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Effect of grazing on the abundance of functional genes associated with N cycling in three types of grassland in Inner Mongolia

K. Ding · L. Zhong · X. P. Xin · Z. H. Xu · X. M. Kang · W. J. Liu · Y. C. Rui · L. L. Jiang · L. Tang · Y. F. Wang

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Abstract
Purpose The aim of the study was to investigate the patterns of soil nitrogen (N)-cycling functional gene abundance along a precipitation gradient on the Mongolian Plateau, and the effects of grazing on the population size of microbial functional group under different precipitation regimes.
Materials and methods Soil samples were taken from grazing and non-grazing plots of meadow steppe, typical steppe, and desert steppe plots on the Mongolian Plateau for soil gravimetric moisture content, pH, and soil organic carbon (SOC), total N, and inorganic N (NH_4^+-N and NO_3^--N) concentrations, and the abundance of functional genes associated with N_2 fixation (*nifH* gene), nitrification (*AOA* and *AOB* genes), and denitrification (*narG*, *nirS*, *nirK*, and *nosZ* genes) was studied. The relationships between environmental variables, soil physicochemical properties, and functional microbial abundance were examined.
Results and discussion Soil properties (soil moisture, pH, soil organic carbon, total nitrogen, NH_4^+-N, and NO_3^--N content) and abundance of N-cycling groups all varied with precipitation. Compared with desert steppe, precipitation significantly decreased the abundance of *nifH* gene by 1 order of magnitude, but markedly increased the abundance of *AOA* and *AOB* genes by 1.32 to 4.72 times and denitrifying genes *narG*, *nirS*, *nirK*, and *nosZ* by 0.66 to 9.02 times in meadow steppe. Grazing significantly decreased the abundance of functional groups in desert steppe and typical steppe ($p<0.001$), while there was no difference between grazing and non-grazing treatments in meadow steppe which had the highest precipitation level. Soil pH was the main factor affecting the abundance of *nifH* gene according to simple linear regression ($R^2=0.934$, $p<0.001$), while moisture was positively related with population sizes of nitrifier and denitrifier groups, explaining 53.8–92.34 % of the variation in the abundance of *AOA*, *narG*, *nirS*, and *nosZ* genes in all three steppes.
Conclusions Soil pH was the major factor that significantly affected the gene abundance of nitrogen fixation process, and soil moisture was the dominant factor controlling the gene abundance of nitrification and denitrification process along the precipitation gradient. Grazing had no effect on the gene abundance of N-cycling process in meadow steppe but decreased it in desert and typical steppe. Our results suggest that grazing may not necessarily be associated with a reduction in microbial functional potentials when soil moisture was relatively good but will decrease the soil microbial functional

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K. Ding · W. J. Liu · L. Tang · Y. F. Wang (✉)
College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China
e-mail: yfwang@ucas.ac.cn

L. Zhong
China-Australia Centre for Sustainable Urban Development, School of Environmental Science and Engineering, Tianjin University, Tianjin 300072, China

X. P. Xin
Chinese Academy of Agricultural Sciences, Institute of Agricultural Resources and Regional Planning, Beijing 100081, China

Z. H. Xu
Environmental Futures Centre, Griffith University, Brisbane QLD 4111, Australia

X. M. Kang
Institute of Wetland Research, Chinese Academy of Forestry, Beijing 100091, China

Y. C. Rui
School of Earth and Environment, The University of Western Australia, Crawley WA 6009, Australia

L. L. Jiang
Laboratory of Alpine Ecology and Biodiversity, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101, China

potentials in a more arid environment in northern China grasslands.

Keywords Grazing · Nitrogen cycling · pH · Precipitation gradient · qPCR · Soil moisture

1 Introduction

Nitrogen (N) cycling is one of the most important biogeochemical processes in terrestrial ecosystems. The transfer of nitrogen into, within, and out of soil requires the interplay of microorganisms performing N_2 fixation, nitrification, and denitrification (De Boer and Kowalchuk 2001; Vitousek et al. 2002). For example, N fixer converts atmospheric N_2 into NH_4^+, nitrifier oxidizes it to NO_2^- and then NO_3^-, and denitrifier reduces NO_3^- to NO_2^-, NO, N_2O, and N_2. Therefore, changes in microbial composition or the abundance of special functional groups can alter N availability to plants or N loss from the ecosystem (Lindsay et al. 2010). A number of studies have demonstrated that the abundance of soil microorganisms is regulated by a wide range of biotic and abiotic factors such as soil moisture, temperature, and litter inputs (Fierer and Jackson 2006; Walker et al. 2008; Sheik et al. 2011; Yergeau et al. 2012); however, most studies focused on one process of the N cycle, i.e., either on nitrification or on denitrification (Chroňáková et al. 2009; Di et al. 2010; Philippot et al. 2009), Therefore, they were unable to completely describe the whole N-cycling process.

