Plant Soil (2019) 439:525–539
https://doi.org/10.1007/s11104-019-04045-6

REGULAR ARTICLE

# Grazing and enclosure alter the vertical distribution of organic nitrogen pools and bacterial communities in semiarid grassland soils

**Huanhe Wang · Jiangye Li · Qichun Zhang · Jun Liu · Bo Yi · Yong Li · Jingwen Wang · Hongjie Di**

Received: 17 October 2018 / Accepted: 18 March 2019 / Published online: 6 April 2019


**Abstract**

*Background and aims* Different grazing management practices have a significant impact on the sustainability of grassland ecosystems. This study invested the vertical distribution of soil nitrogen (N) forms and soil microbial community structures in a semiarid grassland ecosystem under different grazing management practices in Inner Mongolia.

*Methods* Soil samples were collected from three semiarid grassland plots subjected to different long-term management practices namely, free grazing (FG) and two different periods of enclosure (E83, enclosed since 1983 and E97, enclosed since 1997). The soil organic nitrogen (N) pools were analyzed by classical methods, and the bacterial community was assessed by PCR-DGGE and high-throughput sequencing.

*Results* The surface soil N-supplying capacity was in the order of E97 ≥ E83 ≥ FG. The soil ammonium N, amino N, and N-supplying capacity were greater in the enclosed plots than in the FG plot. Additionally, the 0–40-cm soil layer showed the influence of different management practices on the soil properties. The structure and diversity of the soil microbial community also varied with the management type. The soil organic N composition was significantly related to the soil bacterial community structure and microbial categories.

*Conclusions* An appropriate number of years of fencing helps to improve the soil surface nutrient status, whereas overgrazing and prolonged enclosure are not conducive to the restoration of soil nutrients. Different grazing management practices can affect the microbial community structure and turnover of soil N in grasslands.

**Keywords** Grazing · Enclosure · Soil microbial community structure · Soil organic nitrogen · Nitrogen-supplying capacity

Huanhe Wang and Jiangye Li are equally important as the first authors.

Responsible Editor: Elizabeth M Baggs.

H. Wang · J. Li · Q. Zhang · J. Liu · B. Yi · Y. Li · H. Di
Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Key Laboratory of Environment Remediation and Ecological Health, Ministry of Education, Zhejiang University, Hangzhou 310058, China

Q. Zhang (✉)
College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China
e-mail: qczhang@zju.edu.cn

J. Wang
Hangzhou Plant Protection and Fertilizer Station Hangzhou, Hangzhou 310020, People's Republic of China

## Introduction

Grasslands are one of the largest terrestrial ecosystems in the world. In China, the grassland areas make up approximately 400 million hectares, accounting for 11.7% of the global grassland area and 41.2% of the national land area (Liu et al. 2008). The grassland ecosystem is an important terrestrial ecosystem influencing

the soil nitrogen (N) cycle in China. Despite their importance, the natural ecosystems, such as those in Inner Mongolia, are experiencing degradation of different degrees (Ye and Van Ranst 2009), with the area of degradation reaching 90% of the national grassland area, which in turn has negative impacts on livestock production (Kang et al. 2007). Grassland enclosure is considered to be an effective and economical practice for restoring degraded areas, as it has been reported that the surface soil nutrients, vegetation coverage and height, plant diversity, and biomass production of the affected grasslands have been gradually restored since the nationwide conservation programs ("Start-up Regrass Program" and "Returning Grazing Lands to Grasslands") were employed (Jiang et al. 2006; Cheng et al. 2011; Deng et al. 2013). Hu et al. (2016) tested enclosure management in grasslands in China, producing results in 2016 before publication, where they found that both the vegetation biomass and soil carbon (C) content reached a steady state after 15 years of enclosure. However, there is not much literature documenting the differences in soil N pools and microbial community composition between enclosure and grazing management practices.

The N cycle is a constant hot topic globally. Soil N pools generally consist of inorganic N and organic N. Over 90% of the N in most surface soils exists in the organic form (Stevenson 1982). It has long been recognized that organic N plays an important role in soil fertility. N mineralization, regulated mainly by microbes, is one of the main processes of the soil N cycle and is also the most important pathway for effective organic N production. To some extent, the composition of organic N pools determines the size of the inorganic N pools as their bioavailability is different (Weintraub and Schimel 2003; Kemmitt et al. 2008). As a natural grassland, the Inner Mongolia grassland is an N-limiting and vulnerable terrestrial ecosystem in this semiarid region (Xu et al. 2012). Therefore, the soil N pool and microbial community composition are vital for providing available N for herbage growth (Kemmitt et al. 2008). Organic N fractions are classically categorized as hydrolyzable (also acid-soluble) N, amino acid N, amino sugar N, ammonium N, acid-insoluble N, and hydrolyzable unknown (also acid-soluble unidentified) N (Bremner 1965). It was reported that amino acid N and amino sugar N were positively correlated with N mineralization (Jones and Kielland 2012; Roberts et al. 2016). However, most studies related to the N cycle were conducted on the surface soils (0–20 cm) and ignored the N pools in the deeper soil layers (An and Li 2015; Liu et al. 2011). Recently, Osterholz et al. (2017) studied gross ammonification over the 0–80-cm depth, based on a long-term cropping system in Iowa, USA. The report showed that 37% of the gross ammonification occurred in the 20–80-cm depth. The mechanisms of this high stratification were probably closely related to the microbial distribution and N pool composition in the different soil layers. In addition, grazing alters the soil permeability and bulk density as a result of trampling by the animals (Daniel et al. 2002; Andrés et al. 2017), and excreta from the grazers contributes fresh C and N to the grassland, which probably further impacts the N distribution and the size of different organic N pools in the soil profile, given that C and N mineralization and the microbial community in deep soil were reportedly changed by fresh C and N inputs (Wang 2014).

The decomposition of organic N is completed by soil degraders. However, soil microbes are key drivers during the N mineralization process. Bonde et al. (1988) found that the soil N mineralization constants (0.45–0.56 g $kg^{-1}$ $week^{-1}$) were correlated to the microbial biomass N (0.36–0.61 $kg^{-1}$ $week^{-1}$). Generally, the available soil organic N can be utilized easily by most microbes, whereas the breakdown of macromolecular polymers requires special extracellular enzymes (e.g., polyphenol oxidase, laccase, and chitinase) produced by specific microbial groups (Talbot and Treseder 2010). Grazing results in changes to the plant species as a result of biting, impacts the physical properties of the soil as a result of trampling, and affects the abundance and structure of the microbial community in the surface soils or in the rhizosphere of plants (Murphy et al. 1998). However, little information is available about how grazing impacts the microbial community at different depths of the soil layer, which is also critical to learning about soil N storage.

In the unimproved grassland ecosystems, the net N mineralization rate is inherently high, and the soluble organic N is comparable to inorganic N in terms of amount (Bardgett et al. 2003). It was reported that the mineralization of polyphenol-protein compounds by saprophytic fungi was a key step of the N cycle in a temperate forest (Wu 2011). In addition, it was recently found that fungi might be better related to the net N mineralization in semiarid grassland soil (Li et al. 2016). This was because fungi processed a better ability to produce depolymerizing enzymes to break down the polymers into monomers, especially in the harsh soil

environment (Zaman et al. 1999; Schimel and Bennett 2004). Compared with fungi, bacteria were more commonly responsible for catalyzing the conversion of monomers into ammonium in the process of transforming recalcitrant organic N into inorganic N (Graeme and Christa 2006). However, the relationships between the organic N fractions and bacterial communities were not examined, which is quite important to know in order to be able to understand more clearly how bacteria take part in the mineralization of organic N, especially in deep soil.

Therefore, to gain a better understanding of how organic N pools in the soil profile are distributed in semiarid grasslands and how the bacterial community responds to the distribution, a long-term enclosure trial with different enclosure periods and free grazing was conducted on the natural Inner Mongolia grassland where organic N is rich and inorganic N is lacking. Additionally, the impacts of grazing on the organic N distribution and on the relationships between different organic N fractions and bacterial communities were investigated. We hypothesized that an appropriate number of years of fencing would help to improve the soil surface nutrient status, whereas overgrazing and prolonged enclosure would not be conducive to the restoration of soil nutrients. The different grazing management practices applied to the grassland affected not only the soil fertility but also the microbial biomass, microbial community structure, and turnover of soil N.

