Geoderma 351 (2019) 49–58

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Decreased temporary turnover of bacterial communities along soil depth gradient during a 35-year grazing exclusion period in a semiarid grassland

Chao Zhang[a,b], Jing Li[c], Jie Wang[a], Guobin Liu[a,b], Guoliang Wang[a], Liang Guo[a], Shouzhang Peng[a,*]

[a] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling 712100, PR China
[b] Institute of Soil and Water Conservation, Chinese Academy of Sciences & Ministry of Water Resources, Yangling 712100, PR China
[c] College of Forestry, Northwest A&F University, Yangling 712100, PR China

ARTICLE INFO

Handling Editor: Naoise Nunan

Keywords:
Grazing exclusion
Bacterial diversity
Temporal turnover
Metabolic function, soil depth

ABSTRACT

The activity and composition of soil microbial communities during natural restoration have been widely studied, but their succession rates and metabolic functions remain unknown, especially with respect to soil depth. Here, we examined a chronosequence of grasslands that were subjected to grazing exclusion for 0, 10, 15, 25, and 35 years (y) on the semiarid Chinese Loess Plateau, and we investigated the succession rate and the metabolic functions of microbial communities at different soil layers (0–10, 10–20, 20–40, and 40–60 cm). Microbial succession rates represented by temporary turnover were assessed using the slope of linear regressions, based on log-transformed microbial community similarity over time. Metagenome functional content was predicted from the sequence data using PICRUSt. Most soil physicochemical properties, microbial biomass, enzyme activity, bacterial diversity, and carbohydrate and amino acid metabolism increased as a result of grazing exclusion but decreased as a function of soil depth. Bacterial communities showed a transition from fast-growing copiotrophic taxa (*Proteobacteria*) to slow-growing oligotrophic taxa (*Actinobacteria*) with soil depth. Notably, a higher temporary turnover rate of bacterial communities in the topsoil than in the subsoil layer indicated a decreased successional rate of bacterial communities along the soil depth profile. *Nitrospirae* had the highest succession rate, followed by *Gemmatimonadetes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*. Variance partitioning and redundancy analysis demonstrated that soil physico-chemical properties had a greater effect on bacterial composition and metabolic functions than plant characteristics (aboveground and root biomasses), and that soil C and N levels and bulk density were the most influential factors. Our study indicated a positive effect of long-term grazing exclusion on the activities, diversity, and functions of the microbial community, and revealed a decreasing successional rate of bacterial communities with soil depth.

1. Introduction

One of the most important issues in ecology is understanding the response of biological communities to potential impacts resulting from human interference such as grassland overgrazing (Fussmann et al., 2014). As an important ecosystem, grasslands cover one-fifth of global land area, and they play a significant role in controlling soil and water loss and supporting stockbreeding in arid and semiarid areas (Jing et al., 2014). However, unsustainable human activities (e.g. cultivation and overgrazing) have degraded most grasslands, consequently leading to a reduction in soil quality and a loss of ecological function (Zhang et al., 2018). Grassland restoration has been promoted as a means for restoring native plant communities and associated wildlife habitats, for preserving the soil ecosystem, and for promoting sustainable agriculture (Jangid et al., 2010). Various restoration approaches for degraded grassland systems (e.g. fertilization, fire control, fencing, exotic plant exclusion, and grazing exclusion) have been developed over the past few decades (Fierer et al., 2013; Harris, 2009). These restoration activities affect plant communities and soil properties in different ways. For example, prescribed fire and the removal of invasive species have been shown to affect bacterial composition in tallgrass prairies (Barber et al., 2017). Fertilizer application increases the abundance of microbial functional groups involved in the soil N cycle of degenerated grasslands in Tibetan alpine meadows (Ma et al., 2016). Also, fencing for natural restoration has been shown to enhance soil nutrient levels and improve the diversity of soil eukaryotes in arid grasslands (Jing et al., 2014).

* Corresponding author.
E-mail address: szp@nwafu.edu.cn (S. Peng).

https://doi.org/10.1016/j.geoderma.2019.05.010
Received 28 June 2018; Received in revised form 5 May 2019; Accepted 8 May 2019

The exclusion of grazing to facilitate natural succession is an efficient approach used to restore the ecological functions of degraded grasslands, including aboveground diversity and biomass, soil structure, and nutrient levels (Liu et al., 2018; Yao et al., 2018). Previous studies have found that plant communities and soil conditions are two important determinants of soil microbial community structure (Lin et al., 2014; Tang et al., 2018; Cui et al., 2018), and that these factors alter the quantity and quality of organic matter (Mushinski et al., 2018), particle-size composition (Monroy et al., 2012), pH values (Siciliano et al., 2014), and redox potential (Pan et al., 2016). Plants affect microbial community composition and activity through the decomposition of litter, turnover of roots, and root exudation (Wardle et al., 2004; Zhang et al., 2019a). High plant diversity and biomass have been reported to be associated with high soil microbial diversity, which was ascribed to a high amount of available resources from litter decomposition and root exudates (Steinauer et al., 2016). Furthermore, extensive studies have affirmed that changes in soil conditions are closely associated with the variability of soil microbial communities (Lauber et al., 2009). For example, some copiotrophic/oligotrophic bacteria (e.g., Verrucomicrobia and Armatimonadetes phyla) responded quickly to soil nitrogen (N) levels (Bru et al., 2011). Thus, exploring the contribution of plants and soils to changes in microbial communities will improve our understanding of the underlying mechanisms that mediate microbial communities in natural grassland ecosystems.

Species' temporal turnover is defined as the number of species eliminated and replaced per unit of time (Liang et al., 2015), and it can be used to evaluate the successional rate of a microbial community. The temporal turnover of species is dependent on the environmental conditions, anthropogenic impacts, and spatial-temporal gradients (Hatosy et al., 2013; Shade et al., 2013). Recently, Liang et al. (2015) attempted to determine the effects of climate change on the temporal turnover of soil bacterial communities using long-term soil transplant experiments, and they observed that warming resulted in a faster microbial community succession rate but lower species richness.

Microbial activity and diversity have been reported to be greatly affected by soil conditions (Shade et al., 2013; Zhang et al., 2019b). Changes in edaphic factors such as organic matter content, pH, moisture, cation exchange capacity, and oxygen concentrations with increased soil depth might affect the activities and composition of soil microbial communities (Fierer et al., 2003; Jobbagy and Jackson, 2000). The diversity, density, and activity of the microbial community in deeper soil are lower, and therefore, temporal turnover is also likely to be lower (Fierer et al., 2003; Bai et al., 2017). Previous studies reported a significant difference in microbial community composition and functions at different soil depths based on the examination of phospholipid fatty acid profiles (Fierer et al., 2003), and hypervariable regions of rRNA gene sequences (Eilers et al., 2012), and specific genes primers (Tian et al., 2014). However, these previous studies on soil microbial communities focused mainly on diversity and some specific functional genes. Therefore, the succession rate of these microbes along the soil profile and edaphic factors that drive the temporary turnover and the function of these microbial groups remain unclear.

To understand the temporal turnover and potential function of microbial communities in soil profiles during natural succession, we performed experiments in a successional gradient of five grasslands on the Loess Plateau, where grazing was prohibited for 0 to 35 years, to investigate the effects of grazing exclusion on the soil microbial communities in semiarid areas. Soil samples were collected from four soil depths (0–10 cm, 10–20 cm, 20–40 cm, and 40–60 cm) over time to test the following hypotheses: (i) bacterial succession rates will decease with soil depth, and different microbial groups will exhibit different succession rates; (ii) microbial potential functions, including the metabolism of carbohydrates, amino acids, lipids, energy, and xenobiotics, will increase as a result of grazing exclusion and decease with soil depth, and (iii) soil conditions (e.g., C, N, bulk density) contribute more to shaping bacterial community composition and function than plant attributes do (aboveground and root biomasses).

2. Materials and methods

2.1. Description of sites

The experiment was conducted at the Yunwushan National Natural Grassland Protection Zone (106°21′–106°27′E, 36°10′–36°17′N) in the Ningxia province of China. Yunwushan covers 6660 hm^2, and it has been under grazing exclusion since 1982 to recover the degraded grassland ecosystems caused by overgrazing. This area was mainly composed of a key conservation region (1100 hm^2), secondary conservation region (1200 hm^2), and experimental region (4360 hm^2). The study area has a typical semiarid climate, and the mean rainfall is 425 mm annually, most of which occurred between July and September. The mean annual temperature is 7.01 °C, and annual evaporation is 1020–1750 mm. The soil in the study area was a montane gray-cinnamon soil classified as a Calci-Orthic Aridisol (Chinese taxonomic system) and is equivalent to a Haplic Calcisol in the FAO/UNESCO soil classification system (Wang et al., 2019). The vegetation community contained > 250 species, and the *Stipa* genus was a dominant group, including *S. bungeana*, *S. grandis*, and *S. przewalskyi*.

