slow down their growth rate, which ultimately affects the microbial population structure (Barboza et al. 2018).

We conducted our research on the representative area of Eurasian Steppes in Inner Mongolia, China. We analyzed the community structure and diversity of soil bacteria and fungi, and the impact of environmental factors on the community structure. Those analyses were to explore three aspects. First, the composition of soil microbial communities in this area. Second, the affect the time of year (month) and soil depth has upon the structure of soil bacterial and fungal communities. And third, the relationship between soil properties and the soil bacterial or fungal communities.

Materials and methods

Site and soil sampling

The experiment site is located at Erlitu Ranch of Zhengxiangbai Banner, XilinGol, Inner Mongolia, China (42° 9′ 14′′ N, 115° 14′ 39′′ E, altitude: 1405 m), which is typical of Eurasian Steppes. Over the years, the area has been used for grazing. Winter is long and spring is short, summer is hot and autumn is cool. The average annual temperature is 1.9 °C, mean annual precipitation is 314 mm, and the average annual evaporation is 2126 mm. The soil type is mainly chestnut soil and soil nutrient is of medium nitrogen and low phosphorus. The main vegetation types are *Artemisia frigida Willd*, *Leymus chinensis*, *Stipa krylovii Roshe*, and *Cleistogenes squarrosa* (*Trin.*) (*Keng*).

We collected soil samples every month from June to October in 2018 (named May, Jun., Jul., Aug., Sep.). We

collected each sample at depths of 0–10 cm, 10–20 cm, and 20–30 cm (named 10, 20, and 30) with a soil drill of 8 cm in diameter. We selected six points for soil collecting which were mixed into composite samples (3.02×10^{-2} m^3). Animal and plant residues and other impurities such as gravel were removed by sieving (2 mm). Each composite sample was then divided into 3 subsamples as repeats for later DNA extraction (stored at − 80 °C) and other soil properties analysis (stored at − 20 °C).

Soil measurements

We used dried soil to measure the soil properties of soil samples taken at depths of 10, 20, and 30 cm, respectively. We measured pH (soil-water ratio 1:5) and soil organic matter (SOM, potassium dichromate external heating method) (Bao 2000). We also measured the soil NH_4^+ and NO_3^- concentrations (reagent kit method). We used a data acquisition unit (Campbell Scientific Inc., Logo, UT, USA, http://www.campbellsci.com) and sensor (Helsinki, Finland, https://www.vaisala.com/) for soil temperature (ST) and volume water content (VWC) measurements.

DNA extraction, PCR amplification, and sequencing

The total DNA of soil microorganisms was extracted from 0.5 g of each soil sample using a DNeasy PowerSoil Kit (QIAGEN Benelux B.V., Venlo, The Netherlands), each sample run in triplicate. We assessed DNA concentration and quality using an ultramicro nucleic acid quantifier, and checked the DNA integrity using 1% agarose gel electrophoresis.

The primers used in PCR were universal primers of bacterial 16S rRNA V3–V4 hypervariable region (338F, 806R) and fungal ITS1 region (ITS1-F, ITS2R), based on the Hiseq sequencing platform (Illumina Int., San Diego, CA, USA). The PCR reaction mixtures contained genomic DNA 40–60 ng, PCR buffer 15 μL, dNTP (10 mM) 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 ddH_2O to a final 50 μL. The following program was used for gene amplification: initial denaturation at 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 40 s. 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. We used QIIME v1.8.0 (Caporaso et al. 2010) to cluster the obtained Effective Tags at a similarity level of 97%, and obtain OTU (Operational Taxonomic Units). The representative sequences of OTU were then compared with the Silva (bacteria) and UNITE (fungal) databases to obtain the classification and taxonomic annotation information for the species corresponding to each OTU. We then counted the composition of each sample community at each level (phylum, class, order, family, genus, species).

Statistical analysis

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

We analyzed Alpha diversity after the taxonomic annotation of OTU using Mothur (Schloss et al. 2009), and we used the Shannon-Wiener Index ($H' = -\sum_{i=1}^{S} Pi \ln Pi$, where S is the total number of species and Pi is the proportion of individuals of this species to the total number of individuals) to measure species. We used Beta diversity analysis to compare the similarity of species diversity among different groups. Principal coordinates analysis (PCoA) uses dimensionality reduction to observe the differences in microbial community composition between groups (Anderson and Willis 2003). PERMANOVA (Adonis) can test for significance the difference of Beta diversity between samples of different groups. We performed PCoA and PERMANOVA and used the vegan package of the R language (Anderson and Walsh 2013; Team RC 2014) to plot them.