## Materials and methods

### Study site description

The study site, Baiyinxile Ranch, is located in the northeast of Inner Mongolia (N43° 33′ 12″ to 43° 33′ 35″, E116° 42′ 26″ to 116° 42′ 31″), at an elevation of 1000–1500 m. The area is characterized by a temperate semiarid climate with a mean annual temperature of − 0.4 °C, where the minimum and maximum monthly mean temperatures range from − 19.5 °C in January to 20.8 °C in July. The average annual precipitation is 350 mm, distributed unevenly among the seasons but falling mainly during June–August, which coincides with the plant-growing season. The annual mean evaporation is 3–4 times more than the precipitation. From March to May, it is windy, with a monthly mean wind speed of 4.9 m $s^{-1}$. The soil type is classified as dark chestnut by Chinese soil classification, or calcic-orthic aridisol by US soil taxonomy. The most common vegetation species of this region is *Leymus chinensis*.

### Soil samples collection

Three long-term management practice treatments were carried out at the research site, each with three replicates and all randomly allocated in the field. One treatment was the enclosure of a 600 m × 400 m area since 1983 (designated as the E83 plot), whereas another treatment was the enclosure of an 80 m × 400 m area since 1997 (designated as the E97 plot). The third treatment was chronic free grazing in a 600 m × 50 m area, where sheep were allowed to graze freely throughout the year since 1983 (designated as the FG plot). The grazing intensity was 12 sheep $ha^{-1}$ (GI 12), which exceeded the very heavy grazing rate (the moderate grazing intensity is GI4) (Schönbach et al. 2011; Ren et al. 2015). Soil samples were collected from the three different plots in October 2015. Five samples from each of three depths (0–20, 20–40, and 40–60 cm) at each plot were bulked into a single composite sample, packed with ice packs, and transported to the laboratory. The soil samples were then passed through a 2-mm sieve and stored at 4 °C for soil analysis. A subsample of each composite sample was stored at − 80 °C for later nucleic acid extraction. The methods used to measure the basic physicochemical properties of the soil samples were carried out according to the descriptions given by Bao (2000).

### Soil N-supply capacity determination

The potential N-supplying capacity in the different layers of soils under different management practices was determined according to the incubation methods described by Jin (2007) and by Stanford and Smith (1976) and Gianello and Bremner (1986), with slight modifications. In brief, 10 g of fresh soil with a 75% water field capacity (WFC) was first incubated at 30 °C for 1 week. Then, the soil was dried at 50 °C for 24 h in an incubator. Thereafter, the sample was watered again to adjust the moisture to 75% WFC and incubated at 30 °C for 1 more week. Following the incubation, the acidic potassium permanganate ($KMnO_4$) method and the potassium chloride (KCl) water bath method were used to determine the soil N-supplying capacity by measuring the $NH_4^+$-N and $NO_3^-$-N concentrations

before and after incubation. The $NH_4^+$-N and $NO_3^-$-N concentrations were measured using an automated flow injection analyzer (San++, Skalar Analytical BV, Netherlands).

### Soil organic N fractionation

Total nitrogen (TN) content was analyzed using Kjeldahl acid digestion method with an auto-analyzer (Foss Inc., Hillerod, Sweden). Before determining the organic N fractions, the soil was hydrolyzed in hydrochloric acid in order to release N from the soil clay minerals and organic colloids. The hydrolysate was further used to determine the concentrations of different organic N fractions namely, the total acid-soluble unidentified N, ammonia N, amino sugar N, amino acid N, and acid-insoluble N. The concentrations of these different organic forms of N were determined according to the classical methods described by Bremner (1965).

### Soil microbial biomass and bacterial community abundance analyses

The microbial biomass C (MBC) and microbial biomass N (MBN) in the samples were determined by the fumigation extraction method (Brookes et al. 1985). DNA was extracted from 0.50 g of the evenly mixed three soil replicates that had been freeze-dried and kept at − 80 °C, using the Fast DNA Spin Kit for Soil (MP Biomedical, USA) according to the manufacturer's instructions. In the last step, 100 μL of Tris-EDTA buffer was used to elute all the DNAs to the catch tube. After the extraction, the DNA concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., USA) and the quality of the extracted DNA was evaluated by the index of the $OD_{260}/OD_{280}$ ratio shown on the spectrophotometer. This ratio, ranging from 1.8 to 2.0, indicated that the quality of the extracted DNA was suitable for analysis. The real-time quantitative polymerase chain reaction (qPCR) was used to determine the bacterial 16S rRNA gene abundance. The universal primer pair 515/806 was used to amplify the V4 hypervariable region of the 16S rRNA gene (Caporaso et al. 2011). A typical 20 μL reaction contained 10.0 mL of SYBR® Premix Ex Taq™ II (Takara, Japan), 0.4 μM of each primer, 1–10 ng of template DNA, and sterile water to make up the final volume. All reactions were run on the LightCycler® 480 II apparatus (Roche, Germany), and qPCR analysis was performed using LightCycler148® SW 1.5.1 software (Roche). The standard curve used in the qPCR analysis was prepared as follows: The 16S rRNA gene was PCR-amplified and the PCR product was purified using the EasyPure® Genomic DNA Kit Clean-up Kit (TransGen, China) according to the manufacturer's instructions. The purified PCR products were cloned into the pEASY-T1 vector (TransGen), which was then transformed into Trans1-T1 phage-resistant chemically competent cells (TransGen) according to the manufacturer's protocol. The plasmids were extracted from overnight cultures using the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific, Germany), then purified with the TaKaRa MiniBEST Plasmid Purification Kit Ver. 4.0 (TaKaRa, Japan), and finally used as a template in the PCRs with the M13 and SR primers. The concentration and quality of the amplicons were estimated using the NanoDrop 2000 spectrophotometer, and the initial concentration of the plasmid used for the standard curve was 425.6 ng $\mu L^{-1}$. The standard curve was generated using a 10-fold serial dilution of the evaluated plasmid across eight orders of magnitude (9.19E+09 to 9.19E+02 copies $g^{-1}$ dry soil). The reaction was conducted on a LightCyclerTM 480 II real-time qPCR system (Roche Diagnostics International Ltd., Switzerland), using a previously described run program (Li et al. 2016). The efficiency was 96% for all gene amplifications, and the R value was 0.999.

### Soil bacterial community diversity analysis

The diversity of the soil bacterial community was determined by denaturing gradient gel electrophoresis (DGGE) and next-generation sequencing. The PCR and DGGE were carried out using the same methods as described by Li et al. (2016). For high-throughput sequencing, the universal primers 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′) with 10-nt barcodes were used to amplify the V4 region of the 16S rRNA gene for pyrosequencing using the MiSeq sequencer (Caporaso et al. 2011; Caporaso et al. 2012). The PCR mixture (25 μL) contained 1X PCR buffer, 1.5 mM of $MgCl_2$, 0.4 μM each of deoxynucleoside triphosphate, 1.0 μM of each primer, 0.5 U of TransStart FastPfu DNA Polymerase (TransGen), and 10 ng of soil genomic DNA. The PCR amplification program was as follows: an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 40 s, 56 °C for

60 s, and 72 °C for 60 s, and a final extension at 72 °C for 10 min. The PCR for each sample was conducted twice and the products were combined after the second amplification. The combined PCR products were subjected to electrophoresis on a 1.0% agarose gel. The band of correct size was excised, following which the DNA in the band was purified using a gel extraction kit (Omega Bio-Tek, USA) and quantified with the NanoDrop 2000 spectrophotometer. All DNA samples were pooled together, with an equal molar amount for each DNA sample. The sequencing samples were prepared using the TruSeq DNA Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. The purified library was diluted, denatured, re-diluted, mixed with PhiX (equal to 30% of the final DNA amount) as described in the Illumina library preparation protocols, and then applied to an Illumina MiSeq system for sequencing with the Reagent Kit ver. 2 (Illumina) as described in the manufacturer's manual.

