Pak. J. Bot., 57(6): DOI: http://dx.doi.org/10.30848/PJB2025-6(29)

SOIL NUTRIENT LEVELS DETERMINE THE FACTORS THAT INFLUENCE CHANGES IN THE SOIL BACTERIAL COMMUNITY IN THE XILINGOL STEPPE, INNER MONGOLIA, CHINA

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Abstract

Soil bacteria play key roles in various ecosystems and mediate several important ecological processes. Understanding the factors determining soil bacterial community composition is critical for evaluating and forecasting ecosystem functions. However, the factors affecting soil bacterial community changes vary widely across different studies. Aims: Spatiotemporal heterogeneity and scale, the plant community, and soil properties are considered the factors driving changes in soil bacterial communities. However, the impacts of these factors on soil bacterial communities remain unclear. Therefore, the aim of this study was to elucidate the impact of these factors on soil bacterial communities. Three different grasslands were selected, and the abundance, diversity and composition of the soil bacterial communities were determined at three soil depths (topsoil: 0-10 cm, middle soil: 10-20 cm, and subsoil: 20-30 cm), as well as the corresponding vegetation and soil properties of the grasslands. Our results suggest that vegetation and soil properties can collectively influence soil bacterial communities; however, the dominant factors affecting soil bacterial changes differed at different soil nutrient levels. In low-nutrient grasslands (G1 and G3), changes in soil bacterial communities were closely linked with soil properties or vegetation characteristics; in high-nutrient grasslands (G2), changes were collectively affected by vegetation and soil properties. Conclusions: The findings of this study indicate that close and highly positive associations among plants, bacteria, and soil can be found only under nutrient-sufficient conditions.

Key words: Soil bacterial abundance, Soil bacterial diversity, Soil properties, Soil nutrient limitation.

Abbreviations: GE: gazing exclusion; FG: free grazing; AB: aboveground biomass; GAB: aboveground biomass of gramineous species; AAB: aboveground biomass of annual and biennial species; FAB: aboveground biomass of forbs; SAB: aboveground biomass of shrubs; LM: litter mass; SR: species richness; GSR: richness of gramineous species; ASR: richness of annual and biennial species; FSR: species richness of forbs; SSR: species richness of shrubs; BB: belowground biomass; SBD: soil bulk density; FW: fresh weight; DW: dry weight; SWC: soil water content; PH: soil pH value; AHN: alkali-hydrolyzable nitrogen; TN: total nitrogen; AP: available phosphorus; TP: total phosphorus; AK: available potassium; TK: total potassium; SOM: soil organic matter; PHO: phosphatase activity; SUC: sucrase activity; URE: urease activity; H: Shannon-Wiener diversity; E: Pieou evenness; ANOVA: analysis of variance; SRA: sequence read archive; AQ: absolute quantitation; RQ: relative quantitation; RDA: redundancy analysis; PCA: principal component analysis; PCoA: principal coordinate analysis; PLS-DA: partial least squares discriminant analysis; AASBC: absolute abundance of soil bacterial community; HSBC: Shannon-Wiener diversity index of soil bacterial community; TPM: transcripts per million.

Introduction

Soil bacteria play key roles in ecosystems and mediate many ecological processes that are central to ecosystem functions, including nutrient cycling (Balser & Firestone, 2005), litter decomposition (Johnson *et al.,* 2003), and the regulation and maintenance of plant biodiversity (Zak *et al.,* 2003). Previous studies have indicated that soil microbial communities can be influenced by various biotic and abiotic factors, such as vegetation biomass, diversity and composition (Waymouth *et al.,* 2020) and soil physical and chemical properties (Liu *et al.,* 2010; Zhong *et al.,* 2019). However, the patterns of changes in soil microbial communities and the determinants of these changes are still unclear.

Numerous findings indicate that many factors can cause changes in soil microbial communities. Soil microbial communities can differ across various spatial and temporal scales due to differences in the interactions between soil microbial communities and environmental factors (Lindström & Langenheder, 2011). Soil properties, such as available moisture, soil pH, organic carbon content, total nitrogen content, available phosphorus content, and available potassium content, are also considered key factors affecting the composition and diversity of soil bacterial communities at different scales (Fierer & Jackson, 2006; Serna-Chavez *et al.,* 2013; Zhong *et al.,* 2019). Soil pH is considered the most important factor influencing soil bacterial community composition and diversity at the continental scale (Griffiths *et al.,* 2011), regional scale (Wang *et al.,* 2015; Yang *et al.,* 2018), and plot scale (Yao *et al.,* 2014). Other evidence suggests that plants have the capacity to drive and shape soil bacterial community structure through the release of exudates (Liu *et al.,* 2010; Yang *et al.,* 2018; Liu *et al.,* 2020). In particular, plant diversity is considered to promote the diversification of soil microbes, resulting in increased diversity of soil microbial communities at different scales (Prober *et al.,* 2015; Ren *et al.,* 2018). Different plant species release various types of root exudates; thus, the growth of different plants results in different soil bacterial community structures (Ladygina & Hedlund, 2010). Inconsistencies in the dominant factors driving soil bacterial community changes are usually attributed to the spatial heterogeneity of study sites with

different abiotic and biotic conditions (Lindström & Langenheder, 2011; Yang *et al*., 2018). However, little information is available on the factors driving soil bacterial community changes and the relative importance of abiotic and biotic factors. To address this gap in knowledge, a comprehensive analysis of the quantitative linkages between soil microbial communities and biotic and abiotic properties is needed, which would provide a greater understanding of the dominant factors driving soil microbial community changes and patterns.

In our previous research, we reported that soil nutrient levels are an important factor driving changes in soil bacterial communities (Huang *et al*., 2022, 2023; Wang *et al*., 2023). To explore whether soil nutrient levels also cause the observed inconsistencies in the driving factors of changes in soil bacterial communities and the corresponding patterns, we studied the driving factors and patterns of soil bacterial community changes in three grasslands with different soil nutrient levels. These three grasslands had similar aboveground vegetation communities, climatic conditions, and soil types but had different vegetation characteristics and soil nutrient levels (Tables 1, 2 and S1). In this study, we aimed to further clarify the dominant factors driving soil bacterial community changes in three different grasslands and determine the effects of changes in vegetation and soil properties on soil bacterial communities. This work contributes to our understanding of the forces driving soil bacterial community changes and corresponding patterns at different scales.

Table 1. Vegetation biomass and species richness of the three grasslands.