We used LEfSe analysis (Linear Discriminant Analysis effect size) to analyze the significant differences of soil bacterial and fungal communities' abundance under different treatments (Segata et al. 2011). We first determined the microbial species with significant differences in relative abundance between groups based on the rank sum test, then a linear discriminant analysis (LDA) was performed on the different species to obtain statistically significant biomarkers. We analyzed and plotted them on the LefSe website (http://huttenhower.org/galaxy/). An LDA score of 2.0 indicates a significant difference, and a score of 4.0 indicates extremely significant difference. In addition, we performed correlation analysis based on the Pearson algorithm in R to assess the correlation between environmental factors and the OTU abundance of bacterial and fungal community. We performed structural equation modeling (SEM) using the software IBM SPSS Amos 24.0 (Chicago, IL: Amos

Development Corporation) based on the results of the correlation analysis. This model was optimized according to our parameters (Lefcheck 2016).

Results

Variation of soil physicochemical properties

Our results showed that soils pH and NH_4^+ concentration reached a maximum in September, while VWC reached a maximum in August and September, and ST reached a maximum in July ($p < 0.05$, Table S1). Soil pH and NO_3^- increased significantly with soil depth, while SOM, NH_4^+, VWC, and ST decreased significantly with soil depth ($p < 0.05$, Table S1). Soil characteristics (except SOM) and bacterial richness and evenness varied significantly from month to month, while bacterial diversity, fungal richness, and fungal evenness changed non-significantly from month to month. Also, all indicators are significantly affected by depth (Table S2). The results of multivariate analysis of variance showed that the interaction of time of year (month) and depth significantly affected the soil physical and chemical indexes (except NO_3^-) ($p < 0.05$, Table 1).

Variation in bacterial and fungal community richness

The bacterial communities of the soil samples were mainly Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, and Verrucobacteria (95%). Ascomycota, Basidiomycota, Unclassified, and Mortierellomycota (99%) dominated the fungal community (Figure S1). GLMM analysis revealed that the abundance and diversity of bacteria and fungus, as well as the fungal evenness, decreased significantly with increased sampling depth. Both bacterial evenness and fungal diversity increased continually over time (Table S1). Our results showed that depth and time of year (month) significantly affected the changes in the abundance of phyla of bacteria and fungi (Table 2). The abundance of Actinobacteria, Ascomycota, and Basidiomycota increased with the increase of depth, while the abundance of Proteobacteria, Acidobacteria, and Chloroflexi decreased significantly with the increase of depth (Figure S1). The abundance of Ascomycota increased gradually from May to September, while the abundance of Verrucomicrobia and Basidiomycota showed a decreasing trend (Figure S2).

Beta diversity of the bacterial and fungal community

According to PCoA results, the bacterial and fungal community at 10 cm was different from 20 cm and 30 cm soil layers (Fig. 1a, b). As shown in Fig. 1c, d, the bacterial community composition in August was quite different from other months, and the fungal community composition was different in August and September. Moreover, according to PERMANOVA's analysis, depth had a significant effect on bacterial communities (R^2 = 0.487, p = 0.001) but not fungal communities (R^2 = 0.177, p = 0.001). The time of year (month) variable had no significant effect on fungal communities (R^2 = 0.155, p = 0.001) and bacterial communities (R^2 = 0.074, p = 0.087) (Fig. 1b–d, Figure S3).