## Sequence data analysis

The sequence data were processed using QIIME Pipeline ver. 1.7.0 (http://qiime.org/tutorials/tutorial.html). All sequence reads were trimmed and assigned to each sample based on their barcodes. Multiple steps were required to trim the sequences (e.g., removal of sequences < 300 bp), and 4332 reads (the lowest read number in all samples) were chosen randomly for each sample. Sequences were clustered into operational taxonomic units (OTUs) at the 97% identity threshold. The aligned 16S rRNA gene sequences were chimera-checked using the UCHIME algorithm (Edgar et al. 2011). The alpha-diversity (for analyzing the phylogenetic distance in the whole phylogenetic tree), Chao1 estimator (characterizing the species richness), and Shannon index (reflecting the bacterial community diversity) were also calculated. Rarefaction curves were generated from the observed species. Taxonomy was assigned using the Ribosomal Database Project classifier (Wang et al. 2007).

## Statistical analysis

Samples were clustered based on Bray–Curtis similarities, using the hclust function in the R vegan package. The redundancy analysis (RDA), canonical correspondence analysis (CCA), and Mantel test were calculated using the R vegan package (Oksanen et al. 2012). The RDA was carried out to explore the effect of topsoil organic N on the topsoil microbial species.

All statistical data were submitted to one-way analysis of variance followed by Duncan's multiple-range test, performed using SPSS ver. 20. $P < 0.05$ was considered to be statistically significant.

**Table 1** Physicochemical properties of the soil samples

| Treatment | pH ($H_2O$) | AK mg $kg^{-1}$ | SOM g $kg^{-1}$ | TN g $kg^{-1}$ | CEC cmol $kg^{-1}$ | WHC % | C/N ratio |
|---|---|---|---|---|---|---|---|
| 0–20 cm | | | | | | | |
| E83 | 6.75 ± 0.03 a | 141.5 ± 1.88 c | 16.62 ± 0.53 b | 1.33 ± 0.12 b | 11.2 ± 0.12 a | 29.8 ± 0.86 a | 7.2 ± 0.36 a |
| E97 | 6.67 ± 0.02 b | 178.1 ± 1.29 a | 18.61 ± 0.42 a | 1.53 ± 0.08 a | 10.6 ± 0.31 b | 30.0 ± 1.33 a | 7.1 ± 0.33 a |
| FG | 6.90 ± 0.04 a | 164.4 ± 1.15 b | 12.22 ± 0.21 c | 1.18 ± 0.05 c | 8.8 ± 0.26 c | 27.0 ± 0.68 b | 6.0 ± 0.29 b |
| 20–40 cm | | | | | | | |
| E83 | 7.42 ± 0.04 b | 44.5 ± 0.81 b | 9.85 ± 0.22 b | 0.76 ± 0.14 a | 8.4 ± 0.16 b | 24.2 ± 0.59 a | 7.5 ± 0.33 b |
| E97 | 7.56 ± 0.04 b | 58.7 ± 0.90 a | 9.39 ± 0.31 b | 0.71 ± 0.09 a | 8.4 ± 0.11 b | 23.7 ± 1.17 a | 7.6 ± 0.37 b |
| FG | 8.11 ± 0.05 a | 58.6 ± 1.45 a | 11.89 ± 0.17 a | 0.64 ± 0.21 b | 9.2 ± 0.25 a | 23.2 ± 1.03 a | 10.8 ± 0.53 a |
| 40–60 cm | | | | | | | |
| E83 | 7.85 ± 0.05 b | 32.6 ± 0.70 c | 5.79 ± 0.19 c | 0.44 ± 0.07 a | 7.6 ± 0.29 b | 18.1 ± 0.92 a | 7.6 ± 0.37 b |
| E97 | 8.20 ± 0.08 a | 41.8 ± 0.99 b | 6.11 ± 0.27 b | 0.47 ± 0.11 a | 7.8 ± 0.34 b | 19.1 ± 0.82 a | 7.5 ± 0.29 b |
| FG | 8.38 ± 0.06 a | 61.4 ± 1.41 a | 7.43 ± 0.31 a | 0.49 ± 0.15 a | 8.0 ± 0.17 a | 19.1 ± 0.9 a | 8.8 ± 0.43 a |

Soil basic physical-chemical properties under different management practices. AK, available potassium; SOM, soil organic carbon; TN, total nitrogen; CEC, cation exchange capacity; WHC, water holding capacity; C/N ratio, carbon to nitrogen ratio. The statistics analysis was conducted in the same soil layer and different lowercase letters in the same column indicated the difference reached significant level of $p < 0.05$. Data are presented as means ± SE, $n = 3$

## Results

### Soil N-supplying capacity and organic N fraction

The physicochemical properties of the soil samples are shown in Table 1. N was extracted from the soil samples using either the acidic $KMnO_4$ method or the KCl water bath method. For both extraction methods and for each of the three managed plots (E83, E97, and FG), the N concentrations were decreased with increasing soil depth after the last 30 °C incubations (Fig. 1). In the 0–20 cm layer, the $NH_4^+$-N concentrations in E97 soil were significantly higher than those in E83 and FG soils ($P < 0.05$), whereas there was little difference in $NO_3^-$-N concentrations among the three plots ($P < 0.05$). The N-supplying capacity of the soil at 0–20 cm ranged from

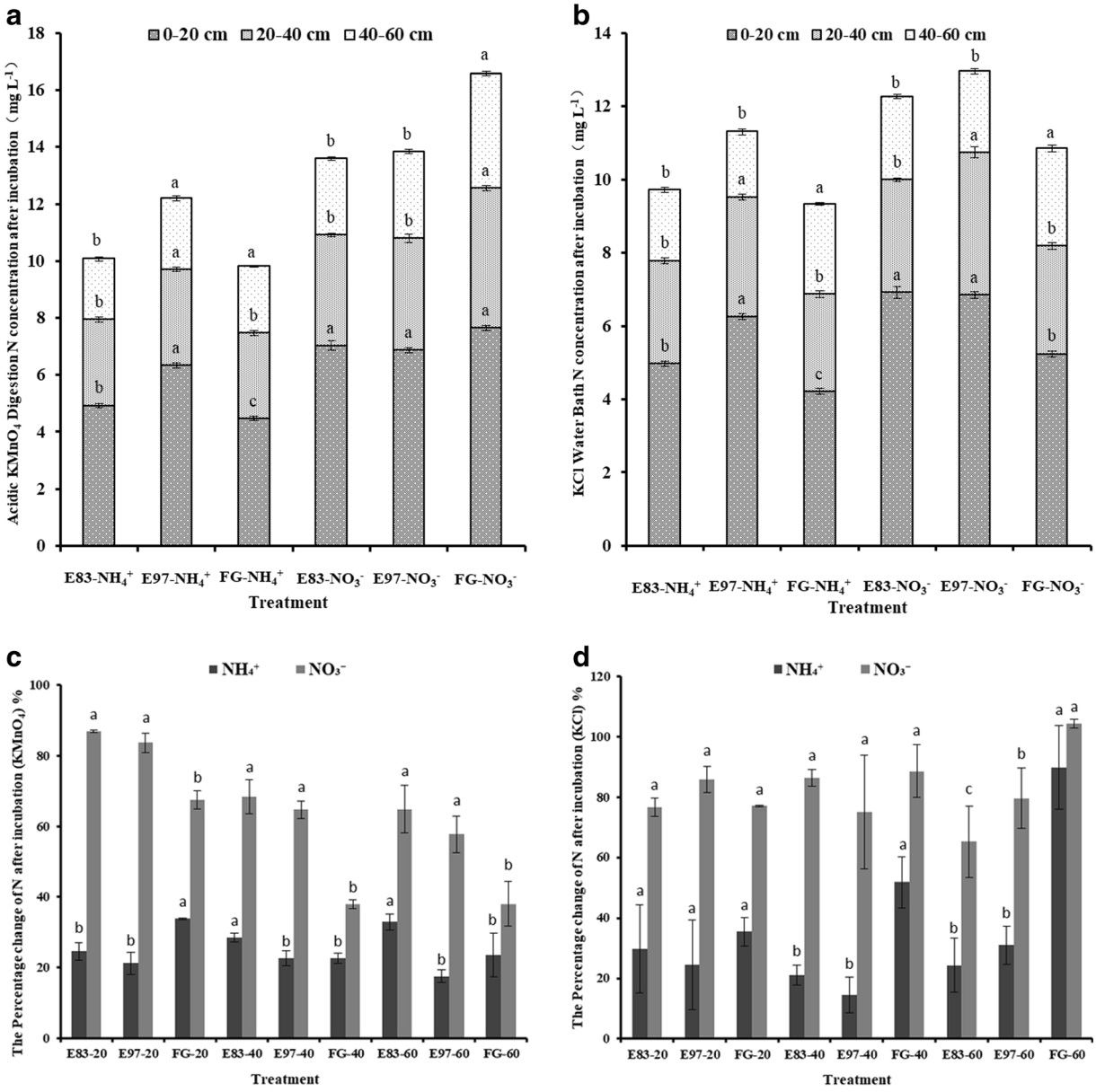


**Fig. 1** NH4 $^+$-N and NO3$^-$-N concentrations after incubation by KMnO4 digestion method **a** or KCl water bath method **b** and the percentage change of N after incubation **c d** in the soil profile under different management practices