Study site	Vegetation biomass						
	AB	**BB**	**LM**	**GAB**	**FAB**	**SAB**	**AAB**
Grassland 1	300.83 ± 27.29	28.35 ± 3.14	43.02 ± 4.07	97.26 ± 9.98	84.86 ± 8.06	118.55 ± 9.42	0.16 ± 0.01
Grassland 2	268.79 ± 66.22	53.84 ± 6.88	91.37 ± 7.56	141.87 ± 6.87	97.18 ± 9.49	21.13 ± 1.89	8.61 ± 0.34
Grassland 3	171.17 ± 14.86	55.88 ± 5.25	151.21 ± 10.60	159.94 ± 10.12	10.73 ± 1.58	0.00 ± 0.00	0.50 ± 0.03
Study site	**Vegetation species richness**						
	SR	**H**	**E**	**GSR**	**FSR**	**SSR**	**ASR**
Grassland 1	12.00 ± 0.67	1.14 ± 0.04	0.46 ± 0.04	2.67 ± 0.02	6.67 ± 0.61	2.00 ± 0.17	0.67 ± 0.01
Grassland 2	15.67 ± 2.22	2.07 ± 0.13	0.76 ± 0.08	5.67 ± 0.06	6.00 ± 0.50	1.67 ± 0.14	2.33 ± 0.14
Grassland 3	7.33 ± 1.11	1.00 ± 0.19	0.51 ± 0.04	3.00 ± 0.00	3.67 ± 0.11	0.00 ± 0.00	0.67 ± 0.04

Table 2. Soil properties of the three grasslands.

Study site	AHN	AK	AP	TN	TK	TP	SOM
Grassland 1	61.02 ± 2.96	16.29 ± 0.72	4.29 ± 0.31	0.63 ± 0.04	34.25 ± 1.28	0.22 ± 0.03	6.13 ± 0.26
Grassland 2	92.42 ± 5.74	17.39 ± 0.94	6.53 ± 0.36	0.90 ± 0.12	35.14 ± 1.87	0.29 ± 0.02	10.75 ± 0.84
Grassland 3	56.23 ± 2.33	13.72 ± 0.21	2.02 ± 0.15	0.83 ± 0.08	35.39 ± 1.02	0.24 ± 0.00	8.31 ± 0.10
Study site	**SBD**	**SWC**	**PH**	**PHO**	**SUR**	**URE**	**AASBC**
Grassland 1	1207.00 ± 14.31	6.45 ± 0.20	6.98 ± 0.17	17.36 ± 1.16	19.72 ± 1.44	48.87 ± 2.03	$0.68\times10^8 \pm 0.02$
Grassland 2	1080.15 ± 81.74	4.81 ± 0.48	7.18 ± 0.04	12.92 ± 0.99	67.37 ± 3.96	271.90 ± 4.57	$1.76\times10^8 \pm 0.51$
Grassland 3	1316.45 ± 17.07	3.60 ± 0.03	7.97 ± 0.02	10.55 ± 0.74	22.21 ± 1.92	129.45 ± 4.18	$1.69\times10^8 \pm 0.24$

AHN: Alkali-hydrolyzable nitrogen content; AK: Available potassium content; AP: Available phosphorus content; TN: Total nitrogen content; TK: Total potassium content; TP: Total phosphorus content; SOM: Soil organic matter content; SBD: Soil bulk density; SWC: Soil water content; PH: Soil pH value; PHO: Phosphatase activity; SUC: Sucrase activity; URE: Urease activity; AASBC: Absolute abundance of the soil bacterial community

Materials and Methods

Study sites: Three grasslands (Grassland 1: 43°33-34' north latitude, 116°40-41' east longitude, 1210-1241 m above sea level; Grassland 2: 43°35-36' north latitude, 116°44-45' east longitude, 1168-1211 m above sea level; and Grassland 3: 43°32-34' north latitude, 116°32-33' east longitude, 1158-1184 m above sea level) in the Xilingol steppe of Inner Mongolia, China, were selected as the study sites. In each grassland, four plots with different vegetation productivity levels were selected, including one no-grazing (GE) plot and three free-grazing (FG) plots. The three FG plots in each grassland represented different degrees of degradation, defined according to the judgment criteria established by Li (1997). The plots were designated as slightly degraded (keeping 1 sheep per hectare, FG1), moderately degraded (keeping 5 sheep per hectare, FG2), or severely degraded (keeping 10 sheep per hectare, FG3). A large sampling site (600×400 m^2) was delineated in each grassland, and three plots (100×100 m^2) were randomly selected in each site. Three quadrats (1×1 m^2) were randomly selected in each plot.

Soil sampling: Sampling was conducted in the GE and FG plots in June 2019. Sampling was performed on a sunny day. Before soil sampling, there had been no rain for more than one week in the sampled grasslands. The soil was sampled by drilling a 7 cm diameter soil core into the 0-10 cm, 10-20 cm, and 20-30 cm soil layers in each quadrat. Each soil sample was divided into two portions. One portion was preserved in -80°C liquid nitrogen for DNA extraction to measure the abundance, diversity,

composition, and ecological functions of the soil bacterial communities. The other portion was air-dried at room temperature, and ground to measure the belowground biomass and the physical, chemical, and enzymatic properties of the soil.

Measurements: The aboveground biomass (AB, g m^{-2}) of different types of plant species [including gramineous species (G), annual and biennial species (A), forbs (F) and shrubs (S)] and litter mass (LM) were reported as the weight of the aboveground parts of the corresponding plant species in each quadrat. The plant species richness (SR) of different grassland types was reported as the number of corresponding plant species in each quadrat. The vegetation belowground biomass (BB, g m^{-3}) was calculated as the weight of the total vegetation roots in each layer of soil taken from a cylindrical soil block with a diameter of 7 cm. The soil bulk density (SBD) was calculated by dividing the weight of a cylindrical soil block with a diameter of 5 cm and height of 5 cm by its volume. The cylindrical soil block was then weighed to obtain fresh weight (FW), oven-dried at 105°C for 24 h, and weighed again to obtain the dry weight (DW). The soil water content (SWC) was calculated as (FW-DW) 100%/FW. The soil pH value (PH) was determined via potentiometry. Seven soil nutrient parameters were measured in this study: alkali-hydrolyzable nitrogen (AHN), total nitrogen (TN), available phosphorus (AP), total phosphorus (TP), available potassium (AK), total potassium (TK), and soil organic matter (SOM). AHN was determined via the alkaline hydrolysis diffusion method; TN was determined via the Kjeldahl nitrogen determination method (NY/T53-1987); AP was determined via the sodium bicarbonate leaching-Mo-Sb colorimetric method (LY/T1233-1999); TP was determined via the alkali fusion-Mo-Sb colorimetric method (NY/T88-1988); AK was determined via the ammonium acetate extraction method (Jones, 1973); TK was determined via the HF-$HClO_4$ heating digestion method (Jackson, 1958); and SOM was determined via the $K_2Cr_2O_7$ oxidation volumetric method (Li, 1983). The phosphatase activity (PHO), sucrase activity (SUC), and urease activity (URE) of all the soil samples were determined according to the methods of Guan (1986).