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	141.026	**0.000**	21.398	**0.000**	25.361	**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
Fungal OTU richness	1.157	0.337	72.667	**0.000**	0.603	0.773
Fungal ACE estimator	1.135	0.347	17.069	**0.000**	0.399	0.918
Fungal Chao1 estimator	1.274	0.288	29.850	**0.000**	0.398	0.918
Fungal Simpson index	0.258	0.904	22.016	**0.000**	1.173	0.327
Fungal Shannon index	5.841	**0.000**	2.564	0.084	3.257	**0.003**

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. $p < 0.05$

Taxonomic composition

The abundance of Actinobacteria, Thermoleophilia, MB_A2_108, and Gaiellales increased significantly with soil depth, while the abundance of Acidobacteria, Proteobacteria, Rhizobiales, and Alphaproteobacteria decreased with soil depth (irrespective of time of year) (Fig. 2a). Similarly, the abundance of Ascomycetes, Basidiomycetes, and Agaricomycetes increased significantly with the increase of soil depth, while the abundance of Dothideomycetes, Hypocreales, Pleosporales, and Eurotionmycetes were significantly reduced (Fig. 2b).

If one does not consider soil depth, time of year (month) significantly affected the abundance of soil bacteria and fungi. We performed monthly analysis of the abundance of soil microbial communities. As shown in Fig. 2c, d, the abundances of Firmicutes, Mortierellomycetes, and Phaeosphaeriaceae (belonging to Ascomycota) increased significantly in September; the abundance of Gemmatimonadetes, Gammaproteobacteria, Subgroup_6 (belonging to Acidobacteria), and Fusarium (also belonging to Ascomycota) increased in August; the abundances of Cantharellus and Ceratobasidiaceae (all belonging to Basidiomycetes) were highest in May; and the abundance of Hypocreales (belonging to Ascomycota) increased in June.

Factors driving bacterial and fungal communities' composition

Through Pearson correlation analysis ($p < 0.05$), we found that time of year (month) and depth were significantly correlated with microbial communities (bacteria and fungi) (Fig. 3). TOC and VWC were significantly related to the bacterial community, while TOC and ST were significantly related to fungal aggregation ($p < 0.05$, Fig. 3). We built a SEM model based on the correlation analysis (Fig. 4). The result showed that time of year (month), depth, pH, TOC, and AN significantly impacted the bacterial communities ($p < 0.05$, Fig. 4a). As shown in Fig. 4b, TOC and AN positively affected fungi, while soil depth and ST influenced fungi negatively ($R^2 = 0.35$). The time of year (month) had a negative effect on AN and ST, leading to a reduced positive effect of AN upon fungal communities relative to the enhanced the negative effect of ST (Fig. 4b).

Discussion

This study took typical degraded grassland in Inner Mongolia as the basis for multiple sampling at a fixed point. The time of year (month) and depth were used to represent time and space. We analyzed the variation of pH, VWC, and ST to understand the soil physical chemistry and nutrients variation as time and space changed. We used next-generation sequencing technology to investigate the changes of microbial diversity and the factors of microbial community structure transformation with temporal and spatial variation. Our results provide strong evidence that spatial heterogeneity (depth) is more significant than time of year (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. The change of soil microbial community composition caused by spatiotemporal variation would inevitably lead to the change of its ecological function, which will then affect the ecosystem function.

Vertical spatial variation of microbial communities and soil properties

Our results indicate that soil pH increased significantly with increasing depth, while ST, VWC, SOM, and AN decreased significantly with increasing soil depth because the top soil layer (0–10 cm) was extensively influenced by external environment conditions. The grasslands of Inner Mongolia in particular had been influenced by human activities like grazing and mowing for a long time, which has resulted in the decline of the productivity and diversity of grassland vegetation (Xun et al. 2018). Furthermore,