2.2. Chronosequence selection and sample collections

In August 2017, five grasslands with different grazing exclusion times were selected in the experimental area when the aboveground biomass peaked. These grasslands have been fenced since 1982, 1992, 2002, and 2007, and the corresponding grazing exclusion times were 35 years (GE35), 25 years (GE25), 15 years (GE15), and 10 years (GE10), respectively. Moreover, the grassland has been continuously grazed at a 50 sheep/ha throughout the year in the area selected as a reference (GE0). Prior to grazing exclusion, all of the investigated sites had been grazed by over 50 sheep/ha. All sites were established within the experimental region; therefore, the sites had similar topographical characteristics, including altitude, latitude, slope degree, and direction (Table 1).

A transect of 500 m × 200 m was established for each selected site, and three plots (100 m × 50 m) were randomly established in each. After aboveground biomass harvest, soil samples were collected from depths of 0–10, 10–20, 20–40, and 40–60 cm from each plot using a soil auger (5 cm in diameter). Soil samples were collected randomly from nine points within the plot and were then mixed together to make a

Table 1
Geographical characteristic of study sites. The soil of all the sites is a montane gray-cinnamon soil (Song et al., 2019).

Sites	Latitude (N)	Longitude (E)	Altitude (m)	Slope gradient (°)	Slope aspect	Dominant species
GE 0	36°17′06″	106°23′28″	2017	18	E26°N	*Potentilla bifurca* Linn., *Stipa przewalskyi* Roshev., *Carex tangiana* Ohwi
GE10	36°16′57″	106°23′28″	2034	20	E38°N	*Leymus secalinus* Tzvel., *Carex tangiana* Ohwi, *Stipa grandis* P. Smirn
GE15	36°16′21″	106°23′15″	2025	20	E32°N	*Carex tangiana Ohwi.*, *Carex tangiana* Ohwi, *Stipa grandis P. Smirn*
GE25	36°16′31″	106°23′27″	2070	18	W21°N	*Stipa grandis* P. Smirn. *Artemisia sacrorum* Ledeb, *Oxytropis bicolor*
GE35	36°15′05″	106°23′10″	2071	21	E29°N	*Stipa przewalskyi* Roshev., *Carex tangiana* Ohwi

composite after removing the aboveground litter. The collected soil was divided into three parts after removing roots, stones, litter, and debris. One sample was immediately stored at −80 °C for microbial community analysis, another was stored at 4 °C for the determination of microbial biomass and enzyme activities, and the third was air-dried for physico-chemical analysis. Furthermore, five 1 m × 1 m subplots in each plot were randomly established for the measurement of aboveground and belowground biomass. Root biomass at different soil layers was collected using a root auger (10 cm in diameter). Live roots (coarse and fine) were selected using tweezers after soil was removed via water-flushing. Roots that could not be selected by hand were soaked in a sodium hydroxide solution, and floating roots were collected using tweezers. Roots from six points within the plot were composited based on depth and were subsequently oven-dried at 60 °C for 36 h to weigh the root biomass. Aboveground biomass was determined by drying the aboveground tissues, including shoots, leaves, and litter, at 60 °C for 36 h.

2.3. Measurement of soil properties

Soil chemical properties were measured using the methods described below. pH was measured using an automatic titrator (Metrohm 702, Swiss) in a water-to-soil ratio of 2.5:1 (v/w). Nitrate (NO_3^-−N) and ammonium nitrogen (NH_4^+-N) were measured using a continuous-flow auto-analyzer (Alpkem, OI Analytical, USA), following sample extraction with 2 M KCl at a soil:KCl ratio of 1:5. Available phosphorus (P) was extracted using sodium bicarbonate, and was verified using the molybdenum blue method. Soil organic carbon (C) was measured using the potassium dichromate oxidation method. Soil moisture was measured gravimetrically by drying the samples at 105 °C for 24 h and was then calculated as % dry weight. Bulk density was determined using a soil core (stainless steel cylinders with a diameter and a height of 5 cm each) along the soil depth profile at each sampling point. Soil microbial biomass C was determined using the fumigation extraction method (Vance et al., 1987). Briefly, 25 g of oven-dried equivalent field-moist soil was fumigated for 24 h at 25.8 °C with $CHCl_3$. Soil was added with 100 mL of 0.5 M potassium sulfate by shaking for 1 h at 200 rpm and was then filtered after fumigant removal. Simultaneously, the other non-fumigated 25 g of soil was subjected to extraction. The organic carbon content of the extracts was determined using a Liqui TOCII analyzer (Elementar Analyses system, Hanau, Germany). Soil particle composition was determined using a laser particle-size analyzer (Mastersizer 2000; Malvern Instruments, Malvern, UK). The clay (< 0.002 mm), silt (0.002–0.05 mm), and sand (0.05–2 mm) contents were calculated based on the classification of the size fractions.

Three enzymes involved in C, N, and P cycling, namely β-1,4-glucosidase (BG), b-1, 4-*N*-acetylglucosaminidase (NAG), and alkaline phosphatase (ALP), were measured using a fluorescent micro-plate assay with 4-methylumbelliferone (MUB) labeled substrates. In brief, 1.0 g fresh soil and 125 mL deionized water were fully mixed for 2 h at 25 °C with an oscillator to prepare soil buffer suspensions, and 1 mL of each soil suspension and 250 μL substrate solution were subsequently pipetted into a 2-mL centrifuge tube using a multichannel pipette. The well containing the negative control received 6 mL buffer, 150 μL substrate, and 180 μL NaOH (0.5 M). The reference standard well received 6 mL buffer, 150 μL standard (10 mM 4-MUB), and 180 μL NaOH. The blank control well received 1 mL sample suspensions and 250 μL deionized water. The quench control well received 1 mL sample suspensions and 250 μL standard. After the centrifuge tube was incubated at 25 °C for 4 h in the dark, 50 μL NaOH was added to terminate the reaction. Then, 250 μL of mixed liquor from each sample (including sample, negative control, reference standard, blank control, and quench control) was pipetted into a 96-well black plate with a multichannel pipette, and activities were examined fluorometrically using a Fluorescence Plate Reader (SpectraMax M2, Molecular Device, California, US) at 365 nm excitation and 450 nm emission. The activities were expressed as nmol substrate converted per mL of sample (nmol h^{-1} g^{-1}). Six replicate wells were used for each treatment.

2.4. Illumina sequencing of bacterial 16S rRNA

Total DNA was extracted from of 0.5 g soil samples using the FastDNA SPIN kit (MP Biomedicals, Cleveland, USA) according to the manufacture's protocol. The quality and quantity of DNA were examined using 2% agarose gel electrophoresis and a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). Primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3) were used to amplify the V4-V5 region of bacterial 16S rRNA. The PCR reaction mixture (20 μL) contained 4 μL 5× FastPfu Buffer, 2 μL 2.5 mM dNTPs, 0.8 μL forward and reverse primers (5 μM), 0.4 μL FastPfu Polymerase, and 10 ng template DNA. The amplification procedure was as follows: initial denaturation at 98 °C for 1 min; 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and 72 °C for 45 s; and a final extension at 72 °C for 5 min. All samples were amplified in triplicate, purified using a xyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.), and pooled in equimolar concentrations. Purified DNA products were sequenced on an Illumina HiSeq2500 PE250 system (Illumina Inc., USA), and raw sequences (FASTQ format) were uploaded to the NCBI SRA database (Accession Number: SRP149460).

Paired-end reads were merged using FLASH (http://ccb.jhu.edu/software/FLASH/), and quality sequences were filtered using the method described by Caporaso et al. (2010). Chimeric sequences were removed using the UCHIME algorithm (Edgar et al., 2011), and quality-filtered sequences were clustered into OTUs using complete-linkage clustering implemented in UCLUST based on a 97% similarity cutoff. In total, we obtained 2,916,421 bacterial sequences after quality control measures were conducted on all soil samples, and there were on average 48,607 sequences, which were approximately 397 bp in length per sample. Operational taxonomic units (OTUs) with less than two counts were excluded. The most abundant sequence in each OTU was chosen as a representative sequence and was then aligned using PyNAST. The taxonomic identity was identified in the Silva reference database (http://www.arb-silva.de) using RDP Classifier. Community diversity, including observed species, Shannon-Wiener index ($H = -\Sigma P_i \ln P_i$), and the Chao1 estimator, was calculated using 40,800 reads per sample (the minimum number of sequences required to normalize the differences in sequencing depth) using QIIME software.

2.5. Predictive potential functions

We focused on the metagenome functions related to the degradation of the main C, N, and exogenous compounds. The PICRUSt 1.0.0-dev software package was used to predict the metagenome functional content from the 16S rRNA dataset (Chen et al., 2016). The function 'pick_reference_otus_through_otu_table.py' in QIIME was used to select OTUs against a reference collection with OTUs assigned at a 97% identity level. The OTUs were selected using the Greengenes version 12. The module 'predict metagenome' was used to create a square matrix, containing normalized-OTU abundance multiplied by each predicted functional trait abundance per sample. Finally, the module 'categorize by function' was used to generate a table of the predicted metagenome functional content based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) classification at level 3.