DNA extraction and PCR amplification: The genomic DNA of each soil sample was extracted via the FastDNA™ SPIN Kit for Soil DNA Extraction (MP Biomedicals, LLC, OH, USA) according to the manufacturer's instructions. The DNA quality was evaluated via agarose gel electrophoresis (MultiDoc-It Digital imaging system, UVP, Cambridge, UK), a concentration assay (NanoDrop2000, Thermo Fisher Scientific, USA), and the OD_{260}/OD_{280} ratio (NanoDrop2000, Thermo Fisher Scientific, USA). Samples with satisfactory DNA concentration and quality were subsequently subjected to high-throughput 16S rRNA PCR amplification (Genesky Biotechnologies Inc., Shanghai, 201315, China). The bacterial V3-V4 (forward primer, 341F: 5'-CCTACGGGNGGCWGCAG-3', reverse primer, 805R: 5'-GACTACHVGGGTATCT AATCC-3') region of the 16S rRNA gene was the target (Klindworth *et al.,* 2013). The PCR products were purified with a PCR Clean-Up™ Kit (MO BIO Labs, Solana Beach, CA, USA) and then sequenced via an Illumina MiSeq 2×250 bp double-terminal sequencing strategy (Jiang *et al.,* 2019).

High-throughput absolute quantitative sequencing was achieved by adding a certain amount of a synthetic 'spike-in standard' sequence to the sample DNA, constructing a 16S amplicon library and sequencing, and then constructing a standard curve according to the 16S amplicon read number and the absolute copy number of the 'spike-in standard' sequence. Using the standard curve, the absolute copy number of 16S rRNA genes of each species was calculated. 16S rRNA operational taxonomic units (OTUs), with an identity threshold of 97% were processed via the Usearch software platform (version 10.0 http://www.drive5.com/usearch/, Edgar, 2013). Chimeric OTUs were then removed via the UCHIME algorithm (Edgar *et al.,* 2011). After the absolute copy numbers of the bacterial OTUs were calculated, the spike-in sequence was removed prior to subsequent analyses. Rarefaction curves based on the observed species were generated for soil bacterial diversity analysis. The raw sequences have been published in the Sequence Read Archive (SRA) database of NCBI servers (https://www.ncbi.nlm.nih.gov/sra) with the accession number PRJNA646998.

Then, the software FastQC was used to evaluate the quality of the raw sequencing data. Sequences containing N bases were removed, as were sequences with a proportion of high-quality bases (Phred score≥20, Q20) less than 60%. Low-quality base sequences at both ends of the sequences were trimmed, and sequences with a length less than 100bp were discarded. The software metaSPAdes (--only-assembler -k 33,55,77,99,127) was employed to assemble the Clean Reads of each sample into Contig. Subsequently, bwa-mem (with default parameters) was utilized to align the Clean Reads to the Contig, and the sequence assembly efficiency was then calculated. The software MMseqs2 (--min-seq-id 0.95 --cov-mode 1 -c 0.9) was used to remove redundancy from the assembly results, and only the Contigs with a length longer than 500bp were retained. The software MetaGeneMark (with default parameters) was used to predict gene structures, and the Genes with the sequence length longer than 100bp were retained. The software bwa-mem (with default parameters) was used to align the Clean Reads to the Contigs, and the R software was used to calculate the TPM (transcripts per million) value of each Contig. Based on the predicted relations between Contig and Gene, the TPM value of each gene was obtained. The software MMseqs2 (-s 7) was used to align sequences to the GTDB database, and the software DIAMOND (blastx --fast) was used to align the gene sequences to the species protein sequence database.

Data analysis: The Shannon-Wiener diversity (H) and the Pielou evenness (E) indices of the vegetation species were calculated according to the methods of West (West, 1993). One-way analysis of variance (ANOVA) based on Duncan's multiple-range test was performed in SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) to examine differences among the different study sites.

The 16S rRNA gene sequences were processed via the QIIME pipeline (http://qiime.org/, version 1.7.0). All sequence reads were trimmed and assigned on the basis of gene barcodes and were clustered into OTUs at a 97% identity threshold. The bacterial α diversity indices (Shannon diversity, Simpson, Chao1 richness, and ACE richness indices) were determined on the basis of the 16S rRNA gene sequence results in Mothur (Schloss *et al.,* 2011). Bacterial β diversity was examined in terms of the taxonomy-based Bray-Curtis distances, phylogeny-based UniFrac distances, and phylogeny-based βNT1 distances with the began or picante package in R; partial least squares discriminant analysis (PLS-DA), principal component analysis (PCA), principal coordinate analysis (PCoA), and permutational multivariate analysis of variance (ADONIS) were also performed. Redundancy analysis (RDA) of the soil bacterial community was conducted on the basis of the taxonomic and phylogenetic distances to identify potential relationships between soil bacterial community properties and environmental factors.

Results

Vegetation characteristics of the three grasslands: To determine the factors driving soil bacterial community structure under different biotic and abiotic conditions, we first aimed to determine the vegetation characteristics of the three grasslands on the basis of the dominant species, community richness, productivity, and community structure. The three grasslands investigated in this study presented similar vegetation communities but different vegetation productivity levels and structures. The dominant species in Grassland 1 were *Stipa grandis*, *Carex korshinskyi*, and *Kochia prostrata*; those in Grassland 2 were *Leymus chinensis*, *Stipa grandis*, and *Achnatherum sibiricum*; and those in Grassland 3 were *Leymus chinensis* and *Stipa grandis* (Table S1). In the severely degraded plots (FG3), the dominant species in Grassland 1 shifted to *Salsola collina* and *Carex korshinskyi*, those in Grassland 2 shifted to *Artemisia sieversiana*, and those in Grassland 3 shifted to *Cleistogenes squarrosa* (Table S1). There were significant differences in vegetation productivity among the three grasslands: Grassland 1 had the highest aboveground biomass (300.83±27.29 g m^{-2}) among the three grasslands; the aboveground biomasses in Grassland 2 and Grassland 3 were 268.79±66.22 g m^{-2} and 171.17±14.86 g m^{-2}, respectively (Table 1). There was also a significant difference in species richness among the three grasslands: G2 had the highest species richness, followed by G1, and G3 had the lowest species richness (Table 1). On the basis of a comprehensive comparison of the aboveground vegetation community structure and productivity, it was found that G2 had higher productivity and the highest proportion of gramineous and forb species among the three grasslands. The vegetation community of G3 was composed mainly of gramineous species, but the vegetation community structure was relatively singular and imbalanced. Although the productivity of the G1 vegetation community was the highest, the vegetation community structure was not ideal for a grassland ecosystem with a low proportion of high-quality forage species and a high proportion of shrub species. The results indicated that the G2 grassland had greater forage productivity, and a better vegetation community structure than those of the G1 and G3 grasslands.