a high of 46.63 mg $L^{-1}$ in E97 to a low of 36.33 mg $L^{-1}$ in FG, with the level in E97 being significantly higher than that in E83 and FG (Table 2). After incubation, the concentrations of $NH_4^+$-N and $NO_3^-$-N were increased by 17.5–33.8% and 37.9–87.0%, respectively, with the $NO_3^-$-N increment being the highest in the 0–20 cm soil layers and reaching 67.5–87.0% by the acidic $KMnO_4$ method (Fig. 1c). At the same soil depth, the percentage increase of $NO_3^-$-N was in the order of E83 > E96 > FG (Fig. 1c). In the 20–40 cm soil layers, the N-supplying capacity of the three plots switched to FG > E97 ≥ E83 (Table 2). This observation was the same for the 40–60 cm layers. In addition, the $NO_3^-$-N concentrations in the 20–40 and 40–60 cm soil layers of FG were significantly higher than those of E97 and E83, but there was little difference between E83 and E97(Fig. 1a, Fig. 1b, $P < 0.05$). Both in 0–20 cm and 20–40 cm, the $NH_4^+$-N concentrations switched to E97 > E83 ≥ FG, whether by acidic KMnO4 (Fig. 1a) or KCl water bath extraction (Fig. 1b).

Soil organic nitrogen is the main source of available nitrogen, so the amount of soil organic nitrogen and its biological stability determine the capacity of soil nitrogen supply. The concentrations of the different forms of N at each soil layer in the three different plots were clearly in the order of amino acid N > acid-insoluble N > ammonia N > acid-soluble unidentified N > amino sugar N (Fig. 2a), accounting for 31.8–39.4%, 23.1–35.6%, 12.8–25.0%, 5.6–15.1%, and 3.7–5.7% of the TN, respectively (Fig. 2b). There was no difference in the percentage of ammonia sugar N to TN among the different plots at the same soil depth, with the percentage decreasing slightly only in the 40–60-cm layer relative to the other two layers in the same plot. The percentages of acid-insoluble N to TN and acid-soluble unidentified N to TN increased with increasing soil depth in the same plot, whereas the percentage of ammonia N to TN decreased (Fig. 2b). At the same soil depth (e.g., 0–20 cm), the percentages of amino acid N to TN varied from 39.41 to 35.36%, following the order E83 ≥ E97 ≥ FG, and the trend was similar for ammonia N. On the other hand, the acid-soluble unidentified N varied from 5.63% to 10.75%, following the order E83 < E97 < FG, whereas the change of acid-insoluble N followed the order E83 ≈ E97 < FG. Compared with the E83 treatment, FG significantly changed the percentages of the different forms of organic N to TN, except for amino sugar N in the 0–20-cm soil layer (Fig. 2).

## Soil microbial biomass and bacterial community diversity

The MBC and MBN amounts in the 0–20-cm soil layer were in the order of E83 ≥ E97 > FG (Table 3). The results generally showed that the surface soil microbial biomass had been significantly decreased by the chronic grazing, whereas it was increased by the enclosure treatment. On the other hand, in the 40–60-cm soil depth, the microbial biomass was in the order of FG > E83 ≥ E97.

The bacterial 16S rRNA gene abundance ranged from 4.82E+10 to 5.35E+10 copies $g^{-1}$ dry soil (Fig. 3) at all soil layers in E97, being significantly higher than that in E83 and FG, whereas it was the lowest in FG at all soil layers ($P < 0.05$). The bacterial 16S rRNA gene abundance at the 40–60 cm soil layer was significantly lower than that at the other two soil depths for all three plots ($P < 0.05$). For example, in FG, the gene abundance was only 5.32E+8 copies $g^{-1}$ dry soil, which was less than 25% of that at 0–20 cm and 55.4% of that at 20–40 cm.

The results showed that the band number and the density of each band in the E83 soil were similar to those in the E97 soil (Fig. 4a). For both enclosure treatments (E83 and E97), the Shannon diversity index for the bacterial 16S rRNA gene was 1.17 for the 0–20 cm

**Table 2** N-supplying capacity of the soil

| Treatments | TN<br>g $kg^{-1}$ | Mineralizable N<br>mg $kg^{-1}$ | N-supplying capacity<br>mg $kg^{-1}$ |
|---|---|---|---|
| 0–20 cm | | | |
| E83 | 1.23 ± 0.03 a | 23.13 ± 0.68 b | 35.85 ± 0.69 b |
| E97 | 1.21 ± 0.03 a | 26.91 ± 0.84 a | 39.63 ± 0.56 a |
| FG | 0.79 ± 0.02 b | 23.7 ± 1.05 b | 36.33 ± 0.49 b |
| 20–40 cm | | | |
| E83 | 0.66 ± 0.02 a | 13.98 ± 0.58 a | 20.73 ± 0.42 b |
| E97 | 0.61 ± 0.02 b | 15.36 ± 0.47 a | 21.84 ± 0.85 b |
| FG | 0.52 ± 0.01 c | 18 ± 0.95 b | 23.7 ± 0.56 a |
| 40–60 cm | | | |
| E83 | 0.36 ± 0.01 a | 9.69 ± 0.32 a | 14.43 ± 0.37 a |
| E97 | 0.46 ± 0.01 b | 12.18 ± 0.47 b | 16.65 ± 0.46 b |
| FG | 0.38 ± 0.01 a | 14.49 ± 0.95 c | 19.17 ± 0.36 c |

N-supplying capacity in the soil profile by the acidic potassium permanganate method. TN, total nitrogen. The statistics analysis was conducted in the same soil layer and different lowercase letters in the same column indicated the difference reached significant level of $p < 0.05$. Data are presented as means ± SE, $n = 3$