2.6. Time-decay relationship and other data treatments

The time-decay relationship model, which is usually used to describe variation in community similarity over time, was used to illustrate the changes in microbial community composition (Liang et al., 2015). Differences in microbial community composition were calculated based on the Bray-Curtis distance. Arrhenius (log–log) ordination

was applied to simulate the species-time relationship in the following model: $\ln(Ss) = \text{constant} - w\ln(T)$, where Ss is the pairwise similarity in community composition; T (year) is the time interval between the two compared samples; w is the regression coefficient of $\ln(Ss)$ with $\ln(T)$ and a constant intercept; w is the turnover rate of the microbial species over time. The significance of w values was tested using a one-sample t-test between the original slope and the mean of bootstrapped slopes, which was determined using random pairing of the original set (permuted 999 times) (Horner-Devine et al., 2004). The significance of w values among different estimations was achieved by bootstrapping (999 times), followed by a pairwise t-test. Differences in w values between microbial communities were tested using the diffslope function in the package "samba" (Jurasinski, 2012).

The effects of exclusion time and soil depth on root biomass, soil properties, microbial biomass, enzyme activities, microbial diversity, and potential microbial functions were determined using a one-way analysis of variation (ANOVA), followed by post-hoc comparisons using a least significant difference (LSD) test. Significance was established at the 0.05 level. The significance of the differences in bacterial composition between soil depths was tested using analysis of similarities (ANOSIM). Variance partitioning, redundancy analysis (RDA) and Pearson correlation tests were used to determine the effects of environmental factors on bacterial composition and functions. Adjusted R^2 in variation partitioning was used to determine the proportion of variation in bacterial communities explained by factors, and the significance was tested by an ANOVA permutation at the 0.05 level. All analyses were conducted in the R program (version 3.0.2; http://www.r-project.org/) with vegan packages.

3. Results

3.1. Plant biomass and soil physico-chemical properties

Grazing exclusion increased the aboveground and root biomasses compared to the continuous grazing site (Table S1). Aboveground biomasses significantly increased during the initial 25 years, followed by a sharp decrease thereafter. With the exception of the 40–60 cm layer, where root biomass did not change, root biomass at the 0–10 cm, 10–20 cm, and 20–40 cm layers increased during the first 25 years and peaked at the GE25 site. Root biomass decreased along soil depth. Organic C, total N, and NH_4^+-N behaved similarly in the different layers, increasing with time and peaking at the GE25 site (Table S1). The highest NO_3^--N content occurred at the GE15 site at 0–10 cm and 10–20 cm, and at 20–40 cm and 40–60 cm at the GE35 site. The available P content did not change with time. Soil moisture increased with time but did not show consistent changes with soil depth. The soil pH increased from 8.39 to 8.49 at the 0–10 cm layer and from 8.19 to 8.26 at the 40–60 cm layer. Soil bulk density decreased with time and increased with soil depth. Content of clay, silt, and sand showed no significant changes with exclusion time nor with soil depth.

3.2. Soil microbial biomass and enzyme activities

The effects of grazing exclusion on the microbial biomass and enzyme activities are shown in Table 2. Grazing exclusion significantly increased the microbial biomass C and BG, NAG, and ALP activities compared with those at the GE0 site throughout the soil depth profiles, and these properties were highest at GE25 and then decreased at depths of 0–10 cm and 10–20 cm. Content of microbial biomass C and BG, NAG, and ALP activities exhibited a decreasing trend along soil depth across the exclusion stages.

3.3. Bacterial community composition and potential functions

A total of 5132 bacterial OTUs were identified based on a 97% similarity threshold. Indicators of bacterial community diversity (Table 3), including the number of OTUs, Shannon index values, and Chao1 estimator values, also exhibited an increasing trend in the initial 25 years, followed by a drastic decrease. These three indicators that reflected microbial diversity exhibited an obvious decreasing trend along the soil depth. ANOSIM test confirmed that OTU-based taxonomic bacterial-community composition differed significantly among the four layers (Table S2). *Actinobacteria* was the most abundant class across the grassland soils, accounting for 36.2% of all sequences on average, followed by *Proteobacteria* (20.2%), *Acidobacteria* (14.5%), *Chloroflexi* (7.0%), and *Planctomycetes* (6.2%), respectively (Fig. 1). The abundance of *Actinobacteria* decreased along soil depth while the abundance of *Proteobacteria* decreased. The abundance of *Acidobacteria* did not change along soil depth.

A significant time-decay relationship was observed for bacterial communities at different layers (Fig. 2). Slopes of the communities ($w = 0.0505$, 0.0403, $P < 0.001$) in the upper layer (0–10 cm, 10–20 cm) were significantly steeper than that of the deeper layer ($w = 0.0329$ and 0.0189 for 20–40 cm and 40–60 cm, respectively; $P < 0.001$), indicating a decreased successional rate in bacterial communities along the soil depth. At the phylum level (Table 4), with the exception of *Firmicutes* and *Tectomicrobia*, significant variation in w values was observed for these taxa along the soil depth, ranging from 0.0189 to 0.1109. The highest temporary turnover was found in *Nitrospirae* ($w = 0.0715$, $P < 0.0001$), followed by *Gemmatimonadetes*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Planctomycetes*, and *Chloroflexi*. Interestingly, these taxa presented obvious decreasing turnover rates along the soil depth.

Grazing exclusion significantly improved the abundance of KEGG orthologs (KOs) assigned to carbohydrate-, amino acid-, lipid-, energy-, and xenobiotic-metabolizing KO compared to the grazing site in the topsoil (0–10 cm) (Fig. 3), and the highest values were found at GE25. The abundance of carbohydrate- and amino acid-metabolizing KO at sites of GE15, GE25, and GE 35 was higher than that at sites of GE0 and GE10 at the above 40 cm depth, and lipid-, energy- and xenobiotic-metabolizing KO did not change below 10 cm across all the sites. The abundance of carbohydrate- and amino acid-metabolizing KO decreased along soil depth.

3.4. Possible drivers of the variation in the bacterial community

Soil pH and particle composition were not taken into account in the evaluation of the relationships among the soil and the plant and microbial communities because of the low variation. Variance partitioning indicated that plant and soil properties were the significant factors affecting the bacterial composition and functions ($P < 0.01$), and sampling distance was not significant ($P > 0.05$) and played a minor role. Soil physico-chemical properties and plant biomasses purely explained 20.69% and 13.04% of variation in bacterial composition, respectively, and 29.35% and 17.74% of variation in the metabolic functions, respectively (Fig. 4). RDA analysis explained 73.0% of bacterial community variation, and root biomass, soil organic C, bulk density, NO_3^--N, and total N drove changes in bacterial community composition. Among these variables, organic C, bulk density, and NO_3^--N are the most influential factors (Fig. 5). Therefore, soil properties were more important than the plant characteristics in determining the bacterial community. The correlations between environmental parameters and the dominant microbial phylum are also summarized in Table S3. Evidently, root biomass, contents of soil organic C, total N, NH_4^+-N and NO_3^--N were positively correlated with bacterial diversity (including Shannon index and Chao estimator values), the abundance of *Proteobacteria*, and the abundance of carbohydrate- and amino acid-metabolizing KO. However, these values were negatively correlated with *Actinobacteria*, *Nitrospira*, and *Gemmatimonadetes*. Bulk density was positively correlated with *Actinobacteria* and *Nitrospira*, and negatively correlated with the Shannon index, Chao estimator, the abundance of *Proteobacteria*, and the abundance of carbohydrate- and amino acid-

Table 2
Changes in soil microbial biomass and enzyme activities in the soil profile during process of the grazing-exclusion.

Parameters	Depth (cm)	GE0	GE10	GE15	GE25	GE35
Microbial biomass C ($mg\ kg^{-1}$)	0–10	685.1 ± 47.9 Ca	968.0 ± 77.7 Ba	973.0 ± 53.5 Ba	1047.4 ± 80.2 Aa	924.2 ± 32.3 Ba
	10–20	449.0 ± 47.1 Bb	742.1 ± 34.5 Ab	761.6 ± 57.2 Ab	825.2 ± 17.3 Ab	770.2 ± 35.1 Ab
	20–40	260.5 ± 33.7 Cc	489.5 ± 13.2 Bc	388.5 ± 25.4 Bc	412.4 ± 29.5 Bc	600.5 ± 35.8 Ac
	40–60	55.2 ± 12.9 Cd	216.3 ± 15.4 Bd	216.1 ± 18.3 Bd	196.4 ± 17.1 Bd	327.4 ± 32.0 Ad
β-1,4-glucosidase ($nmol\ h^{-1}\ g^{-1}$)	0–10	223.6 ± 14.7 Ca	355.2 ± 8.6 Ba	418.8 ± 35.9 ABa	444.7 ± 31.0 Aa	401.2 ± 71.0 ABa
	10–20	139.2 ± 23.5 Cb	294.8 ± 18.2 Ab	178.7 ± 14.6 BCb	288.4 ± 43.3 Ab	213.3 ± 24.7 Bb
	20–40	98.1 ± 13.0 Bb	169.1 ± 17.8 Ac	97.0 ± 12.8 Bc	94.9 ± 6.7 Bc	99.8 ± 19.9 Bc
	40–60	48.1 ± 1.2 Cc	76.5 ± 2.1 Ad	50.3 ± 2.0 Cd	63.6 ± 5.7 Bd	57.0 ± 3.1 BCd
β-1,4-*N*-acetylglucosaminidase ($nmol\ h^{-1}\ g^{-1}$)	0–10	31.1 ± 2.9 Ba	50.8 ± 7.4 ABa	57.1 ± 13.3 ABa	63.1 ± 12.5 Aa	37.6 ± 3.3 Ba
	10–20	23.9 ± 9.1 Bab	27.1 ± 3.4 ABb	35.1 ± 1.6 ABb	38.5 ± 7.6 Ab	37.2 ± 2.7 Ba
	20–40	17.9 ± 1.4 Bab	24.8 ± 4.3 ABb	26.6 ± 0.2 ABb	21.4 ± 3.2 Bc	32.7 ± 4.4 Aa
	40–60	12.0 ± 0.9 Cb	19.6 ± 3.6 Cb	16.3 ± 1.1 BCc	20.8 ± 3.6 Bc	17.2 ± 2.1 Ab
Alkaline phosphatase ($nmol\ h^{-1}\ g^{-1}$)	0–10	431.2 ± 27.5 Ba	544.9 ± 105.8 ABa	625.8 ± 36.0 Aa	613.3 ± 18.9 Aa	551.2 ± 13.3 ABb
	10–20	442.7 ± 34.7 Ba	516.7 ± 51.1 ABa	643.1 ± 50.3 Aa	553.7 ± 77.5 ABa	554.3 ± 10.4 ABb
	20–40	475.6 ± 30.0 Ca	534.4 ± 13.2 BCa	581.6 ± 10.4 ABa	543.6 ± 38.3 BCa	620.8 ± 23.7 Aa
	40–60	396.9 ± 10.7 Da	476.2 ± 5.2 Aa	572.6 ± 5.8 Ca	516.9 ± 21.7 Ba	536.3 ± 6.3 Bb