Soil properties of the three grasslands: To determine the factors driving soil bacterial community structure under different biotic and abiotic conditions, we then aimed to determine the main soil nutrient contents, enzyme activities, and other soil properties. A comprehensive comparison of the available and total main nutrient contents and other soil properties revealed that among the grasslands, G2 had relatively high AHN, AP, AK, TN, TP, and TK contents; a higher SOM content; a lower SBD; and higher SUC and URE. G3 had lower available nutrient contents, a lower SWC, a higher SBD, and higher URE. G1 had a lower SOM content, SUC and URE, and higher PHO (Table 2). These data indicate that the air permeability of the soil in the G2 grassland was greater than in the G1 and G3 grasslands, with higher SOM and main N, P, and K contents.

Soil bacterial communities of the three grasslands: To determine the variations in soil bacterial communities among the three grasslands, we analyzed the relative and absolute abundance, composition, and structure of the soil bacterial communities in the grasslands. The relative and absolute quantitation analysis indicated that the abundance, diversity, and composition of the soil bacterial communities greatly varied among the three grasslands (Fig. 1). Among the three grasslands, Grassland 1 had the lowest absolute abundance and diversity, and Grassland 3 had the highest absolute abundance and diversity (Fig. 1a and Table 2). Bacterial diversity was significantly positively related to the absolute abundance of the soil bacterial communities ($r=0.480$, $p<0.01$). The ANOVA results demonstrated that four bacterial phyla, including Armatimonadetes, Gemmatimonadetes, Ignavibacteriae, and Planctomycetes, presented significant differences in both absolute abundance and relative abundance (Figs. 2a and 2b). Acidobacteria and Latescibacteria were significantly different only in terms of the absolute quantitation (AQ) results, and Firmicutes, Nitrospirae, Elusimicrobia, Thaumarchaeota, Microgenomates, Candidatus-Saccharibacteria, Candidate-divison-WPS-1, Proteobacteria, Omnitro-phica, and Chlamydiae were significantly different only in terms of the relative quantitation (RQ) results (Figs. 2c and 2d). Thus, the three grasslands could be completely separated from each other by partial least squares discriminant analysis (PLS-DA, Fig. 1b).

Table S1. Important values of plant species in different studying plots of three grasslands.

Genus	Plant species	Grassland 1				Grassland 2				Grassland 3			
		GE	FG1	FG2	FG3	GE	FG1	FG2	FG3	GE	FG1	FG2	FG3
Gramineae	*Stipa grandis*	63.79	31.81	38.07	30.53	58.38	68.43	34.7	19.91	82.48	55.19	43.95	54.57
	Agropyron cristatum	10.21	81.18	28.91	32.24	21.67	36.05	65.74	25.65	11.79	41.47	3.81	4.44
	Cleistogenes squarrosa	3.95	44.84	49.21	36.83	7.04	31.26	63.68	15.28	8.36	137.99	139.06	140.79
	Leymus chinensis	9.18	5.45	13.46	25.71	54.55	122.09	72.27	42.21	130.61	29.68	57.13	49.63
	Achnatherum sibiricum	-	-	-	2.34	31.26	-	-	-	-	-	-	-
	Koeleria cristata	-	-	-	-	2.54	1.85	4.04	-	-	-	-	-
	Poa annua	-	-	-	-	6.98	5.25	-	-	-	-	-	-
Cyperaceae	*Carex korshinskyi*	58.84	97.06	55.39	28.92	10.77	2.62	21.76	20.89	22.87	-	18.23	4.68
Liliaceae	*Allium bidentatum*	6.97	-	35.62	2.00	7.63	1.64	3.56	2.86	4.57	5.34	14.82	14.59
	Allium tenuissimum	14.07	-	11.62	-	5.77	2.65	8.3	3.62	19.35	4.13	2.51	-
	Allium ramosum	4.91	-	3.80	-	-	2.11	2.63	-	5.58	-	-	-
	Allium condensatum	-	-	-	5.69	-	-	-	-	-	-	-	-
	Allium anisopodium	-	-	-	-	4.35	-	-	-	-	-	-	-
	Anemarrhena asphodeloides	-	-	-	-	-	-	-	-	-	-	-	10.88
Scrophulariaceae	*Cymbaria dahurica*	3.29	-	-	-	4.73	-	-	-	-	-	-	-
Chenopodiaceae	*Kochia prostrata*	57.42	-	-	22.27	7.25	-	-	-	-	-	-	-
Compositae	*Artemisia frigida*	8.22	-	45.8	2.00	-	-	-	-	-	-	-	-
	Heteropappus altaicus	2.35	-	-	-	-	-	-	-	-	-	-	-
	Artemisia scoparia	9.17	15.83	-	14.83	-	-	-	-	-	-	-	-
	Lappula redowskii	-	-	-	4.98	7.38	-	2.69	-	-	-	-	-
	Artemisia sieversiana	-	-	-	-	14.68	-	-	149.46	2.15	-	-	-
	Scorzonera mongolica	-	-	-	-	-	-	-	-	-	-	5.50	-
	Serratula centauroides	-	-	-	-	9.55	-	-	-	-	-	-	-
Rosaceae	*Potentilla acaulis*	0.37	-	2.8	-	-	-	3.12	-	-	-	-	-
	Potentilla tanacetifolia	0.92	-	-	3.31	-	-	-	-	-	-	-	-
	Potentilla bifurca	32.97	-	-	-	4.04	-	-	-	-	-	-	-
	Sibbaldia procumbens	-	1.11	2.43	-	-	-	-	-	-	-	-	-
Ranunculaceae	*Thalictrum petaloideum*	1.44	-	1.16	-	1.89	10.77	8.08	2.63	-	-	-	-
Leguminosae	*Caragana microphylla*	5.64	-	-	-	11.24	-	-	-	-	-	-	-
	Melissitus ruthenica	-	1.51	-	4.80	-	-	-	-	-	-	-	-
	Astragalus adsurgens	-	-	1.50	4.99	-	-	-	-	1.95	-	-	5.38
	Gueldenstaedtia multiflora	-	-	-	1.06	-	-	-	-	-	-	-	-
Brassicaceae	*Dontostemon dentatus*	2.57	-	-		13.52	8.42	2.99	-	-	-	-	-
	Lepidium apetalum	-	2.75	-	12.25	-	-	-	5.60	-	-	-	-
Chenopodiaceae	*Chenopodium glaucum*	2.26	1.24	7.25	17.48	-	-	-	-	-	-	-	-
	Axyris amaranthoides	1.45	-	-	-	-	-	-	-	-	-	-	-
	Salsola collina	-	9.63	2.97	47.78	8.09	1.69	3.90	11.88	-	34.03	-	2.23
Convolvulaceae	*Convolvulus arvensis*	-	5.41	-	-	-	-	-	-	-	-	-	-
	Convolvulus ammannii	-	-	-	-	-	-	-	-	5.98	-	6.28	5.93
Melanthiaceae	*Veratrum nigrum*	-	2.17	-	-	-	-	-	-	-	-	-	-
	Veratrum nigrum	-	-	-	-	32.05	-	-	-	-	-	4.42	-
Labiatae	*Phlomis umbrosa*	-	-	-	-	1.26	-	-	-	-	-	-	-
Crassulaceae	*Sedum aizoon*	-	-	-	-	2.94	-	-	-	-	-	-	-
Apiaceae	*Saposhnikovia divaricata*	-	-	-	-	3.78	-	-	-	-	-	-	-
Iridaceae	*Iris tenuifolia*	-	-	-	-	-	-	2.54	-	4.31	-	-	-
Primulaceae	*Androsace umbellata*	-	-	-	-	-	-	-	-	-	-	-	6.89