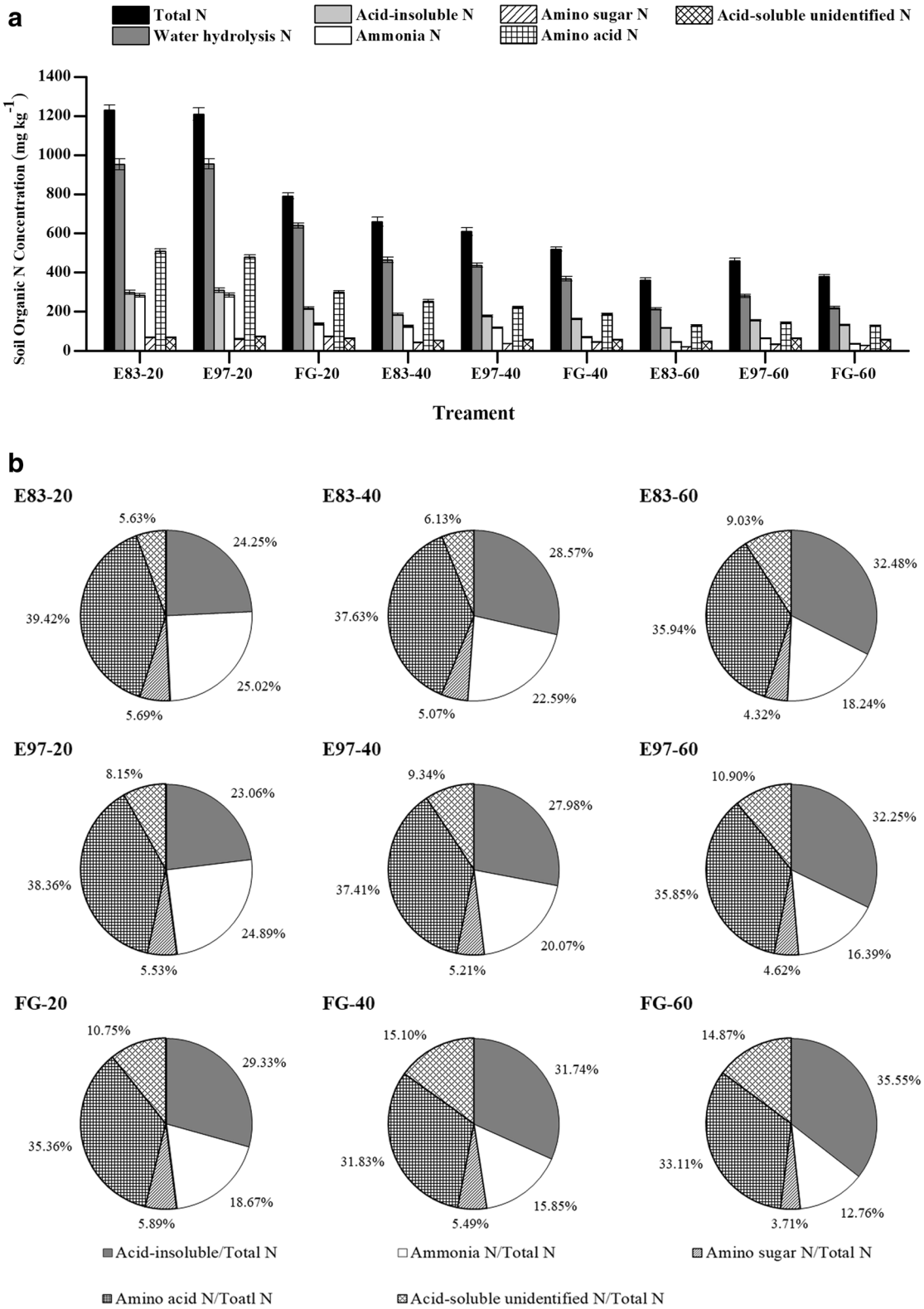


**Fig. 2** The concentrations of different forms of organic N **a** and their percentages of TN **b** in the soil profile under different management practices

**Table 3** MBC and MBN amounts in the 0–20-cm soil layer

| Treatments | Microbial biomass C mg $kg^{-1}$ | Microbial biomass N mg $kg^{-1}$ |
|---|---|---|
| 0–20 cm | | |
| E83 | 350.61 ± 6.05a | 29.39 ± 0.03a |
| E97 | 304.20 ± 3.51b | 28.85 ± 1.08a |
| FG | 264.33 ± 0.94c | 19.73 ± 0.01b |
| 20–40 cm | | |
| E83 | 225.89 ± 6.34b | 23.35 ± 0.12a |
| E97 | 249.76 ± 1.97a | 23.08 ± 0.41a |
| FG | 190.80 ± 0.83c | 22.45 ± 0.22a |
| 40–60 cm | | |
| E83 | 166.57 ± 7.30b | 20.03 ± 0.79c |
| E97 | 162.24 ± 3.33b | 18.03 ± 0.82b |
| FG | 186.68 ± 1.76a | 27.04 ± 0.75a |

Soil microbial biomass C and biomass N in the soil profile under different management practices Values followed by the same letter are not significantly different at $p \leq 0.05$ for the same depth. Data are presented as means ± SE, $n = 3$

layers, which was not significantly different from the value for the 20–40 cm layers (1.11) but significantly higher than the values for the 40–60 cm layers (0.84 for E83 and 0.80 for E97) (Fig. 4c, $P < 0.05$). The results highlighted that although the long-term exclusion of grazing through enclosure of the area had increased the microbial biomass in the deepest soil layer, it did not harbor higher bacterial diversity compared with the freely grazed site. Compared with the enclosure treatments, FG significantly decreased the bacterial diversity in the 0–20 and 20–40 cm soil layers ($P < 0.05$), for which the Shannon diversity indexes were only 0.82 and 0.80, respectively, which were close to the level for the 40–60 cm layers. Quantity One software was further used to analyze the similarity of the microbial communities (Fig. 4b), where interestingly, it was found that the soil microbial community structures were similar in the two enclosed sites, but different from that of the overgrazed plot.

## Soil bacterial community structure analyzed by high-throughput sequencing

High-throughput sequencing was also used to verify the effects of enclosure and grazing on the microbial community structure in the surface soil, where significant differences with the different management practices were indeed observed. The analysis revealed that the phyla *Crenarchaeota*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, and *Firmicutes* dominated in all soils except in the FG surface soil (FG-20, Fig. 5a). *Crenarchaeota*, a member of the *Archaea*, accounted for 31–35% of the sequences in the 16S rRNA gene libraries, but it was absent in FG-20. *Actinobacteria* was the most abundant bacterial phylum in this study site, and its relative abundance showed a slight increasing trend with increasing soil depth. The relative abundance of *Actinobacteria* in FG soil, which accounted for only 0.78% of the sequences in the 16S rRNA gene libraries, was clearly lower than that in the E83 and E97 soils at 0–20 cm. However, there were no obvious differences among the different soil layers or among the different plots at the same soil depth. However, *Bacteroidetes* and *Firmicutes* were clearly more abundant in the 0–20-cm layer than in the deeper layers. The relative abundance of these two phyla in the 40–60-cm layer was less than half of that in the 20–40-cm layer in the FG soils, whereas there were no differences in

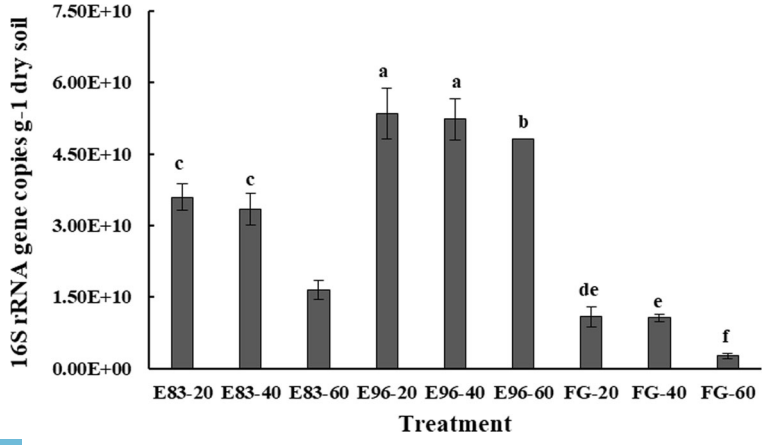


**Fig. 3** Soil bacterial community abundance in the soil profile under different management practices

their abundance between these two layers in the E97 and E83 soils. Compared with the enclosure treatments, FG had clearly increased the relative abundance of *Bacteroidetes* and *Firmicutes* in the soil profile, especially in the 20–40-cm soil layer. At this same soil depth, the relative abundance of *Firmicutes* in E97 was slightly higher than that in E83. In contrast, in the 0–20-cm layer, the relative abundance of *Firmicutes* in E97 was lower than that in E83. As for the other identified bacterial phyla, slight differences were observed among the different soil depths of the same plots and among the same soil depths under different management practices. It was interesting that the composition of bacterial phyla in the 0–20-cm soil layer with FG management was quite different from that in the other soils, and *Proteobacteria* was the key phylum, accounting for more than 50% of the sequences in the 16S rRNA gene libraries.

Given the large difference in *Proteobacteria* abundance in the FG-20 soil (Fig. 5a), we focused on analyzing the *Gammaproteobacteria* by heat map (Fig. 5b), at the genus level. The colors in the heat map from red to blue indicate the gradual reduction in the OTU number. In the FG-20 soil, the relative abundance of the genus *Pseudomonas* reached 77% of all the genera in *Gammaproteobacteria*, whereas that of the genus *Acinetobacter* reached 40%, indicating that these two were the dominant genera. The relative abundance of *Stenotrophomonas* was 22% in the FG-20 soil, whereas it was less than 1% in the other soils. According to the cluster analysis, the composition of *Gammaproteobacteria* species in the FG-20 and FG-60 soils was quite different from that of the others (Fig. 5b).