Result is reported as the mean ± SE (n = 3). Different uppercase in the row indicated the significant difference at the $P < 0.05$ along the grazing-prohibition time at each soil layer. Different lowercase in the column indicated the significant difference at the $P < 0.05$ along soil depth at each site.

metabolizing KO.

4. Discussion

4.1. Soil nutrient and other properties

Plant communities in this grazing-prohibited grassland experienced great increases in aboveground and root biomass compared to the grazed grasslands (Table S1), indicating that grazing exclusion had a positive effect on grassland productivity. However, peak biomass was observed at the GE25 site, and then declined drastically, the cause for this change could be the increased competition and the exclusion of less-competitive species during the late successional stages (Arroyo et al., 2015). This result agreed with a previous study that found that plant productivity was usually highest during the middle-late stage of restoration (Odum, 1969). Grazing exclusion resulted in an increase in soil C and N (total N, NO_3^-, and NH_4^+) contents compared to the grazing grassland (Table S1) because of the increased aboveground and root biomass. Lower soil nutrient levels in GE0 could be attributed to long-term grazing, which accelerated the loss of leaves and roots via ingestion by livestock (sheep), and this then decreased the accumulation of aboveground biomass (Cheng et al., 2016). As the exclusion time increased, enhanced biomass caused nutrient accumulation in the soil via decomposing litter. Along the soil depth profile, soil nutrients, including organic C, total N, NO_3^--N, and NH_4^+-N exhibited a decreasing trend. Higher nutrient levels in the topsoil may be due to the strong correlation with the quantity of root biomass in soil, which obviously decreased with depth, leading to lower C inputs in deep soil. No significant difference was detected in available P content with regard to exclusion time or along the soil depth profile, indicating little impact of natural restoration and soil depth on soil P levels. This finding was consistent with previous result found in studies of loess soils (Zhang et al., 2018), and the outcome could be related to P pool sizes, which are dependent on parent material and controlled by different biological and environmental factors during the pedogenesis. Observed increases in soil bulk density with soil depth could be attributed to decreased penetration of the root system, which is caused by fewer roots in deep layers. Therefore, the results indicated that the soil tended to be more anoxic as the depth increased. Additionally, decreased bulk density over time suggested favorable ventilation conditions and an improvement in soil structure during grazing exclusion.

Better soil conditions, promoted by the increase in plant cover and biomass, positively affected microbial communities in terms of biomass and enzyme activities, as reported during secondary succession (Lozano et al., 2014). Plant biomass, through its effects on soil organic C and N, promoted microbial growth and hence microbial biomass. Plant biomass may promote higher microbial biomass in later successional stages compared to the initial stages when plant biomass was at its lowest. This agrees with our previous study that found that soil microbial biomass in semiarid areas is affected by plants by altering soil nutrients (Zhang et al., 2018). Hydrolytic enzymes are considered to control the rate at which substrates are degraded and become available for microbial or plant uptake. NAG, BG, and ALP are important hydrolytic enzymes involved in the transformation of soil C, N, and P (Roldan

Table 3
Changes in soil microbial diversity in the soil profile during process of the grazing- exclusion.

Parameters	Depth (cm)	GE0	GE10	GE15	GE25	GP35
Observed OTUs	0–10	2217 ± 33 Ba	2276 ± 54 ABa	2279 ± 28 ABa	2392 ± 36 Aa	2246 ± 44 Ba
	10–20	1886 ± 10 Cb	2089 ± 28 BCb	2170 ± 65 ABa	2198 ± 71 Aab	1974 ± 20 BCb
	20–40	1701 ± 37 Cc	1731 ± 15 Cc	1909 ± 17 ABb	2009 ± 97 Ab	1812 ± 45 BCc
	40–60	1526 ± 55 Bd	1489 ± 31 Bd	1695 ± 62 Ac	1787 ± 69 Ac	1527 ± 37 Bd
Chao 1 estimator	0–10	2985 ± 57 Ba	3124 ± 101 ABa	3116 ± 91 ABa	3273 ± 44 Aa	3095 ± 27 ABa
	10–20	2715 ± 75 Cb	3055 ± 165 Aa	3078 ± 29 ABa	3127 ± 56 Aa	2824 ± 45 BCb
	20–40	2398 ± 19 Cc	2581 ± 38 BCb	2745 ± 59 ABb	3003 ± 155 Aa	2632 ± 87 Bb
	40–60	2272 ± 105 BCc	2112 ± 81 Cc	2474 ± 26 ABc	2611 ± 86 Ac	2127 ± 72 Cc
Shannon diversity	0–10	6.46 ± 0.03 Ba	6.51 ± 0.05 ABa	6.51 ± 0.03 ABa	6.60 ± 0.03 Aa	6.44 ± 0.03 Ba
	10–20	6.13 ± 0.02 Bb	6.28 ± 0.10 ABb	6.39 ± 0.07 Aa	6.41 ± 0.08 Aab	6.22 ± 0.01 Bb
	20–40	5.90 ± 0.07 Bc	5.93 ± 0.02 Bc	6.16 ± 0.03 Ab	6.21 ± 0.08 Ab	6.04 ± 0.04 Bc
	40–60	5.70 ± 0.06 Bd	5.64 ± 0.03 Bd	5.89 ± 0.02 Ac	5.99 ± 0.04 Ac	5.74 ± 0.01 Bd

Result is reported as the mean ± SE (n = 3). Different uppercase in the row indicated the significant difference at the $P < 0.05$ along the grazing-prohibition time at each soil layer. Different lowercase in the column indicated the significant difference at the $P < 0.05$ along soil depth at each grazing-exclusion site.