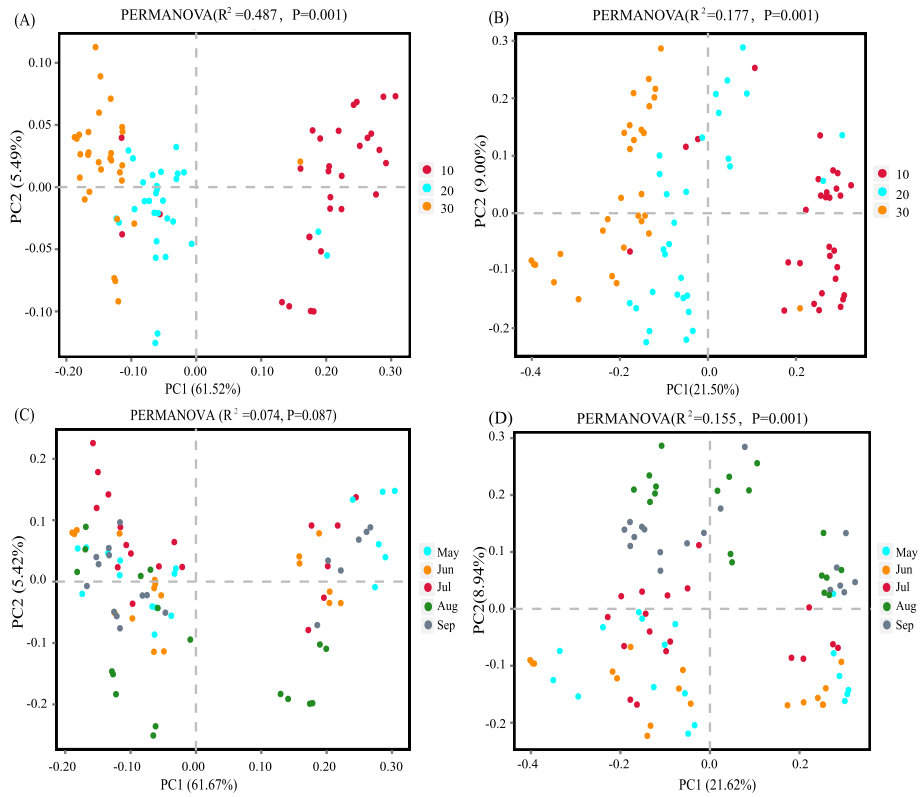

Fig. 1 PCoA analysis of soil microbial community structure based on Bray-Curtis distance algorithm. **a**, **b** represent bacteria and fungi at different soil depths, and **c**, **d** represent bacteria and fungi in different month. Dots represent each sample; colors represent different groupings. The horizontal and vertical coordinates are values that cause the largest difference between samples, and reflect the influence as a percentage.10, 20, and30 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 larger the R^2-value, the higher the interpretation degree of the grouping to the difference, and the greater the grouping difference. When the $p < 0.05$, the reliability of the test is high

human activities also resulted in an increase in bare area, aggravated erosion and coarseness of surface soil, and reduced nutrient content (Liu et al. 2013). The deeper soil (30 cm) environment is more stable than the surface (10 cm). As the soil depth increases, the plant litter and secretions decrease along with distribution of plant roots (Truong and Marschner 2018). This 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). Further, increasing soil depth leads to the decrease of soil carbon and nitrogen availability (Wang et al. 2014a).

According to our results, time of year (month) significantly affected most of the soil physical and chemical indexes, but each index varies without an obvious relationship to time of year (month), which requires further study and discussion. However, we found that NO_3^- content was positively correlated with soil temperature; NH_4^+ was negatively correlated with soil temperature; soil NO_3^- and NH_4^+ were linearly correlated with soil VWC (Fig. 3); all these results were consistent with previous research results (Xie et al. 2020). Note that microorganisms prefer using NH_4^+ while plants prefer to uptake NO_3^- (Xu et al. 2011). Plants use their roots to uptake inorganic nitrogen from the soil and continue to uptake inorganic nitrogen after exuberant plant growth period (Xu et al. 2011). Studies have shown that one-third of soil nitrogen loss (especially NO_3^-) occurs in autumn (Treat et al. 2016), which may explain the decrease of soil NO_3^- content in August and September in this paper. In addition, the VWC increased NH_4^+ content mainly by enhancing microbial activity. Some studies theorize that higher soil water content meant higher nitrification, which then lead to increased NO_3^- content (Osborne et al. 2016). Soil temperature is a major factor in organic decomposition and vegetation growth, which then affects inorganic nitrogen content in