## Relationships among soil microbial community and soil property and soil organic N forms

The typical CCA was conducted to correlate the soil properties (i.e., cation exchange, pH, TN, soil organic matter, and available potassium) with the soil microbial community structure (Fig. 6a). The results showed that the

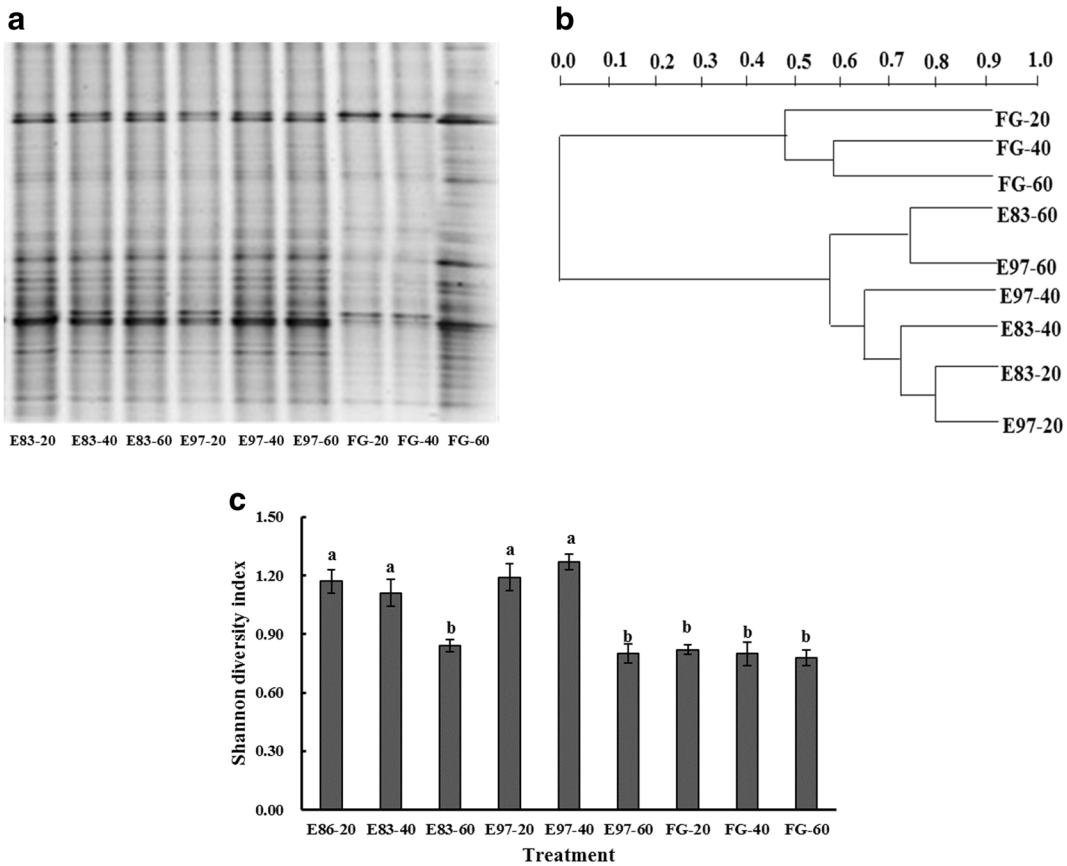


**Fig. 4** DGGE fingerprint **a**, the cluster analysis **b** and Shannon index **c** of bacterial diversity of the soils at different depths under different management practices

microbial communities in different soil depths varied greatly, and the influence of grazing types on the microbial communities was much less than that of the soil depth because the same depth of dots are all clustered together in the graph. However, the microbial community structures under enclosure treatment (E97 and E83) were more similar, and different from those in the overgrazed plots at the same depth (Fig. 6a). The angle between the dots in the 0–20 cm soil layer and the two lines of N and K is very small, and the smaller the Angle is, the greater the correlation will be. It was found that the TN and available potassium was the main reason for the difference in soil microbial community between the 0–20-cm layers.

The relationship between the main category of soil microbes and the soil organic N forms was determined using RDA. The length of each arrow indicated the contribution of the corresponding parameter to the structural variation. It was found that *Firmicutes* and *Bacteroidetes* were closely related to the amino sugar N, whereas *Acidobacteria*, *Crenarchaeota*, *Verrucomicrobia*, *Gemmatimonadetes*, and *Actinobacteria* were closely related to the other organic N forms (Fig. 6b). And the Mantel result indicated that the vital properties that markedly affected topsoil bacterial genera were amino sugar N too (Fig. 6c).

## Discussion

There are many methods available to determine the soil N-supplying capacity. It was reported that the acidic $KMnO_4$ method was the optimal one for calcareous soil (Smith and Li 1993). In this study, not much difference was observed between the N extracted by the acidic $KMnO_4$ method and that by the KCl water bath method, where the N-supplying capacity of the soil at 0–20 cm was in the order of E97 > E83 > FG, suggesting that the management practice of enclosure had increased the N-supplying capacity in the surface soil only, and that excessive enclosure was not better for this soil property in semiarid grassland ecosystems. Hu et al. (2016) synthesized 51 grazing enclosure sites in a grassland area in China and found that both the soil C content and vegetation biomass had reached a steady state after 15 years of enclosure. In our present study, long-term FG management enhanced the soil N-supplying capacity in the 20–60 cm soil layer, which was consistent with the results reported by Qiao (2013). FG management mainly increased the $NO_3^-$-N-supplying capacity in the deep soil layer, as $NH_4^+$-N was relatively more easily oxidized to $NO_3^-$-N by the soil ammonia oxidizers under the natural semiarid conditions (Liu et al. 2011). Moreover, the stocking rate at the FG plot in this study far exceeded very heavy grazing, which resulted in the dramatic reduction of plant productivity, and less plants in the overgrazed plot also led to more $NO_3^-$-N being leached into the deep soil. Moderate mowing and grazing were reported to be more beneficial than no mowing and no grazing for the soil nutrient cycle (Li et al. 2016; Liu et al. 2016), which was possibly because moderate grassland management could efficiently stimulate functional microbial activity in the soil.

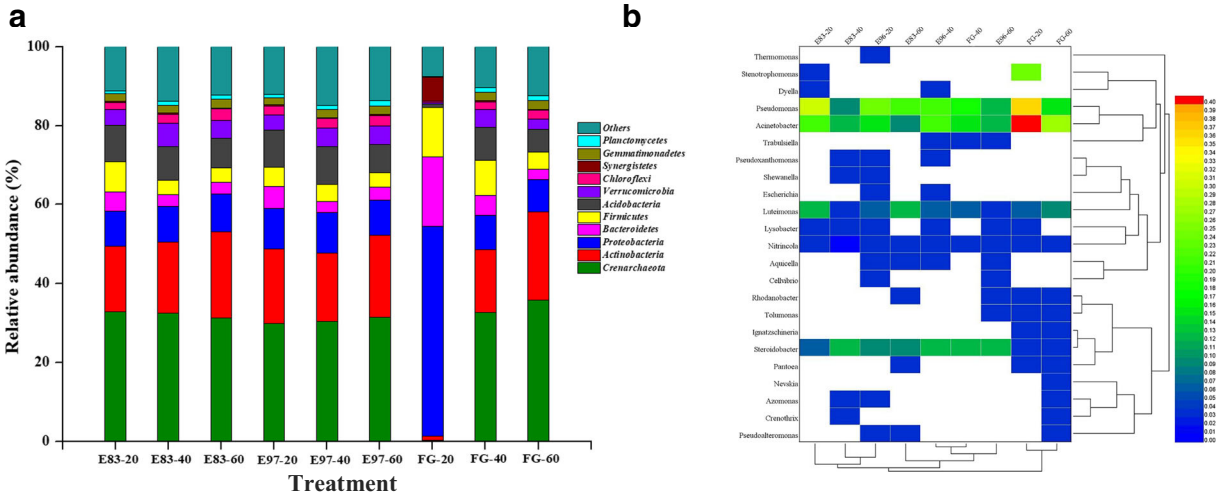


**Fig. 5** Relative abundance of different bacterial phyla **a** and the heat map of *Gammaproteobacteria* **b** in the grassland soils at different depths under different grazing enclosure management practices

Organic N compounds account for well over 90% of the TN in most soils (Parson and Tinsley 1975), and a considerable amount of organic N in soil is continually mineralized into $NH_4^+$ forms through microbial decomposition of the native organic matter, becoming available to plants. Better knowledge about the nature of soil N will allow us to better understand the soil N cycle and to use N in soils more efficiently. Our results showed that FG increased the N-supplying capacity in the deep soil layers, whereas it decreased the percentages of labile N in the corresponding soil layers, which suggested that the higher N-supplying capacity in the deeper FG soils could be related to the increased acid-insoluble N and unidentified N. In fact, it has been often deduced that some recalcitrant organic N occluded in the minerals or absorbed in the organic colloids could be mineralized and released by microbial metabolism to supply N for plant growth in special environmental conditions (Jiao et al. 2010). In particular, the soil organic matter contents in the deeper soil layers (> 20 cm) of the FG plot were