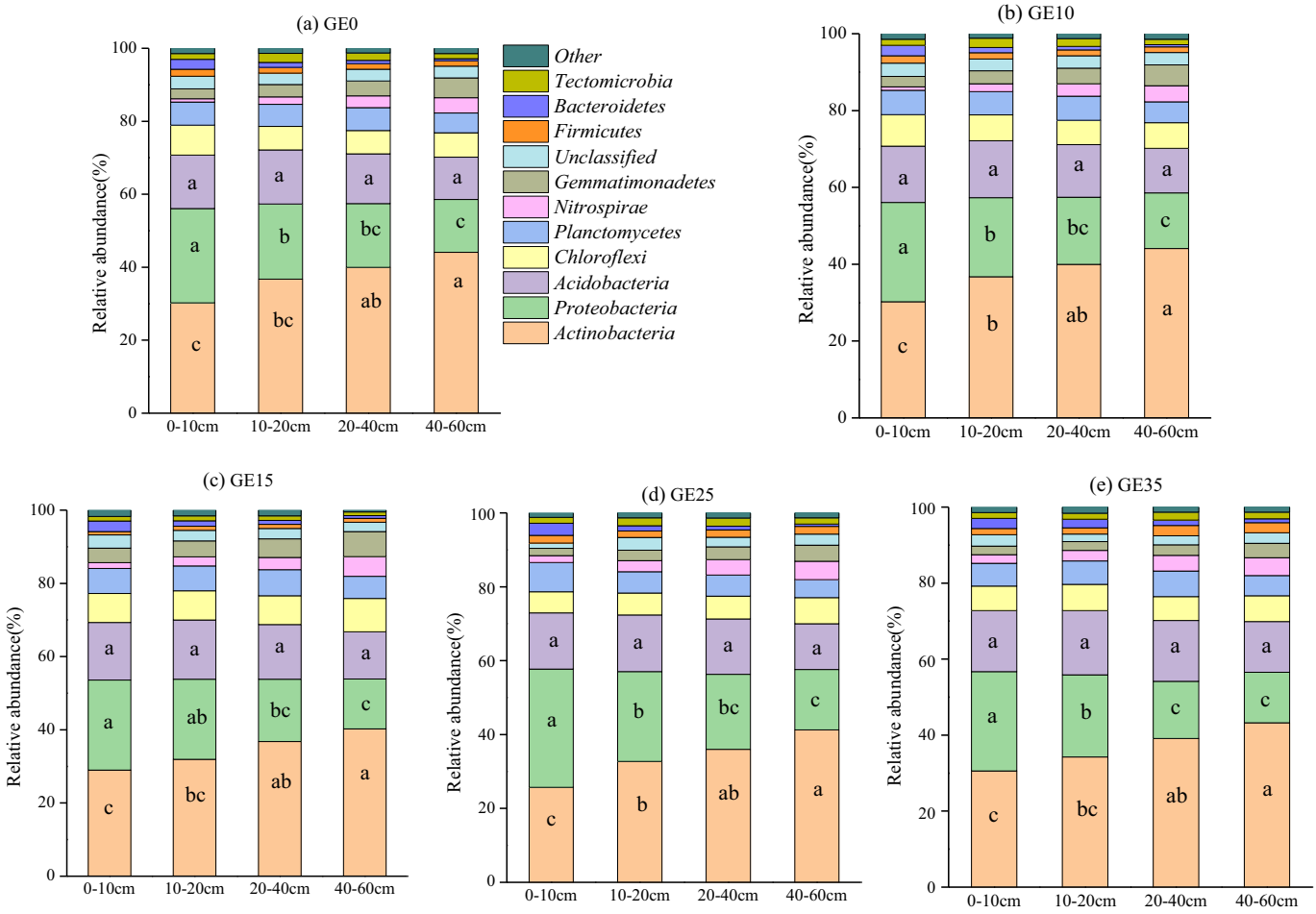

Fig. 1. Relative abundance of bacterial phylum along the soil profile at the different stages. The data for the average relative abundances from three replicates were calculated as the ratio between the abundance of the sequence type and the total number of sequences. Values are means of three replicate. Different letters indicate significant differences ($P < 0.05$) among the exclusion stages.

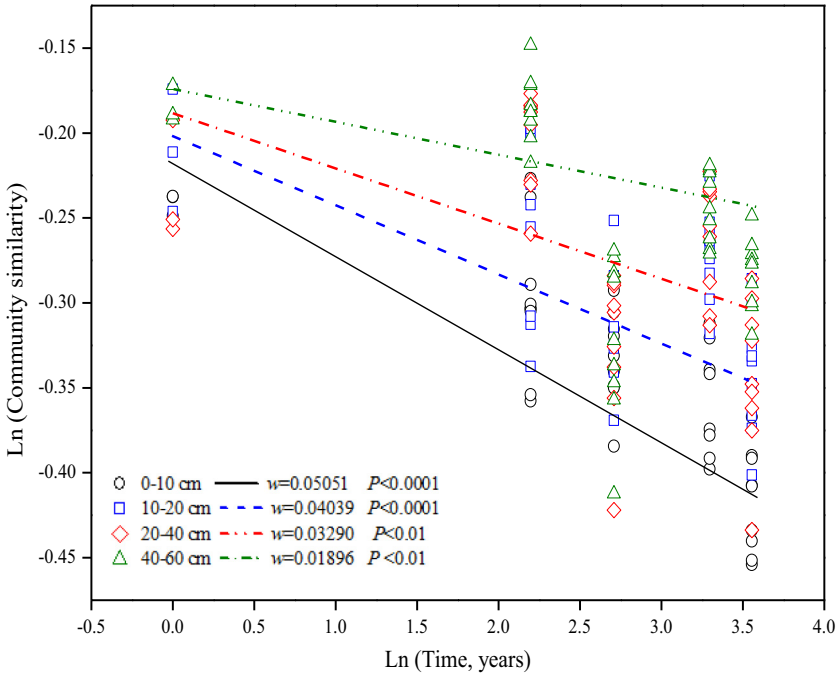

Fig. 2. Time–decay curves for soil bacterial communities at four soil layers along the grazing-exclusion time. The slopes of all lines are significantly less than zero and significantly different for pairwise comparison.

Table 4
Temporary turnover of individual microbial community.

Phylum	Soil depth (cm)	Turnover (*w*)	*F*	*P*
Acidobacteria	0–10	0.0368	28.20	< 0.0001
	10–20	0.0240	23.21	< 0.0001
	20–40	0.0230	15.06	0.0004
	40–60	0.0189	10.08	0.0030
Chloroflexi	0–10	0.0355	23.50	< 0.0001
	10–20	0.0321	8.32	0.0065
	20–40	0.0317	6.04	0.0188
	40–60	0.0263	5.73	0.0218
Bacteroidetes	0–10	0.0463	7.89	0.0079
	10–20	0.0506	10.03	0.0031
	20–40	0.0382	2.38	0.1318
	40–60	0.0609	5.22	0.0282
Actinobacteria	0–10	0.0758	34.17	< 0.0001
	10–20	0.0472	27.25	< 0.0001
	20–40	0.0408	24.76	< 0.0001
	40–60	0.0325	19.97	< 0.0001
Firmicutes	0–10	0.0362	0.47	0.4974
	10–20	0.0065	0.10	0.7533
	20–40	0.0143	0.12	0.7219
	40–60	0.0180	0.902	0.1106
Gemmatimonadetes	0–10	0.074	26.36	< 0.0001
	10–20	0.0577	13.59	0.0007
	20–40	0.0532	7.55	0.0092
	40–60	0.0377	12.91	0.0009
Nitrospirae	0–10	0.1105	37.80	< 0.0001
	10–20	0.0662	41.62	< 0.0001
	20–40	0.065	32.52	< 0.0001
	40–60	0.0444	16.68	0.0002
Planctomycetes	0–10	0.0776	31.38	< 0.0001
	10–20	0.0340	16.64	0.0002
	20–40	0.0293	14.49	0.0005
	40–60	0.0283	15.37	0.0004
Proteobacteria	0–10	0.0613	33.55	< 0.0001
	10–20	0.0572	8.19	0.0069
	20–40	0.0514	7.82	0.0082
	40–60	0.0262	5.63	0.0230
Tectomicrobia	0–10	0.0223	3.56	0.0671
	10–20	0.0178	0.42	0.5214
	20–40	0.0191	0.61	0.4402
	40–60	0.0045	0.10	0.7570

et al., 2005). In our study, the observed increase in the levels of these three enzymes with time (Table 2) indicates the presence of enhanced microbial activity along the succession. The increase in NAG and BG levels with time could be attributable to an increase in plant residues and organic C returned to the soil along the successional chronosequence, while ALP could be explained by soil microbes attempting to obtain the scarce P from organic sources (Nannipieri et al., 2018). Decreased microbial biomass C and BG, NAG, and ALP activities with soil depth were due to the reduction in the nutrient substrates for microbes.

4.2. Bacterial community composition and potential functions

NThe investigated soil depth profiles represented strong environmental gradients, with multiple edaphic factors changing with depth. One of the most obvious variations along the profiles was the significant decrease in microbial diversity estimated by observed OTUs, Shannon index richness, and the Chao1 estimator in nearly all of the restored soils (Table 3), and this was in line with the expected depth patterns of soil bacterial diversity found in other studies (Bai et al., 2017; Gu et al., 2017). Our results showed that changes in environmental circumstances with soil depth represent a strong ecological filter, and that many surface-dwelling microorganisms are less likely to thrive in deeper soil horizon environments. Some phyla exhibited specific distribution patterns along soil depth profiles. *Proteobacteria*, appeared to be abundant in the topsoil and less abundant in the subsoil (Fig. 1), and this could be related to their copiotrophic living attributes. In contrast,