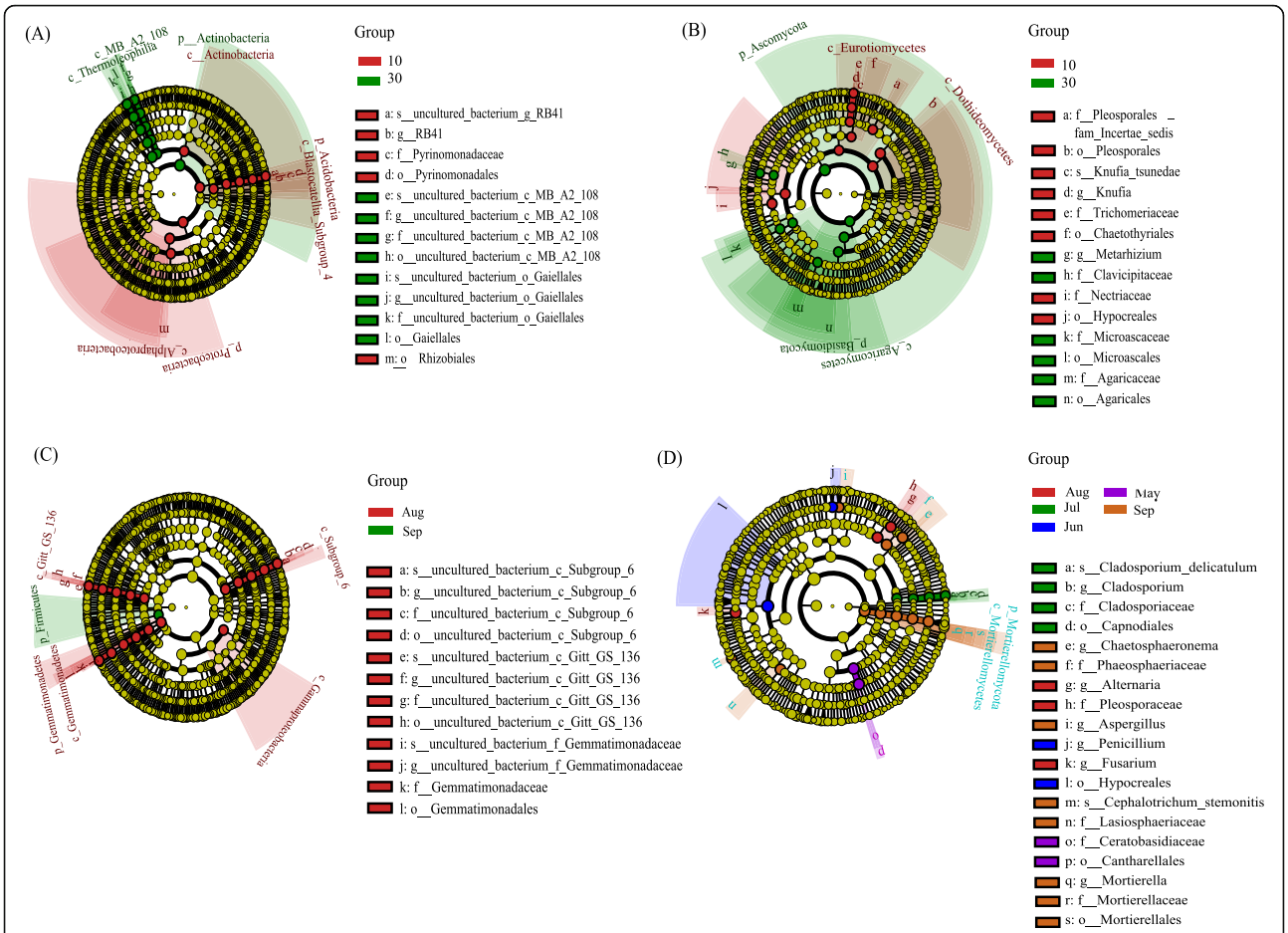

Fig. 2 LEfSe analysis of soil bacteria and fungi. **a**, **b** represent bacteria and fungi at different soil depths and shows species with LDA Score more than 4.0, and **c**, **d** represent bacteria and fungi in different months and shows species with LDA Score more than 3.5. *P* value is less than 0.05, and different colors indicate species in different groups. 10 and 30 respectively represents 10 cm and 30 cm. May, Jun, Jul, Aug, and Sep respectively represent sampling in May, June, July, August, and September

soil (Bilbrough et al. 2000). Soil temperature also affects the adsorption and desorption of nitrogen in the soil solid phase (Davidson and Janssens 2006).