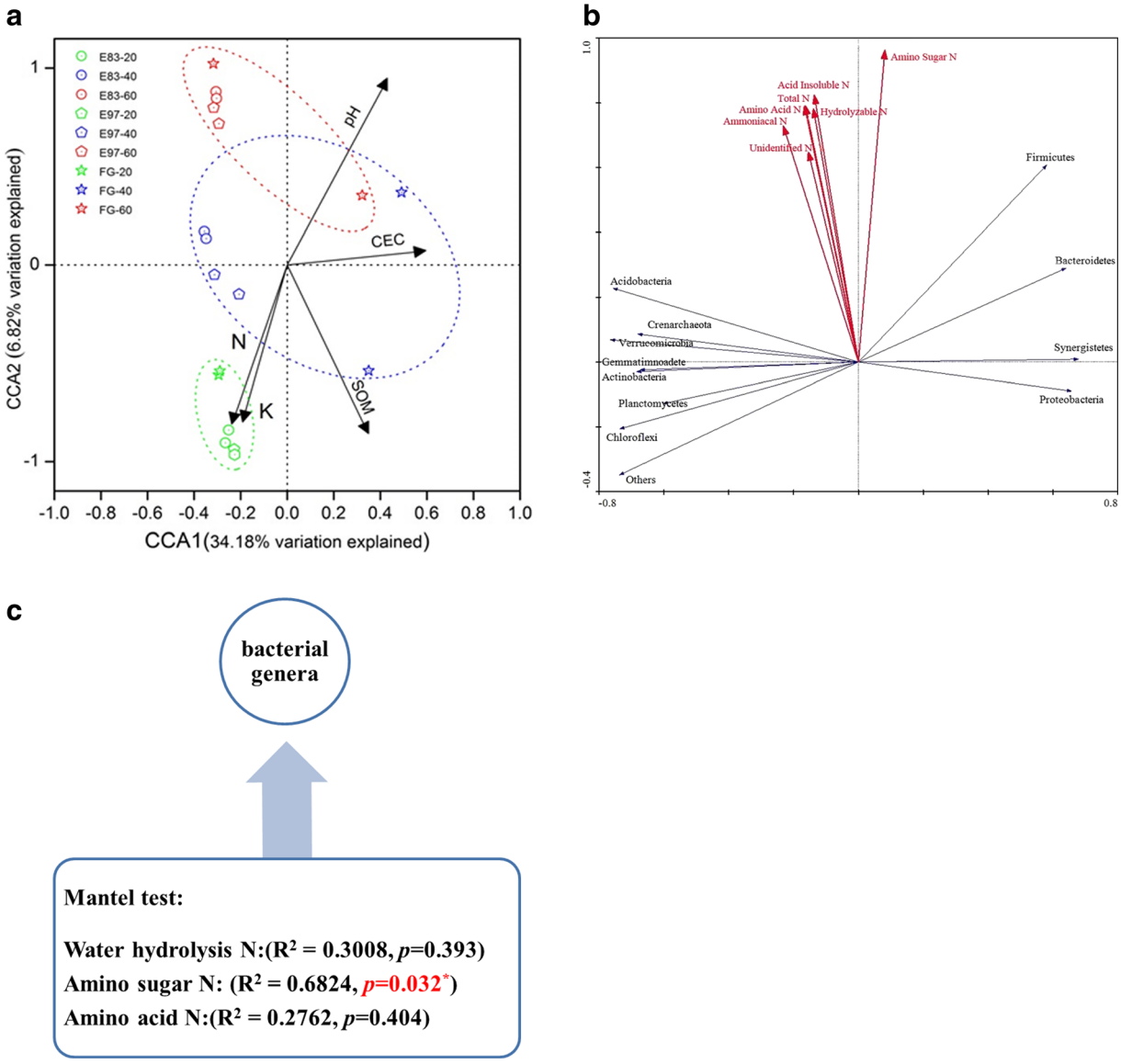


**Fig. 6** The relationship between soil properties and microorganisms under different management practices based CCA analysis **a** and the relationship between topsoil organic nitrogen and topsoil bacterial genera by RDA **b**. Mantel test showed the correlations relating soil organic N forms to the main category of soil microbes **c**. * indicated the significant difference at $p < 0.05$

significantly higher than those in the enclosed plots. The soil C/N ratio showed the same results, indicating that fungal degraders might contribute more to the degradation of recalcitrant organic N than bacterial degraders do (Strickland and Rousk 2010; Li et al. 2016). Li and Zhang (2002) also found that grazing decreased the acid-insoluble N and unidentified N in dark chestnut soil. However, the N-supplying capacity of the surface soil in E97 was significantly higher than that in FG, probably owing to the higher soil bacterial diversity and microbial biomass in the enclosed areas (Figs. 3, 4).

According to the results of the soil microbial community composition, the Shannon diversity index reached a steady level at the same soil layer after 17 years of enclosure. In contrast, long-term FG management had destroyed the physical properties of the topsoil (Table 1) through the constant tramping by sheep and reduced soil aeration, as reported by Pan et al. (2018), which would impact the topsoil bacterial community and subsequently the subsoil properties. *Pseudomonas* and *Acinetobacter* were the dominant genera in the surface soil of the FG site, where the soil nutrients were poor. *Pseudomonas* is characterized by its degradation of protein, where some species with strong degradation ability could live in poor-nutrient conditions (Mina et al. 2003). *Acinetobacter* is a gram-negative bacterial genus belonging to the wider class of *Gammaproteobacteria*. The members are important soil organisms that contribute to the mineralization of substances, such as aromatic compounds (Niepceron et al. 2013). The increase of *Acinetobacter* in the FG plot might be related to changes in the soil environment caused by the overgrazing (Zhang 2010). The soil organic N composition was significantly related to the soil bacterial community structure (Fig. 6). The microbial community structures were more similar in the fenced-off sites, but different from that in the overgrazed plots (Martensson and Olsson 2012; Tan et al. 2015). These results suggest that different management practices significantly affect the soil microbial community structure and further impact the nutrient supply of the surface soil, as also reported by Zhao (2011). Therefore, we recommend the management practice of moderate grazing after some period of enclosure for improving the function of semiarid grassland ecosystems. Moreover, the enclosure period should not be too long but based on the degree of degradation instead.

## Conclusions

In this study, the vertical distribution of organic N pools and bacterial communities under different field management practices in a semiarid grassland ecosystem was studied. We found that the effects of the different management practices on the soil properties of the shallow layer were greater than those on the deep layer. Our work revealed that moderate fencing restoration could increase the soil N-supplying capacity and enhance the soil nutrients. Significant changes in the microbial community diversity and structure occurred under different management practices whether in the surface or deep soil layers; in particular, significant differences in the microbial communities were found between the different soil depths. The impact of the different depths on the microbial communities was much greater than the impact caused by the soil management practice. The microbial categories were closely related to the soil organic N forms, which were positively correlated with amino sugar N.

**Funding information** This work was supported by the National Key Basic Research Program of China (2015CB150502), the National Natural Science Foundation of China (41401266), the Science & Research Program of Zhejiang Province (2016C32084), and The National Key Research and Development Program of China (2016YFD0801103).