GE: Grazing exclusion area; FG: Free grazing area. - Indicated the absence of this plant species

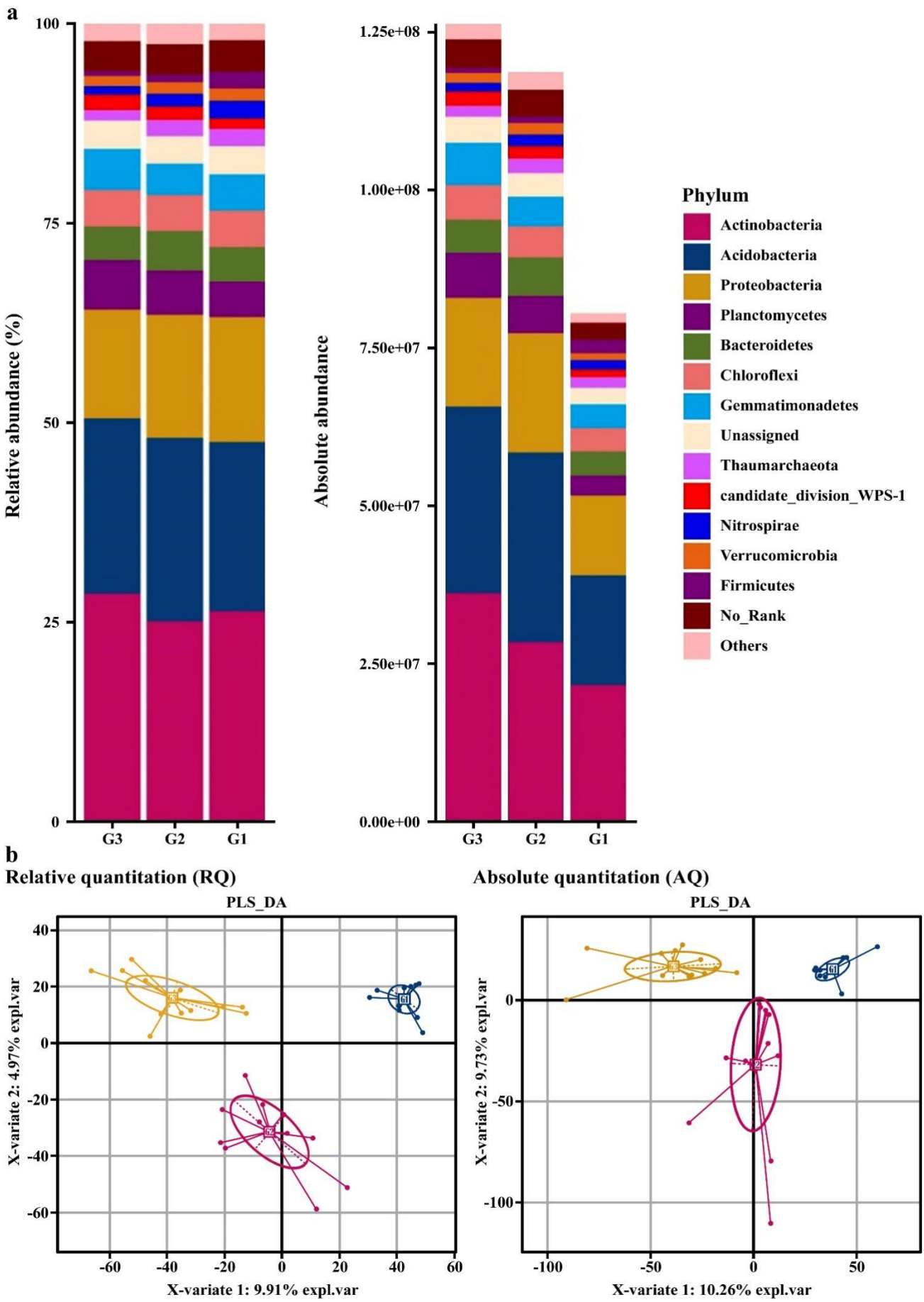

Fig. 1. Abundance of soil bacterial communities: a, partial least squares discriminant analysis (PLS-DA) results; b, for Grassland 1 (G1), Grassland 2 (G2), and Grassland 3 (G3) on the basis of the relative quantitation (RQ) and absolute quantitation (AQ) results.