We found that both soil characteristics and microbial community structure were more significantly correlated with soil depth than with time variations. This is consistent with previous findings, which confirm the importance of spatial heterogeneity (Fierer and Jackson 2006; Lauber et al. 2013), where variance on the scale of meters or even centimeters is significant (O'Brien et al. 2016). Studies have found that microbial community structure and abundance were responsive to changes of environmental factors in equal levels (Bell et al. 2014; Na et al. 2019), but some studies have shown that community composition is more susceptible to change than community diversity (Fierer and Jackson 2006). Our study did not find that response differed between community composition and diversity. Instead, we found that bacterial abundance and evenness were significantly affected by both soil depth and time of year (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 the properties of each, as 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 which are able to withstand sudden environmental changes (Sun et al. 2017). The mycelium structure also assists the fungi to spread, whereas bacteria does not have this advantage and possesses severe transmission limitations (Young 2006; Schmidt et al. 2014). The interaction between time of year (month) and depth weakened the effect of depth upon 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 better understand the observed changes in microorganisms.

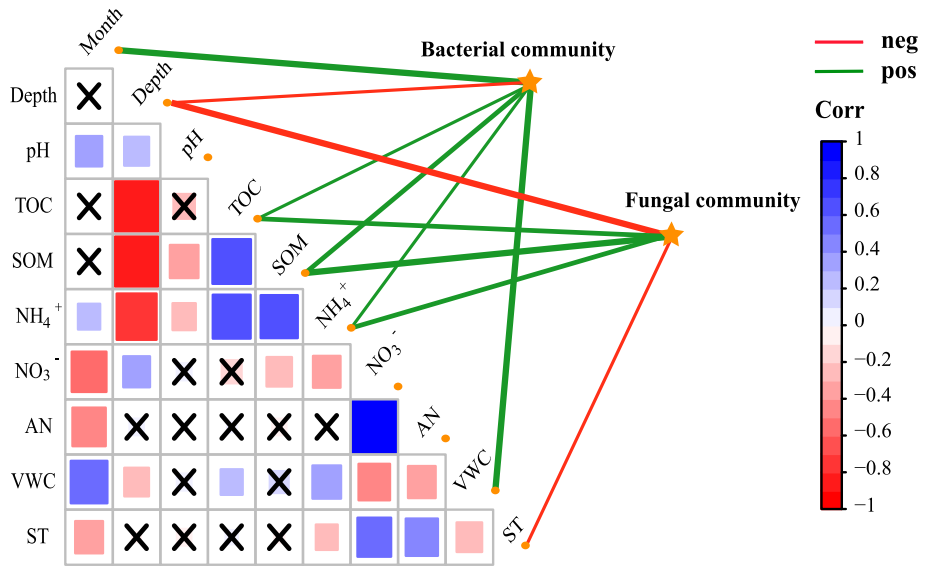

Fig. 3 Pearson correlation analysis between time of year (month), depth and soil properties, and bacterial and fungal community structures. *TOC* total soil organic carbon, *SOM* soil organic matter, *AN* available ammonium (the sum of NO_3^- and NH_4^+), *VWC* volume water content of soil, *ST* soil temperature. Bacterial community was represented by abundance of OTU in bacteria. Fungal community was represented by abundance of OTU in Fungi. "×" indicates non-significance at $p < 0.05$

The interaction of multiple factors may be synergistic, superimposed, or antagonistic, which cannot be predicted by single factor experiment (Zhu et al. 2016). For example, the interaction between precipitation and CO_2 concentration and temperature enhanced the influence of precipitation on microbial biomass (Gray et al. 2011).

Driving factors of soil microbial community structure

Changes of soil properties had impacts on the variation of microorganisms in the vertical section. Soil properties and microbial species abundance varied with soil depth in our results (Table 2, Table S1, Fig. 2). Therefore, the variation of VWC and SOM contributed to the abundance changes of Acidobacteria and Proteobacteria, and the variation of pH contributed to the abundance changes of Actinomycetes, Ascomycetes, and Basidiomycetes. Studies have shown that pH is the driving force in the formation of soil microbial communities that changes in soil fungal communities, and these significantly correlate with soil moisture and pH (Zheng et al. 2009; Wang et al. 2014a, 2014b). On the other hand, different microbial communities have different utilization of nutrients (Chapin et al. 2011). Compared with fungi and Actinobacteria, bacteria more generally use smaller organic matter molecules, while fungi and Actinobacteria can decompose substrates with relatively large molecules by producing lignin-degrading enzymes (Bonanomi et al. 2017; Chapin et al. 2011). Therefore, this characteristic of fungi contributed to its higher diversity in soil layer of 30cm, while bacteria had higher diversity in soil layer of 10 cm.