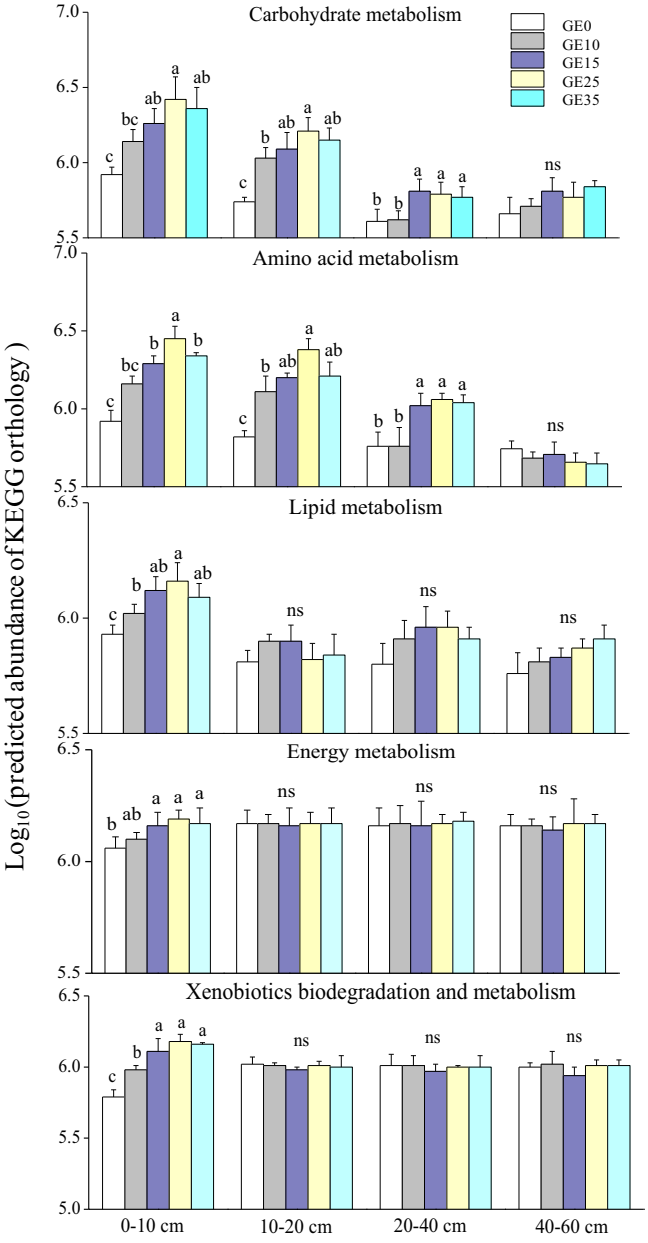

Fig. 3. Predicted abundance of KEGG orthologs (KOs) assigned to the metabolism of carbohydrate, amino acids, lipids, energy, and xenobiotics along the soil profile at the different stages. Values are $\log_{10}$-transformed KO abundances. Metagenome functional content was predicted from the 16S rRNA sequence data against a set of reference genomes using PICRUSt (http://picrust.github.io/picrust/). Result is reported as the mean ± SE (n = 3). Different letters indicate significant differences (P < 0.05) among the exclusion stages. ns: not significant.

Actinobacteria showed increasing abundance as soil depth increased. This result was in accordance with the commonly accepted hypothesis, which posits that the abundant *Actinobacteria* are considered oligotrophic groups (i.e., slow-growing K-strategist bacteria), and they are usually more adapted to growth-limited conditions and tolerant to drought compared to other bacterial groups. Our results indicated a transition from a higher abundance of *Proteobacteria* in the topsoil layer to a higher abundance of *Actinobacteria* in the subsoil layer. *Acidobacteria* exhibited no significant difference in abundance above 40-cm soil depths. This result may partly indicate a stronger adaptation of *Acidobacteria* to changing environmental conditions. However, little is known about the activities and ecology of *Acidobacteria*. Some species

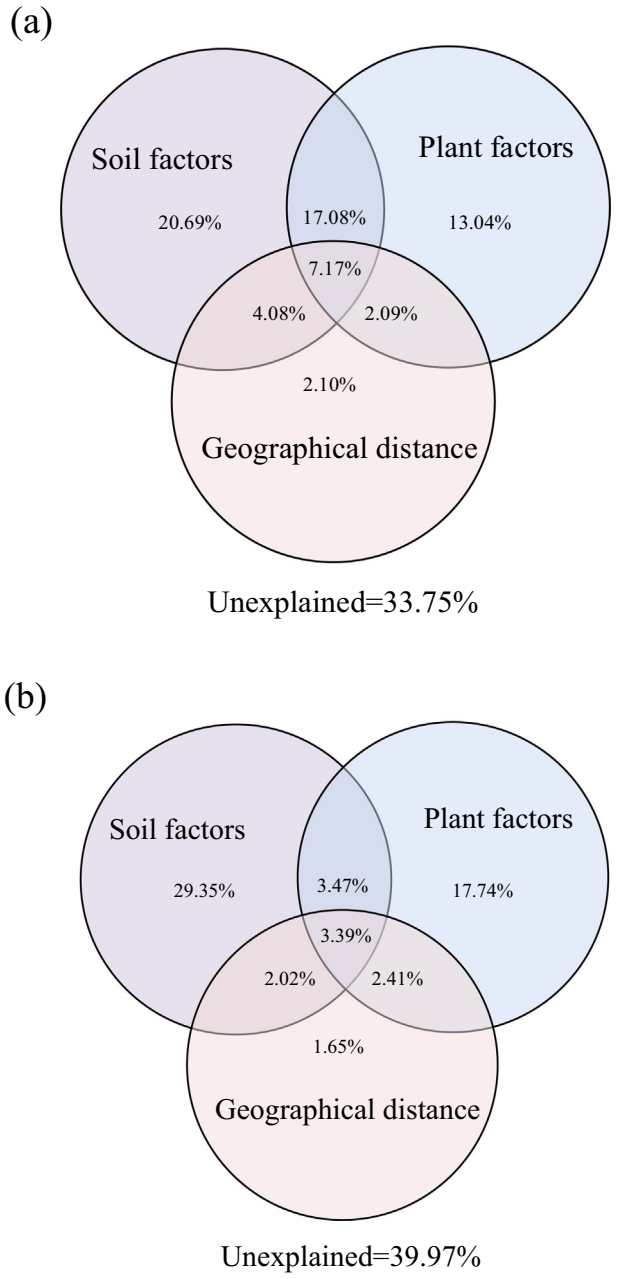

Fig. 4. Variance partitioning analysis to identify the contributions of environmental factors and geographic distance to bacterial composition (a) and metabolic functions (b). Soil factors include organic C, total N, NO_3^--N, NH_4^+-N, available P, moisture, and bulk density. Plant factors include aboveground biomass and root biomass. The bacterial composition was calculated based on the identified OTU, and functions based on the metabolism of carbohydrate, amino acids, lipids, energy, and xenobiotics. The geographic distance was calculated based on Table S5.

in this phylum are heterotrophic, some subdivisions are quite versatile in carbohydrate utilization, and some survive well in environments with poor nutrients (Gu et al., 2017; Kielak et al., 2009; Mushinski et al., 2018). Thus, whether *Acidobacteria* is typically oligotrophic requires further investigation.

Based on the PICRUSt predictive profile, we found a higher abundance of carbohydrate-, amino acid-, lipid-, energy-, and xenobiotic-metabolizing KO at the grazing exclusion sites than at the grazing site at a depth of 0–10 cm (Fig. 3), suggesting that grazing exclusion improved the potential functions regarding the metabolism of C and N in grassland soils (especially carbohydrate and amino acid). In addition, bacterial potential functions related to carbohydrate and amino acid metabolism were much higher in the topsoil than in the subsoil. These results are similar to those of Mushinski et al. (2018) from a forest ecosystem, who found higher metabolism of C and N in the topsoil than in deeper soil. The quantity and quality of C and N can drive belowground ecological functions by modulating bacterial community composition (Chen et al., 2016). In the topsoil, a higher abundance of carbohydrate- and amino acid-degradation genes indicated more active degradation of C and N in that habitat. The topsoil contained a richer variety of organic compounds and higher proportions of heterotrophic bacteria compared to the subsoil. *Proteobacteria* are considered to be important microbes for degrading carbohydrates and amino acids as heterotrophic degraders (Zhu et al., 2018). The positive correlation between the abundance of this phylum and the abundance of carbohydrate- and amino acid-metabolizing KO (Table S4) indicated the importance of Proteobacteria for the metabolism of C and N components.

4.3. Temporary turnover of bacterial communities

Temporal microbial community turnover is a critical indicator of the succession dynamics of biological communities because of its close linkage with ecosystem function (Shade et al., 2013). The turnover values of the bacterial communities obtained in our study (0.023–0.146) had the same magnitude as those of other studies (Hatosy et al., 2013; Liang et al., 2015) ranging from 0 to 0.3. Interestingly, we found decreasing temporary turnover in bacterial communities along the soil depth profile (Fig. 2). This result supported our hypothesis that microbial succession rates will decrease with soil depth during the grazing exclusion process, and this was closely associated with the substrate supplies, such as soil C and N concentrations, and ventilation conditions such as bulk density (Table S3). In contrast to microbes in subsoil, microbes in topsoil exhibited extremely high densities and high generation times due to the more abundant substrates utilized by microbes (Brown et al., 2004). Organisms in topsoil may exhibit fast undulations in population dynamics, because they present large species pools from which local sites can be rapidly colonized, thereby showing fast turnover (Korhonen et al., 2010). Bulk density is another important factor affecting microbial temporary turnover, because bulk density determines the levels of oxygen and compression in soil. It is widely accepted that bacteria, especially aerophilic bacteria, respond positively to high oxygen concentrations, and high oxygen content could promote microbial activity. Previous studies of different soil ecosystems have identified a significant increase in bulk density and a corresponding decrease in oxygen levels and increase in compression along the soil depth profile (Eskandari et al., 2018; Ozpinar et al., 2018). Moreover, an investigation of a similar chronosequence model in the Yunwushan grassland also found increased bulk density with increased soil depth (Zeng et al., 2017). Therefore, we assumed that the higher oxygen content in topsoil than in subsoil could be partly responsible for the decreasing temporary turnover of bacterial communities along the soil depth profile. The replacement of microbial species in our study suggests the decreased stability and reassembling processes of microbial populations during succession. Microbial communities have a great capacity to briefly adapt to changing surrounding conditions for their specific metabolic and genetic attributes (Schmidt et al., 2007). Therefore, the succession rate of microorganisms is likely key to the maintenance of soil ecosystem function.