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

## References

An H, Li GQ (2015) Effects of grazing on carbon and nitrogen in plants and soils in a semiarid desert grassland, China. J Arid Land 7(3):341–349

Andrés P, Moore JC, Cotrufo F, Denef K, Haddix ML, Molowny-Horas R, Wall DH (2017) Grazing and edaphic properties mediate soil biotic response to altered precipitation patterns in a semiarid prairie. Soil Biol Biochem 113:263–274

Bao SD (2000) Soil and agricultural chemistry analysis. Nanjing, China

Bardgett RD, Streeter TC, Bol R (2003) Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. Ecology 84(5):1277–1287

Bonde TA, Schnurer J, Rosswall T (1988) Microbial biomass as a fraction of potentially mineralizable nitrogen in soils from long-term field experiments. Soil Biol Biochem 20:447–452

Bremner JM (1965) Total nitrogen. In: Black CA (ed) Methods of soil analysis, part 2. Soil Science Society American, Madison, pp 1149–1178

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chlorofrom fumigation and the release of soil nitrogen: a rapid extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci 108:4516–4522

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J6:1621–1624

Cheng J, Wu G, Zhao L (2011) Cumulative effects of 20-year exclusion of livestock grazing on above- and belowground biomass of typical steppe communities in arid areas of the Loess Plateau, China. Plant Soil Environ 57(1):40–44

Daniel JA, Potter K, Altom W, Aljoe H, Stevens R (2002) Long–term grazing density impacts on soil compaction. Transactions of the ASAE 45(6):1911

Deng L, Zhang Z, Shangguan Z (2013) Long-term fencing effects on plant diversity and soil properties in China. Soil Tillage Res 137:7–15

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200

Gianello C, Bremner JM (1986) Comparison of chemical methods of assessing potentially available organic nitrogen in soil. Commun Soil Sci Plant Anal 17(2):215–236

Graeme WN, Christa S (2006) Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle? Trends Microbiol 14(5):207–212

Hu ZM, Li S, Guo Q, Niu S, He N, Li L, Yu G (2016) A synthesis of the effect of grazing exclusion on carbon dynamics in grasslands in China. Glob Chang Biol 22:1385–1393

Jiang GM, Han XG, Wu JG (2006) Restoration and management of the Inner Mongolia grasslands require a sustainable strategy. Ambio 35:269–270

Jiao N, Herndl GJ, Hansell DA, Benner R, Kattner G, Wilhelm SW, Azam F (2010) Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nat Rev Microbiol 8(8):593–599

Jin FH (2007) A comparative study of soil N-supplying capacity measurement method. Northwest Agriculture and Forestry University

Jones DL, Kielland K (2012) Amino acid, peptide and protein mineralization dynamics in a taiga forest soil. Soil Biol Biochem 55:60–69

Kang L, Han X, Zhang Z, Sun OJ (2007) Grassland ecosystems in China: review of current knowledge and research advancement. Philosophical Transactions of the Royal Society B: Biological Sciences 362(1482):997-1008

Kemmitt SJ, Lanyon CV, Waite IS, Wen Q, Addiscott TM, Bird NR, Brookes PC (2008) Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass-a new perspective. Soil Biol Biochem 40(1):61–73

Li X, Zhang S (2002) Nitrogen forms and effects of grazing in dark chestnut soil of Inner Mongolia grassland. Acta Pratacul Sin 2:15–21

Li J, Zhang Q, Li Y, Liu J, Pan H, Guan X, Xu X, Xu J, Di H (2016) Impact of mowing management on nitrogen mineralization rate and fungal and bacterial communities in a semi-arid grassland ecosystem. J Soils Sediments:1–12

Liu JW, Zhang YJ, Li YJ, Deli W (2008) Overview of grassland and its development in China. World Grassland and Rangeland Congress 2008:3–10

Liu T, Nan Z, Hou F (2011) Grazing intensity effects on soil nitrogen mineralization in semi-arid grassland on the loess plateau of northern China. Nutr Cycl Agroecosyst 91(1):67–75

Liu J, Zhang Q, Li Y, Di H, Xu J, Li J, Guan X, Xu X, Pan H (2016) Effects of pasture management on soil fertility and microbial communities in the semi-arid grasslands of Inner Mongolia. J Soils Sediments 16(1):235–242

Martensson LM, Olsson PA (2012) Reductions in microbial biomass along disturbance gradients in a semi-natural grassland. Appl Soil Ecol 62(6):8–13

Mina D, Yoshiyuki O, Akinori O (2003) The dual functions of biphenyl-degrading ability of pseudomonas sp. KKS102: energy acquisition and substrate detoxification. Biosci Biotechnol Biochem 67(9):1970–1975

Murphy DV, Sparling GP, Fillery IRP (1998) Stratification of microbial biomass C and N and gross N mineralization with soil depthin two contrasting Western Australian agriculture soils. A us J Soil Res 36:45–55

Niepceron M, Martinlaurent F, Crampon M, Portetkoltalo F, Akpavinceslas M, Legras M et al (2013) Gammaproteobacteria as a potential bioindicator of a multiple contamination by polycyclic aromatic hydrocarbons (pahs) in agricultural soils. Environ Pollut 180(3):199–205

Oksanen J, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara, et al (2012) Package 'vegan'. Community Ecology Package, 2, 129

Osterholz WR, Rinot O, Liebman M, Castellano MJ (2017) Can mineralization of soil organic nitrogen meet maize nitrogen demand? Plant Soil 415(1–2):73–84

Pan H, Liu H, Liu Y, Zhang Q, Luo Y, Liu X, Liu Y, Xu J, Di H, Li Y (2018) Understanding the relationships between grazing intensity and the distribution of nitrifying communities in grassland soils. Sci Total Environ 634(1):1157–1164

Parson JW, Tinsley J (1975) Nitrogenous substances. In Gieseking, J. E. (ed.) Soil Components. Volume 1. Springer Verlag, New York. pp. 263–304

Qiao J (2013) Negative feedback regulation of Inner Mongolia typical grassland ecosystem nitrogen

Ren HY, Han GD, Ohm M, Schönbach P, Gierus M, Taube F. (2015) Do sheep grazing patterns affect ecosystem functioning in steppe grassland ecosystems in Inner Mongolia?. Agriculture, Ecosystems & Environment 213:1-10

Roberts TL, Norman RJ, Slayton NA, Wilson CE (2016). U.S. Patent No. 9,354,235. Washington, DC: U.S. Patent and Trademark Office

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602

Schönbach P, Wan H, Gierus M, Bai Y, Müller K, Lin L, Susenbeth A, Taube F (2011) Grassland responses to grazing:

effects of grazing intensity and management system in an innzer mongolian steppe ecosystem. Plant Soil 340(1–2): 103–115

Smith KA, Li S (1993) Estimation of potentially mineralisable nitrogen in soil by KCl extraction. Plant and Soil 157(2):167–174

Stanford G, Smith SJ (1976) Estimating potentially mineralizable soil nitrogen by acid permanganate extraction. Soil Sci 122: 71–76

Stevenson FJ (1982) Organic forms of soil nitrogen (no. nitrogeninagrics, pp. 67-122). American Society of Agronomy, Crop Science Society of America, Soil Science Society of America

Strickland MS, Rousk J (2010) Considering fungal: bacterial dominance in soils-methods, controls, and ecosystem implications. Soil Biol Biochem 42:1385–1395

Talbot JM, Treseder KK (2010) Controls over mycorrhizal uptake of organic nitrogen. Pedobiologia 53:169–179

Tan H, Yan R, Yan Y, Chen B, Xin X (2015) PLFA analysis of soil microbial community structure in warm meadow steppe under different grazing intensities. Acta Pratacultural Sci 3

Wang QK (2014) Fresh carbon and nitrogen inputs alter organic carbon mineralization and microbial community in forest deep soil layers. Soil Biol Biochem 72:145–151

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267

Weintraub MN, Schimel JP (2003) Interactions between carbon and nitrogen mineralization and soil organic matter chemistry in arctic tundra soils. Ecosystems 6(2):0129–0143

Wu TH (2011) Can ectomycorrhizal fungi circumvent the nitrogen mineralization for plant nutrition in temperate forest ecosystems? Soil Biol Biochem 43:1109–1117

Xu Z, Wan S, Ren H, Han X, Jiang Y (2012) Influences of land use history and short-term nitrogen addition on community structure in temperate grasslands. J Arid Environ 87:103–109

Ye L, Van Ranst E (2009) Production scenarios and the effect of soil degradation on long-term food security in China. Glob Environ Chang 19:464–481

Zaman M, Di HJ, Cameron KC, Cameron KC, Frampton CM (1999) Gross nitrogen mineralization and nitrification rates and their relationships to enzyme activities and the soil microbial biomass in soils treated with dairy shed effluent and ammonium fertilizer at different water potentials. Biol Fertil Soils 29:178–186

Zhang Yongsheng (2010) The influence of grazing on the physicochemical properties and bacterial diversity of the baikal impermeable soil. (Doctoral dissertation, Chinese academy of agricultural sciences)

Zhao Shuai (2011) Overgrazing and enclosed original of Hulun Buir steppe soil microbial diversity. Chinese Academy of Agricultural Sciences