We also found that the soil temperature is highest in July and August, and the soil moisture content is highest in June and July. In this area, soil pH is alkaline, and NO_3^-, NH_4^+ content is limited. The contents of NH_4^+ and NO_3^- are the lowest in August, and the pH is highest in August. Through analysis of correlation and taxonomic composition, we found that time of year (month) had a significant positive effect on abundance of bacterial community (Figs. 2 and 3). According to the result of SEM, time of year (month) has a direct and significant positive effect on bacteria, and it promotes its significant positive effect on bacteria by indirectly affecting pH and VWC. The time of year (month) has no direct effect on fungi, but indirectly affects fungi through AN and ST. This indirect effect causes significant changes in species abundance in the 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 (Cmic, Nmic), soil TOC, TN, and NH_4^+ all increase with the increase of precipitation, while pH value decreases with the increase of precipitation (Yao et al. 2017)

Nevertheless, time of year (month), soil depth, and environmental variables did not contribute to 65–70% of microbial composition variation in our study. The possible reason is the existence of other unmeasured environmental factors that vary in space and time (Bahram et al. 2015; Hanson et al. 2012), including biotic interactions such as competition, mutualism, and predation

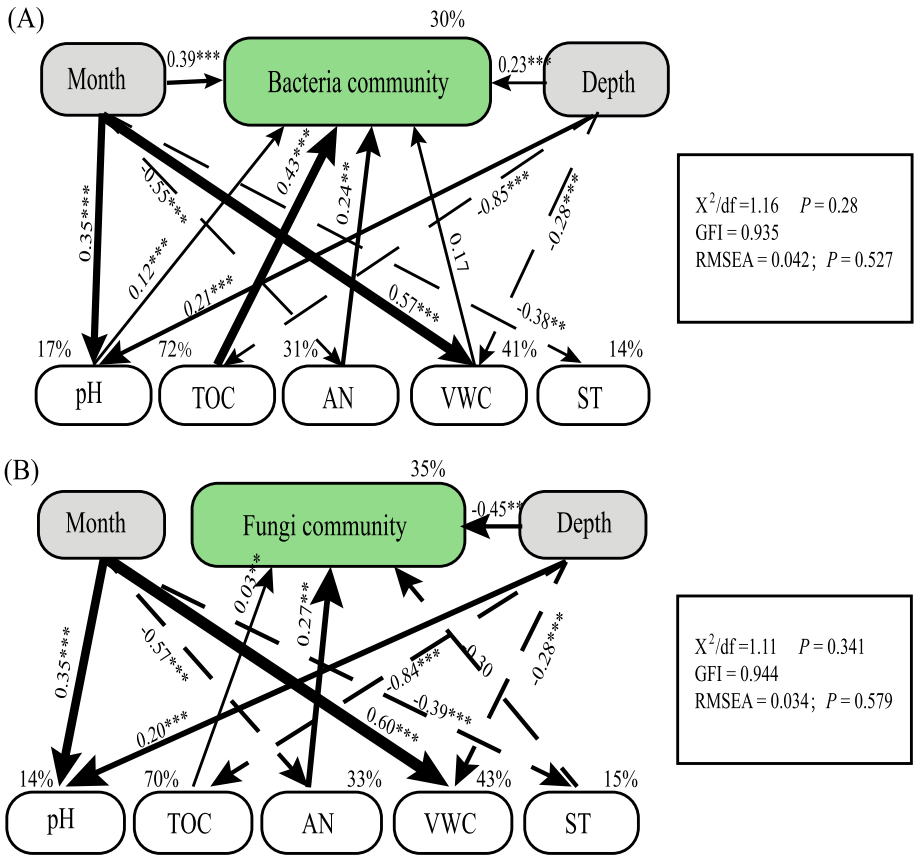

Fig. 4 SEM analysis of bacteria and fungus with environmental factors. **a**, **b** represent structural equation model analysis of bacteria and fungi. TOC, AN, VWC, ST represent as above; *BC* bacterial community, *FC* fungi community. Solid arrows indicate positive correlations, and dotted arrows indicate 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 (R^2) associated with response variables represent the proportion of contributed to variation by other variables

between microbial taxa (Zhou and Ning 2017) and ecological processes such as dormancy and persistence traits of microbial communities and their members (Averill et al. 2019).