Different taxonomic groups exhibited variable temporal turnover in response to grazing exclusion. Some populations, such as *Nitrospirae*, *Gemmatimonadetes*, *Actinobacteria*, and *Proteobacteria* phyla, exhibited higher turnover than other microbes (Table 4), suggesting these microbes are more sensitive to environmental change. Our result was accordance with the finding of Zhang et al. (2017) who investigated the stability and succession of sediment microbial communities in response

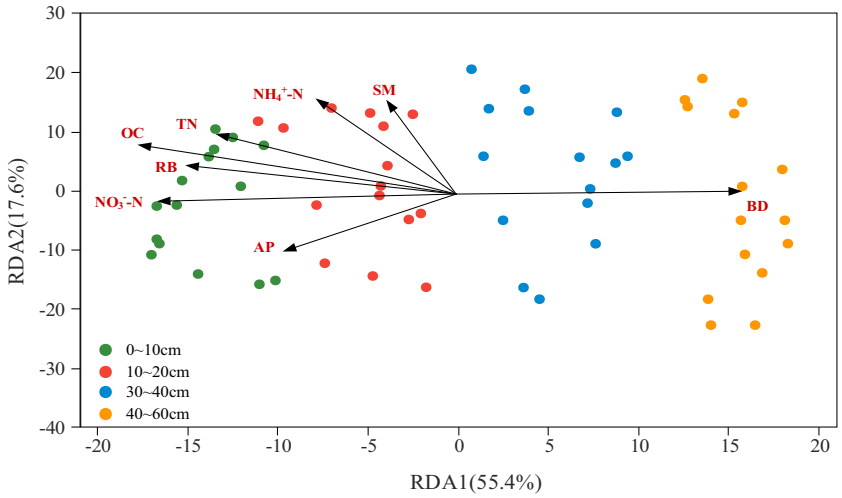

Fig. 5. Ordination plots of the redundancy analysis to identify the influence of plant and soils on the bacterial community based on the identified OTU. AB: aboveground biomass, RB: root biomass, OC: organic C, TN: total N, AP: available P, SM: soil moisture, BD: bulk density.

to dredging disturbance. The extensive variation in temporal turnover between microbial groups could be attributed to the differences in adaptation to the changing environmental conditions (e.g., soil nutrient levels and aggregated status) caused by grazing exclusion, which might alter interspecies and intraspecific interactions (e.g., symbiosis, competition, parasitism, and predation) (Liang et al., 2015), thus leading to variation in microbial taxon assemblage.

4.4. Effects of plants and soils on bacterial community structure

The composition of microbial communities in natural ecosystems is dependent on plant and soil conditions. Variation partitioning analysis showed that a high proportion (20.69% and 29.35%) of the variation in bacterial community composition and functions can be explained by soil physico-chemical properties (Fig. 4). This indicated that soil conditions were a major factor in structuring microbial communities. This is comparable with a few previous studies (Ding et al., 2017; Yao et al., 2018). The unexplained variation may be due to unmeasured additional factors and ecologically-neutral processes of diversification. RDA suggested that soil organic C, NO_3^--N, and bulk density were the most important determinants of bacterial community structure. Soil organic C and N supplies were strongly driven by depth. The decreased amount of C and N available to microorganisms as the depth increased caused the community to transition to groups with variable survival strategies that occupied different parts of the profile. Similarly, Hartman et al. (2008) suggested that the availability of resources is a main determinant of soil microbial community composition along depth profiles, and copiotrophic and oligotrophic microbes favored surface and deeper soils, respectively. In addition to nutrients, bulk density is an important factor that forcefully regulates cell metabolism in soils. In this study, a significant negative relationship between bulk density and bacterial diversity signified the negative impact of bulk density on the quantity and variety of microbial communities. Decreasing bulk density along the soil depth profile indicated the accumulation of small-size particles and increase in compression in the anoxic deep layer, leading to the stimulation of anaerobic populations and stress of aerobic groups. For example, a significant positive correlation was observed between bulk density and the bacterial groups *Deltaproteobacteria* and *Anaerolineae* (Table S3), of which the strictly anaerobic families *Geobacteraceae*, *Syntrophaceae*, and *Desulfobulbaceae* were dominant. In addition, the strong effect of available P on bacterial communities and strong associations between available P and dominant taxa suggested the important role of P in the formation of microbial communities.

It is worth mentioning that the results obtained in our study were based on an approach of substituted space for time. Although this approach has been used to investigate changes in plant-soil ecosystem over time (Wardle et al., 2004; Harris, 2009; Barber et al., 2017; Zhang et al., 2018; Wang et al., 2019), it is not perfect as it is difficult to avoid the risk of pseudo replication, such as the influence from the sampling distance (Ramette and Tiedje, 2007). For that, we examined the contribution of sampling geographical distance to the changes in bacterial community and found a non-significant role of sampling distance in determining the bacterial composition and functions (Fig. 4), therefore, changes in bacterial community could be drove by plant and soil properties caused by grazing exclusion.

5. Conclusions

Long-term grazing exclusion increases soil nutrients, microbial biomass C, enzyme activities, bacterial diversity, and the metabolism of carbohydrates and amino acids, but each of these decreased as the soil depth increased. Decreased abundance of *Proteobacteria* and increased *Actinobacteria* with soil depth suggested a transition from copiotrophic to oligotrophic groups. Moreover, bacterial communities showed a decreasing succession rate and a declined metabolic along the soil depth profile during natural restoration, which was more ascribed to changes in soil conditions (e.g. C, N, and bulk density) than changes in plant biomass. Our study provides important information and novel insights regarding the succession and functions of microbial communities along a soil profile following long-term grazing exclusion.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (41771554), and National Key Research and Development Program of China (2016YFC0501707).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2019.05.010.

References

Arroyo, A.I., Pueyo, Y., Saiz, H., Alados, C.L., 2015. Plant-plant interactions as a mechanism structuring plant diversity in a Mediterranean semi-arid ecosystem. Ecology and Evolution 5, 5305–5317.

Bai, R., Wang, J.T., Deng, Y., He, J.Z., Feng, K., Zhang, L.M., 2017. Microbial community and functional structure significantly varied among distinct types of paddy soils but