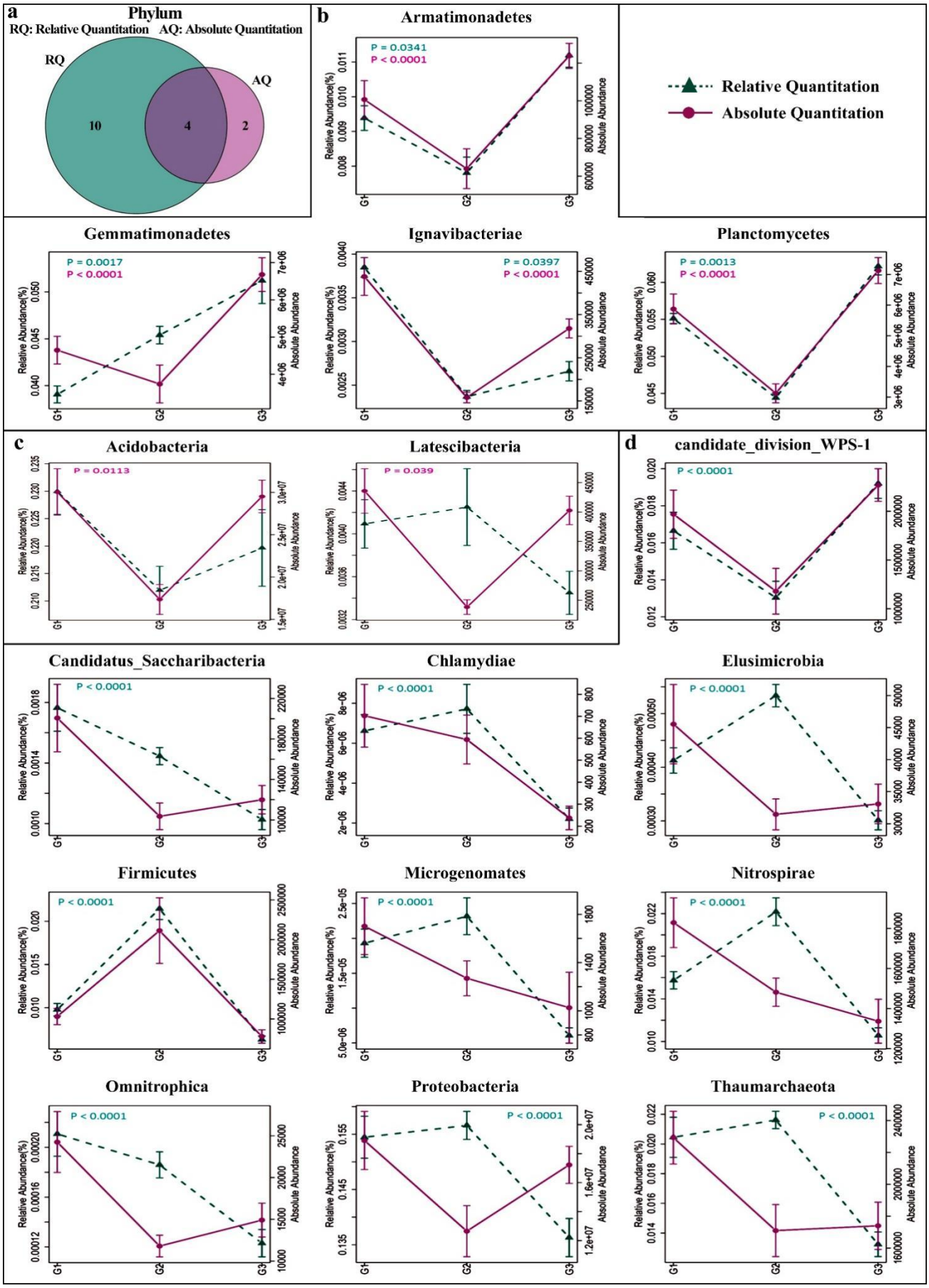

Fig. 2. Venn diagram of the number of bacterial phyla showing significant differences (P<0.05: a, among Grassland 1 (G1), Grassland 2 (G2), and Grassland 3 (G3) on the basis of the relative quantitation (RQ) and absolute quantitation (AQ) results and the specific bacterial phyla showing significant differences on the basis of both RQ and AQ; b, AQ; c, RQ; d, respectively.

Dominant factors driving soil bacterial community changes in the three grasslands: To determine the dominant factors driving soil bacterial community structure under different biotic and abiotic conditions, we analyzed the correlations between soil bacterial community characteristics and biotic and abiotic factors in the three grasslands. According to a correlation analysis of environmental factors at the regional scale, the abundance of soil bacterial communities was determined mainly by soil nutrient levels and soil properties (Table 3). Moreover, the abundance of soil bacterial communities was not correlated with the aboveground biomass or diversity indicators of vegetation communities, except for belowground biomass (BB) and gramineous aboveground biomass (GAB). By further exploring the forces driving changes in the soil bacterial community on a smaller scale, we found that soil properties were not consistently the main factors driving changes in soil bacterial communities (Table 4). At smaller scales, vegetation and soil properties had different effects on soil bacterial abundance: soil properties were key factors driving soil bacterial community changes in G1, mainly vegetation characteristics drove soil bacterial community changes in G3, and vegetation and soil properties collectively affected the soil bacterial communities in G2 (Table 3). These data indicated inconsistencies in the dominant factors driving changes in the soil bacterial community at the local scale. Even at the same scale, the forces driving changes in the soil bacterial community differed. The factors driving changes in soil bacterial communities were related to the productivity and structure of aboveground vegetation communities, as well as soil fertility levels. When there were restricted resources (including biotic and abiotic factors) in some of the studied ecosystems, these resources were significantly correlated with changes in soil bacterial community structure.

Discussion

Soil bacterial communities are widely known to be influenced by biotic and abiotic factors (Liu *et al.,* 2010; Zhong *et al.,* 2019); however, the dominant factors driving changes in the soil bacterial community usually vary (Cassman *et al.,* 2016; Zhong *et al.,* 2019). This variation is generally attributed to spatial heterogeneity and scale differences (Lindström & Langenheder, 2011; Yang *et al.,* 2018). In our study, we investigated the driving factors of changes in soil bacterial community structure in grasslands with different vegetation characteristics and soil properties to study the relationship between the driving forces of soil bacterial community structure and spatial scale, vegetation characteristics, and soil nutrient levels. The results revealed that spatial heterogeneity was the main factor determining changes in soil bacterial communities. At the regional scale, the main factors driving bacterial community changes were determined to be soil properties. However, at a smaller scale, the main factors driving bacterial community changes were related to respective restricted resources, such as vegetation productivity and soil nutrient levels. These findings further proved that scale differences were the main factors driving changes in soil bacterial communities.