Conclusion

This paper analyzed the spatiotemporal variation and driving factors of soil microbial community structure in a typical degraded steppe. The results show that both time of year (month) and soil depth have a significant effect upon microbial community structure and soil properties, but depth has the more significant effect. The abundance and diversity of bacteria and fungi were significantly affected by depth. Specifically, the abundance of Acidobacteria, Proteobacteria, Actinomycetes, Ascomycetes, and Basidiomycetes varies significantly with depth. Because soil pH increased significantly with increasing depth, while SOM, AN, VWC, and ST decreased significantly with increasing depth, we speculate that SOM and VWC account for the abundance variations of Acidobacteria and Proteobacteria, and pH causes the abundance changes of Actinomycetes, Ascomycetes, and Basidiomycota. This study only analyzed the microbial changes in a single year, which may underestimate the real microbial changes over time. 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.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-021-01633-9.

Additional file 1: Figure S1. The variations in relative abundance of dominant bacterial and fungal phyla with depth. (A) and (B) represent bacteria and fungi. 10, 20, and 30 represent 10 cm, 20 cm, and 30 cm of soil depths, respectively

Additional file 2: Figure S2. The variations in relative abundance of dominant bacterial and fungal phyla with depth. (A) and (B) represent bacteria and fungi. May, Jun, Jul, Aug, Sep respectively represent different months of the growing season, namely May, June, July, August and September

Additional file 3: Figure S3. PERMANOVA analysis of soil bacterial and fungal communities based on the Bray-Curtis distance algorithm. (A) and (B) represent bacteria and fungi at different soil depths; (C) and (D) represent bacteria and fungi in different months. Sitting vertically means Beta distance. The box graph above "All between group" represents the Beta distance data of all samples between groups, and the box graphs below are the Beta distance data between samples within different groups. 10, 20, and 30 represent 10 cm, 20 cm, and 30 cm of soil depths, respectively. May, Jun, Jul, Aug, Sep respectively represent different months of the growing season, namely May, June, July, August and September. The larger the *R*-value, the higher the interpretation degree of the grouping to the difference, and the greater the grouping difference. When the *p*-value is less than 0.05, the reliability of the test is high

Additional file 4: Figure S4. The original diagram of the structural equation model. (A) and (B) represent SEM of bacteria and fungi respectively. The structural equation model was build based on the results of Pearson correlation analysis. The results show that our observed data fit well with the bacterial (χ^2 /df = 1.16, p = 0.280, GIF = 0.935, RMSEA = 0.042) and fungal (χ^2 /df = 1.11, p = 0.341, GIF = 0.944, RMSEA = 0.034) models

Additional file 5: Table S1. Variations in soil properties and microbial community α-diversity index with depth and month

Additional file 6: Table S2. GLMM results showing effects of month and depth on soil properties and microbial community α-diversity index

Acknowledgements
None.

Authors' contributions
HZ: the conception of the study (lead), writing—final draft and review, and editing (equal). WZ: conceptualization (equal), data analysis (lead), and writing—original draft (lead). SZ: the conception of the study (lead) and funding acquisition (lead). WG: methodology (supporting). YF: writing—review and editing (supporting). All authors read and approved the final manuscript.

Funding
The National Natural Science Foundation of China jointly supports this work under the grant numbers of 31760154 and 51779116, and the Inner Mongolia Science Fund for Distinguished Young Scholars, under grant number of 2019JQ06. The authors want to thank the anonymous reviewers since their comments are very constructive and helpful for the improvement of this work.

Availability of data and materials
Sequence data have been deposited in the NCBI (https://www.ncbi. nlm.nih.gov/sra) under the accession number PRJNA664840.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 2 February 2021 Accepted: 3 May 2021
Published online: 26 May 2021

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