responded differently along gradients of soil depth layers. Front. Microbiol. 8.
Barber, N.A., Chantos-Davidso, K.M., Peralta, R.A., Sherwood, J.P., Swingley, W.D., 2017. Soil microbial community composition in tallgrass prairie restorations converge with remnants across a 27-year chronosequence. Environ. Microbiol. 19, 3118–3131.
Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. Ecology 85, 1771–1789.
Bru, D., Ramette, A., Saby, N.P.A., Dequiedt, S., Ranjard, L., Jolivet, C., Arrouays, D., Philippot, L., 2011. Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. ISME J. 5, 532–542.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Tumbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.
Chen, L., Brookes, P.C., Xu, J.M., Zhang, J.B., Zhang, C.Z., Zhou, X.Y., Luo, Y., 2016. Structural and functional differentiation of the root-associated bacterial microbiomes of perennial ryegrass. Soil Biol. Biochem. 98, 1–10.
Cheng, J., Jing, G., Wei, L., Jing, Z., 2016. Long-term grazing exclusion effects on vegetation characteristics, soil properties and bacterial communities in the semi-arid grasslands of China. Ecol. Eng. 97, 170–178.
Cui, X.X., Bing, H.J., Fang, L.C., Guo, X.B., Wang, X., Zhang, Y.J., Li, P.F., Zhang, X.C., 2018. Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the northern loess plateau, China. Soil Biol. Biochem. 116, 11–21.
Ding, L.J., Su, J.Q., Li, H., Zhu, Y.G., Cao, Z.H., 2017. Bacterial succession along a long-term chronosequence of paddy soil in the Yangtze River Delta, China. Soil Biol. Biochem. 104, 59–67.
Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.
Eilers, K.G., Debenport, S., Anderson, S., Fierer, N., 2012. Digging deeper to find unique microbial communities: the strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biol. Biochem. 50, 58–65.
Eskandari, S., Guppy, C.N., Knox, O.G.G., Backhouse, D., Haling, R.E., 2018. Understanding the impact of soil sodicity on mycorrhizal symbiosis: some facts and gaps identified from cotton systems. Appl. Soil Ecol. 126, 199–201.
Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. 35, 167–176.
Fierer, N., Ladau, J., Clemente, J.C., Leff, J.W., Owens, S.M., Pollard, K.S., Knight, R., Gilbert, J.A., McCulley, R.L., 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. Science 342, 621–624.
Fussmann, K.E., Schwarzmueller, F., Brose, U., Jousset, A., Rall, B.C., 2014. Ecological stability in response to warming. Nat. Clim. Chang. 4, 206–210.
Gu, Y., Wang, Y., Lu, S.e., Xiang, Q., Yu, X., Zhao, K., Zou, L., Chen, Q., Tu, S., Zhang, X., 2017. Long-term fertilization structures bacterial and archaeal communities along soil depth gradient in a Paddy soil. Front. Microbiol. 8.
Harris, 2009. Soil microbial communities and restoration ecology: facilitators or followers? Science 325, 573–574.
Hartman, W.H., Richardson, C.J., Vilgalys, R., Bruland, G.L., 2008. Environmental and anthropogenic controls over bacterial communities in wetland soils. Proc. Natl. Acad. Sci. U. S. A. 105, 17842–17847.
Hatosy, S.M., Martiny, J.B.H., Sachdeva, R., Steele, J., Fuhrman, J.A., Martiny, A.C., 2013. Beta diversity of marine bacteria depends on temporal scale. Ecology 94, 1898–1904.
Horner-Devine, M.C., Lage, M., Hughes, J.B., Bohannan, B.J.M., 2004. A taxa-area relationship for bacteria. Nature 432, 750–753.
Jangid, K., Williams, M.A., Franzluebbers, A.J., Blair, J.M., Coleman, D.C., Whitman, W.B., 2010. Development of soil microbial communities during tallgrass prairie restoration. Soil Biol. Biochem. 42, 302–312.
Jing, Z.B., Cheng, J.M., Jin, J.W., Su, J.S., Bai, Y., 2014. Revegetation as an efficient means of improving the diversity and abundance of soil eukaryotes in the loess plateau of China. Ecol. Eng. 70, 169–174.
Jobbagy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol. Appl. 10, 423–436.
Jurasinski, G., 2012. Simba: a collection of functions for similarity analysis of vegetation data. https://cran.r-project.org/web/packages/simba.
Kielak, A., Pijl, A.S., van Veen, J.A., Kowalchuk, G.A., 2009. Phylogenetic diversity of Acidobacteria in a former agricultural soil. ISME J. 3, 378–382.
Korhonen, J.J., Soininen, J., Hillebrand, H., 2010. A quantitative analysis of temporal turnover in aquatic species assemblages across ecosystems. Ecology 91, 508–517.
Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75, 5111–5120.
Liang, Y., Jiang, Y., Wang, F., Wen, C., Deng, Y., Xue, K., Qin, Y., Yang, Y., Wu, L., Zhou, J., Sun, B., 2015. Long-term soil transplant simulating climate change with latitude significantly alters microbial temporal turnover. ISME J. 9, 2561–2572.
Lin, Y.T., Whitman, W.B., Coleman, D.C., Chen, T.-H., Chiu, C.Y., 2014. Composition of bacterial communities in sand dunes of subtropical coastal forests. Biol. Fertil. Soils 50, 809–814.
Liu, S., Zamanian, K., Schleuss, P.-M., Zarebanadkouki, M., Kuzyakov, Y., 2018. Degradation of Tibetan grasslands: consequences for carbon and nutrient cycles. Agric. Ecosyst. Environ. 252, 93–104.
Lozano, Y.M., Hortal, S., Armas, C., Pugnaire, F.I., 2014. Interactions among soil, plants, and microorganisms drive secondary succession in a dry environment. Soil Biol. Biochem. 78, 298–306.
Ma, W.B., Jiang, S.J., Assemien, F.A., Qin, M.S., Ma, B.B., Xie, Z., Liu, Y.J., Feng, H.Y., Du, G.Z., Ma, X.J., Roux, X.L., 2016. Response of microbial functional groups involved in soil N cycle to N, P and NP fertilization in Tibetan alpine meadows. Soil Biol. Biochem. 101, 195–206.
Monroy, F., van der Putten, W.H., Yergeau, E., Mortimer, S.R., Duyts, H., Bezemer, T.M., 2012. Community patterns of soil bacteria and nematodes in relation to geographic distance. Soil Biol. Biochem. 45, 1–7.
Mushinski, R.M., Zhou, Y., Gentry, T.J., Boutton, T.W., 2018. Bacterial metataxonomic profile and putative functional behavior associated with C and N cycle processes remain altered for decades after forest harvest. Soil Biol. Biochem. 119, 184–193.
Nannipieri, P., Trasar-Cepeda, C., Dick, P., 2018. Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. Biol. Fertil. Soils 54, 11–19.
Odum, E.P., 1969. The strategy of ecosystem development. Science 164, 262–270.
Ozpinar, S., Ozpinar, A., Cay, A., 2018. Soil management effect on soil properties in traditional and mechanized vineyards under a semiarid Mediterranean environment. Soil Tillage Res. 178, 198–208.
Pan, W., Kan, J., Inamdar, S., Chen, C., Sparks, D., 2016. Dissimilatory microbial iron reduction release DOC (dissolved organic carbon) from carbon-ferrihydrite association. Soil Biol. Biochem. 103, 232–240.
Ramette, A., Tiedje, J.M., 2007. Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. Proc. Natl. Acad. Sci. U. S. A. 104, 2761–2766.
Roldan, A., Salinas-Garcia, J.R., Alguacil, M.M., Diaz, E., Caravaca, F., 2005. Soil enzyme activities suggest advantages of conservation tillage practices in sorghum cultivation under subtropical conditions. Geoderma 129, 178–185.
Schmidt, S.K., Costello, E.K., Nemergut, D.R., Cleveland, C.C., Reed, S.C., Weintraub, M.N., Meyer, A.F., Martin, A.M., 2007. Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. Ecology 88, 1379–1385.
Shade, A., Caporaso, J.G., Handelsman, J., Knight, R., Fierer, N., 2013. A meta-analysis of changes in bacterial and archaeal communities with time. ISME J. 7, 1493–1506.
Siciliano, S.D., Palmer, A.S., Winsley, T., Lamb, E., Bissett, A., Brown, M.V., van Dorst, J., Ji, M., Ferrari, B.C., Grogan, P., Chu, H., Snape, I., 2014. Soil fertility is associated with fungal and bacterial richness, whereas pH is associated with community composition in polar soil microbial communities. Soil Biol. Biochem. 78, 10–20.
Song, Z.L., Wang, J., Liu, G.B., Zhang, C., 2019. Changes in nitrogen functional genes in soil profiles of grassland under long-term grazing prohibition in a semiarid area. Sci. Total Environ. 673, 92–101.
Steinauer, K., Chatzinotas, A., Eisenhauer, N., 2016. Root exudate cocktails: the link between plant diversity and soil microorganisms? Ecology and Evolution 6, 7387–7396.
Tang, Y., Yu, G., Zhang, X., Wang, Q., Ge, J., Liu, S., 2018. Changes in nitrogen-cycling microbial communities with depth in temperate and subtropical forest soils. Appl. Soil Ecol. 124, 218–228.
Tian, X.F., Hu, H.W., Ding, Q., Song, M.H., Xu, X.L., Zheng, Y., Guo, L.D., 2014. Influence of nitrogen fertilization on soil ammonia oxidizer and denitrifier abundance, microbial biomass, and enzyme activities in an alpine meadow. Biol. Fertil. Soils 50, 703–713.
Vance, E.D., Brooks, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass. Soil Biol. Biochem. 19, 703–707.
Wang, Z., Zhang, Q., Staley, C., Gao, H.L., Ishii, S.S., Wei, X.R., Liu, J., Cheng, J.M., Hao, M.D., Sadowsky, M.J., 2019. Impact of long-term grazing exclusion on soil microbial community composition and nutrient availability. Biol. Fertil. Soils 55, 121–134.
Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.
Yao, M., Rui, J., Li, J., Wang, J., Cao, W., Li, X., 2018. Soil bacterial community shifts driven by restoration time and steppe types in the degraded steppe of Inner Mongolia. Catena 165, 228–236.
Zeng, Q., An, S., Liu, Y., 2017. Soil bacterial community response to vegetation succession after fencing in the grassland of China. Sci. Total Environ. 609, 2–10.
Zhang, N., Xiao, X., Pei, M., Liu, X., Liang, Y., 2017. Discordant temporal turnovers of sediment bacterial and eukaryotic communities in response to dredging: non-resilience and functional changes. Appl. Environ. Microbiol. 83 e02526–16.
Zhang, C., Liu, G., Song, Z., Wang, J., Guo, L., 2018. Interactions of soil bacteria and fungi with plants during long-term grazing exclusion in semiarid grasslands. Soil Biol. Biochem. 124, 47–58.
Zhang, C., Song, Z.L., Zhuang, D.H., Wang, J., Xie, S.S., Liu, G.B., 2019a. Urea fertilization decreases soil bacterial diversity, but improves microbial biomass, respiration, and N cycling potential in a semiarid grassland. Biology & Fertiity of Soils 55, 229–242.
Zhang, C., Wang, J., Liu, G.B., Song, Z.L., Fang, L.C., 2019b. Impact of soil leachate on microbial biomass and diversity affected by plant diversity. Plant Soil. https://doi.org/10.1007/s11104-019-04032-x.
Zhu, P., Wang, Y.P., Shi, T.T., Zhang, X.L., Huang, G.Q., Gong, J., 2018. Intertidal zonation affects diversity and functional potentials of bacteria in surface sediments: A case study of the Golden Bay mangrove, China. Appl. Soil. Ecol. 130, 159–168.