Soils are the substrates and living environments of soil bacteria (Chen *et al.,* 2016), and soil physicochemical properties are the primary factors affecting soil bacterial communities. Many studies have shown that the functional diversity of soil microbial communities is controlled mainly by resource availability at the regional scale (Liu *et al.,* 2010), as low soil nutrient availability might facilitate the growth of some bacteria that can readily adapt to nutrient-deficient conditions by adjusting their lifestyles (k- or r-strategists) (Van der Heijden *et al.,* 2008; Zhang *et al.,* 2016). The addition of nutrients to soils can directly affect the soil microbial community composition (Cassman *et al.,* 2016), strongly indicating that soil nutrient levels and resource availability are very important for determining the diversity, structure and composition of soil bacterial communities. Different results have emerged across different studies in terms of the dominant factors driving soil bacterial community changes and specific patterns (Yun *et al.,* 2014). Soil pH and inorganic N are considered the dominant factors driving soil bacterial diversity at regional scales (Yang *et al.,* 2018), and SOC and TN were found to play important roles in determining soil bacterial community composition and diversity at local scales (Zhong *et al.,* 2019) and at larger scales (Ren *et al.,* 2018). In addition, other soil properties, such as soil moisture, temperature, NO_3^-, SBD, and N availability, have been shown to determine soil bacterial diversity (Liu *et al.,* 2010; French *et al.,* 2017; Zhong *et al.,* 2019). By analyzing the results of previous studies, we found that these dominant factors did not show clear driving patterns depending on scale or specific regions. However, in this study, a close link was observed between soil nutrient levels and the factors driving soil bacterial community changes, indicating that soil bacterial abundance and diversity are limited primarily by soil nutrients and can also be regulated and affected by vegetation characteristics through plant–soil feedback. Our study revealed that soil properties were significantly related to soil bacterial changes, and only two vegetation characteristics (BB and GAB) that were significantly related to soil bacterial changes were also closely related to soil N availability. Therefore, the results supported our hypothesis that soil bacterial changes were related mainly to soil nutrient availability and fertility level and that this correlation between soil bacterial changes and soil properties was greater than that between soil bacterial changes and vegetation characteristics. This link is not unique to the present study; soil nutrient availability was also predicted to be the key factor determining the responses of soil bacterial communities to climate change (Cregger *et al.,* 2012; Liu *et al.,* 2020).

However, when analyzing the driving forces of changes in soil bacterial communities at a smaller scale, we often obtain different conclusions. In this study, in G1 which had relatively poor soil fertility, total N, P, and K were limiting soil nutrients and were closely linked with soil bacterial abundance (Tables 2 and 4). The ability of URE to increase ammonia nitrogen (Pettit *et al.,* 1976) is another important factor affecting soil bacterial changes under poor soil fertility conditions (Table 4). Under high

soil nutrient conditions (G2), the soil bacterial communities were not only directly regulated by SBD, SOM, AHN, TN, and TP but also positively related to AB, LM, FAB, SAB, SR, GSR, and SSR (Table 4). Many studies have shown that vegetation biomass, especially litter production, can affect soil nutrient availability (Zhang *et al.,* 2018), and plant diversity promotes an improvement in soil quality, which is reflected mainly in an increase in AP (Zhou *et al.,* 2019). Thus, the positive correlations of vegetation biomass and diversity characteristics with soil bacterial communities in G2 indicated that, on the one hand, vegetation could directly affect soil bacterial abundance; on the other hand, vegetation could indirectly result in soil bacterial community changes through soil properties (Table 4). In cases in which the soil nutrient level could support the growth of the soil bacterial community without resource limitations, for example, in G3, the soil bacterial community became limited by vegetation characteristics. Although more diverse plant communities are expected to occur at more complex nutrient ratios and lead to more diverse soil microbial communities (Zhong *et al.,* 2019), the plant diversity in G3 was relatively low among the three grasslands, and gramineous species were dominant (Table 1). Thus, the present evidence strongly suggests that vegetation and soil properties collectively influence the abundance, composition and diversity of soil bacterial communities, but the dominant factors driving changes in the soil bacterial community depend on soil nutrient availability and the soil fertility level.

Previously, many ecologists tended to believe that more diverse soil bacterial communities could be recruited by highly diverse vegetation communities (Prober *et al.,* 2015; Zhong *et al.,* 2019). However, in the present study, the grasslands with the highest plant species richness and aboveground production level (in G1) presented the lowest soil bacterial abundance and diversity. This was not in line with our previous understanding, but the findings are easily explained by the principle of soil nutrient limitation. Insufficient nutrient levels in soil cannot meet the growth needs of numerous soil bacterial communities. Thus, at regional or larger scales, soil nutrient levels primarily determine the abundance and diversity of soil bacterial communities. Associations among plants, bacteria, and soil are found only at relatively small scales, and soil bacterial communities can be affected by vegetation characteristics, soil management methods, and other human activities.

The limitation of this work is that it does not address the influencing factors of soil bacterial community changes on a global scale and in different types of ecosystems. Due to the significant differences in aboveground vegetation characteristics, soil fertility, and soil microbial communities at a global scale and in different types of ecosystems, the corresponding driving forces behind changes in soil bacterial communities are more complex. However, as can be confirmed from this study, the scale determined by sampling selection indeed leads to different conclusions, and the conclusion we obtained represents the most significant driving force of changes in soil bacterial communities at the current scale. Regardless of the specific vegetation characteristics, soil properties, and soil microbial community structure of a given ecosystem, these factors still interact and influence each other. Soil bacterial communities are also constrained by limiting resources, which become the main driving forces of changes in soil bacterial communities. To explore the driving forces of changes in the soil bacterial community in more depth, a systematic analysis incorporating a more comprehensive range of environmental factors is required at different scales.

Table 3. Correlations between soil bacterial abundance and various environmental factors (vegetation and edaphic factors) at the regional scale.

Vegetation factors	r	*P*	Edaphic factors	r	*P*
AB	0.133	0.441	SWC	0.158	0.356
LM	0.180	0.294	SBD	-0.502**	0.002
BB	0.542**	0.001	PH	-0.004	0.980
GAB	0.401*	0.015	SOM	0.507**	0.002
AAB	-0.168	0.327	AHN	0.566**	0.000
FAB	0.054	0.753	TN	0.535**	0.001
SAB	-0.184	0.282	AP	0.345*	0.039
SR	0.060	0.727	TP	0.533**	0.001
H	0.110	0.524	AK	0.357*	0.033
E	0.113	0.510	TK	-0.029	0.866
GSR	0.224	0.188	HSBC	0.480**	0.003
ASR	-0.147	0.391	PHO	0.248	0.144
FSR	0.058	0.736	SUC	0.406*	0.014
SSR	0.032	0.854	URE	0.398*	0.016

AB: Aboveground biomass; BB: Belowground biomass; LM: Litter mass; GAB: Gramineous aboveground biomass; FAB: Forb aboveground biomass; SAB: Shrub aboveground biomass; AAB: Annual and biennial aboveground biomass; SR: Species richness; H: Shannon–Wiener diversity index; E: Pielou evenness index; GSR: Gramineous species richness; FSR: Forb species richness; SSR: Shrub species richness; ASR: Annual and biennial species richness; AHN: Alkali-hydrolyzable nitrogen content; AK: Available potassium content; AP: Available phosphorus content; TN: Total nitrogen content; TK: Total potassium content; TP: Total phosphorus content; SOM: Soil organic matter content; SBD: Soil bulk density; SWC: Soil water content; PH: Soil pH value; PHO: Phosphatase activity; SUC: Sucrase activity; URE: Urease activity; HSBC: Shannon–Wiener diversity index of the soil bacterial community. Significance levels are denoted with *p<0.05, **p<0.01, and ***p<0.001

Table 4. Correlations between soil bacterial abundance and various environmental factors in each grassland.

	Vegetation factors	r	*P*	Edaphic factors	r	*P*
Grassland 1	AB	-0.105	0.744	SWC	-0.286	0.367
	LM	-0.110	0.733	SBD	-0.199	0.535
	BB	0.559	0.059	PH	-0.513	0.088
	GAB	0.008	0.979	SOM	0.273	0.391
	AAB	-0.040	0.901	AHN	0.231	0.469
	FAB	-0.067	0.836	TN	0.180	0.575
	SAB	-0.141	0.663	AP	0.793**	0.002
	SR	-0.205	0.522	TP	0.178	0.581
	H	-0.051	0.876	AK	0.600*	0.039
	E	0.064	0.844	TK	0.104	0.748
	GSR	-0.035	0.914	HSBC	0.666*	0.018
	ASR	-0.02	0.951	PHO	0.353	0.261
	FSR	-0.155	0.632	SUC	0.456	0.137
	SSR	-0.159	0.622	URE	0.858**	0.000
Grassland 2	AB	0.638*	0.026	SWC	0.655*	0.021
	LM	0.642*	0.024	SBD	-0.662*	0.019
	BB	0.511	0.09	PH	-0.465	0.127
	GAB	0.349	0.266	SOM	0.611*	0.035
	AAB	-0.347	0.268	AHN	0.650*	0.022
	FAB	0.605*	0.037	TN	0.618*	0.032
	SAB	0.619*	0.032	AP	0.276	0.385
	SR	0.595*	0.041	TP	0.711**	0.009
	H	0.572	0.052	AK	0.175	0.586
	E	0.524	0.080	TK	0.051	0.876
	GSR	0.655*	0.021	HSBC	0.613*	0.034
	ASR	0.167	0.603	PHO	0.523	0.081
	FSR	0.521	0.082	SUC	0.462	0.130
	SSR	0.635*	0.026	URE	0.142	0.659
Grassland 3	AB	0.575	0.051	SWC	0.089	0.784
	LM	0.626*	0.029	SBD	-0.184	0.568
	BB	0.441	0.151	PH	-0.156	0.629
	GAB	0.574	0.051	SOM	0.507	0.092
	AAB	-0.427	0.166	AHN	0.559	0.059
	FAB	0.661*	0.019	TN	0.429	0.164
	SAB	ND	ND	AP	0.125	0.700
	SR	0.328	0.298	TP	0.431	0.161
	H	-0.663*	0.019	AK	0.253	0.427
	E	-0.489	0.106	TK	0.028	0.931
	GSR	-0.435	0.158	HSBC	0.197	0.540
	ASR	-0.532	0.075	PHO	0.377	0.227
	FSR	0.568	0.054	SUC	-0.001	0.997
	SSR	ND	ND	URE	0.305	0.335

AB: Aboveground biomass; BB: Belowground biomass; LM: Litter mass; GAB: Gramineous aboveground biomass; FAB: Forb aboveground biomass; SAB: Shrub aboveground biomass; AAB: Annual and biennial aboveground biomass; SR: Species richness; H: Shannon–Wiener diversity index; E: Pielou evenness index; GSR: Gramineous species richness; FSR: Forb species richness; SSR: Shrub species richness; ASR: Annual and biennial species richness; AHN: Alkali-hydrolyzable nitrogen content; AK: Available potassium content; AP: Available phosphorus content; TN: Total nitrogen content; TK: Total potassium content; TP: Total phosphorus content; SOM: Soil organic matter content; SBD: Soil bulk density; SWC: Soil water content; PH: Soil pH value; PHO: Phosphatase activity; SUC: Sucrase activity; URE: Urease activity; HSBC: Shannon–Wiener diversity index of the soil bacterial community. Significance levels are denoted with *p<0.05, **p<0.01, and ***p<0.001. ND indicates not detected

Conclusions

To clarify the factors driving soil bacterial community changes, we selected three grasslands in the Xilingol steppe of Inner Mongolia, China, with different vegetation compositions and productivity levels. As expected, there were large differences in soil properties and in the abundance, diversity and composition of the soil bacterial communities in the three grasslands. A comprehensive analysis of the dominant factors driving changes in soil bacterial communities suggested that vegetation characteristics and soil properties collectively influenced the soil bacterial communities. However, at a local scale, the dominant factors driving soil bacterial community changes varied and depended on soil nutrient levels. Under nutrient-deficient soil conditions with a relatively low abundance and diversity of soil bacterial communities, the dominant factors driving soil bacterial community changes were limiting soil nutrients, whereas under nutrient-rich soil conditions, the factors driving soil bacterial community changes gradually shifted to limiting vegetation characteristics. Thus, at regional scales, soil nutrient availability is the most basic factor determining the abundance and diversity of soil bacterial communities. At local scales, the relative importance of factors influencing the soil bacterial community structure was changed depending on soil nutrient levels. This work could provide a theoretical basis for future explanations of the differences in driving forces behind changes in soil bacterial communities in different ecosystems.

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(Received for publication 18 November 2024)