Precipitation, directly affecting the moisture availability in the soil, is a key factor regulating the spatial and temporal patterns of microbial communities in arid and semiarid terrestrial ecosystems (Reynolds and Stafford Smith 2002; Sorensen et al. 2013). Water availability can directly affect the abundance of soil microorganisms and indirectly affect it through regulating the substrate availability, diffusion of oxygen, and soil pH (Griffiths et al. 2003). N_2 fixation typically occurs at water contents between 20 and 60 % (Belnap et al. 2004); nitrification prefers relatively dry soil condition (Maag and Vinther 1996; Luo et al. 2008), but denitrification usually increases in wetter soils as the soils become more anaerobic (Dobbie and Smith 2001; Bateman and Baggs 2005; Diba et al. 2011). All these researches focused on the effect of soil water on N cycling through observed soil conditions and their effects on carbon (C) and N cycling. Recently, there were some studies that have been considerate on the role that microorganisms play in N cycling especially along a precipitation gradient (Adair and Schwartz 2008; Forbes et al. 2009; Mao et al. 2013). Many researches showed that population size of some functional groups are (e.g., *narG* and *AOB*) significantly related to variations in rainfall amount (Adair and Schwartz 2008; Forbes et al. 2009), while others showed that the N-cycling groups were independent of precipitation gradient (Mao et al. 2013). We still lack a clear understanding on soil N-cycling microbial functional potentials along a precipitation gradient in Inner Mongolia grassland.

Grazing, as a common land-use type in grassland ecosystems, can profoundly impact N cycling through reducing vegetation cover and altering soil water and energy balance (Leriche et al. 2001), increasing soil compaction or reducing soil aeration by tramping (Oenema et al. 2007; Houlbrooke et al. 2008), or changing the quantity and quality of soil organic matter and mineral N content by the deposition of dung and urine (Saggar et al. 2004). Recently, there were large of studies that have been conducted in grasslands on N cycling and its microbial process under different managements (Patra et al. 2006; Le Roux et al. 2008; Xu et al. 2008). These studies showed an interesting result that for most of managed temperate grassland under humid climate, animal grazing would increase N-cycling rates because of the deposition of dung and urine (Le Roux et al. 2008; Chroňáková et al. 2009; Di et al. 2010; Keil et al. 2011), while for most of arid and semiarid grasslands, animal grazing generally decreased N-cycling rates by the grazing-induced reduction in soil organic matter and soil moisture (Xu et al. 2007, 2008; Wolf et al. 2010). This difference of responses to grazing may be partially driven by an interaction between climate and grazing impacting nutrient cycling through changes in microbe population, which has not been explored so far.

In this study, we focus on the effects of animal grazing on the abundance of functional genes associated with N cycling along a low-precipitation gradient set composed of three natural arid and semiarid ecosystem sites across the Inner Mongolia region of China (Fig. 1). The Inner Mongolia grassland covers an area of about 8.67×10^7 hm^2 and is one of the most well-known rangelands in the eastern part of the Eurasian steppe (Coupland 1993), from east to west, presenting a natural precipitation gradient, which is extremely water-limited (Bai et al. 2008). Chen et al. (2014) indicated that the important impact of precipitation on microbial community can be based on phospholipid fatty acid (PLFA), but this technology was limited to detect the biomass of living microorganism; the population size of microbial functional groups has not been explored so far. Grazing-induced grassland degradation has profoundly affected the grassland ecosystems and led to significant depletion in soil organic matter and biomass production (Li et al. 1997, 2008). Previous study only focused on the impact of animal grazing on ecosystem functioning and stoichiometry (Bai et al. 2012). For microbial functional groups, researchers reported the effect of grazing on the abundance of functional microbial genes in one precipitation regime (Zhong et al. 2014), but no information is available along precipitation gradient. Therefore, we address the following research questions: First, how does the abundance of functional genes associated with N-cycling change along a precipitation gradient across the arid and semiarid steppe? Second, how

does grazing affect the population of microbial functional group? And third, does the effect of grazing on the abundance of functional genes vary with different precipitation?

2 Materials and methods

2.1 Site description

This study was conducted at sites for long-term ecological research in Inner Mongolia grassland that represent three different types of grassland: desert steppe, typical steppe, and meadow steppe. We took advantage of a region-scale transect across a low-precipitation gradient on Mongolian Plateau. This transect is approximately 1,200 km long and at 30° longitude.

The desert steppe was located in Siziwang Banner near the Ulanqab Grassland Ecosystem Research Station (41°47′N, 111°53′E, 1,456 m above sea level (a.s.l.)). The climate of this experimental area was semiarid, with annual mean (1982–2008) precipitation of 280 mm and annual mean temperature of 3.5 °C (Lin et al. 2010). The soil type is Kastanozem (FAO soil classification system) with a loamy-sand texture. The dominant species in this desert-steppe grassland are *Stipa breviflora*, and other prevalent species include *Agropyron desertorum*, *Cleistogenes songorica*, *Artemisia frigid*, and *Salsola collina*. Previously, this experimental region had been grazed at a relatively light stocking density before the increasing number of sheep leads to grassland degradation about two decades ago. The enclosure was established in 2004 to investigate the effect of grazing on organisms, including aboveground plants and belowground microorganisms and where grazing density was moderately grazed (1.82 sheep ha^{-1}).

The typical steppe was located in Xilin River Catchment near the Inner Mongolia Grassland Ecosystem Research Station (43°38′N, 116°42′E, 1,200 m a.s.l.). This area is continental and has semiarid climate, with annual mean (1982–2008) precipitation of 335 mm and annual mean temperature of 0.7 °C (Schönbach et al. 2010). The major soil types of this area are calcic chestnuts and calcic chernozems, with fine-sand texture. The dominant species in this typical-steppe grassland are *Stipa grandis*, and other prevalent species include *Leymus chinensis*, *Stipa krylovii*, *Cleistogenes squarrosa*, *Agropyron cristatum*, *A. frigid*, and *Caragana microphylla*. The enclosure was established in 2005 in order to investigate the effect of grazing on organisms, including aboveground plants and belowground microorganisms and where grazing density was moderate (4.5 sheep ha^{-1}).

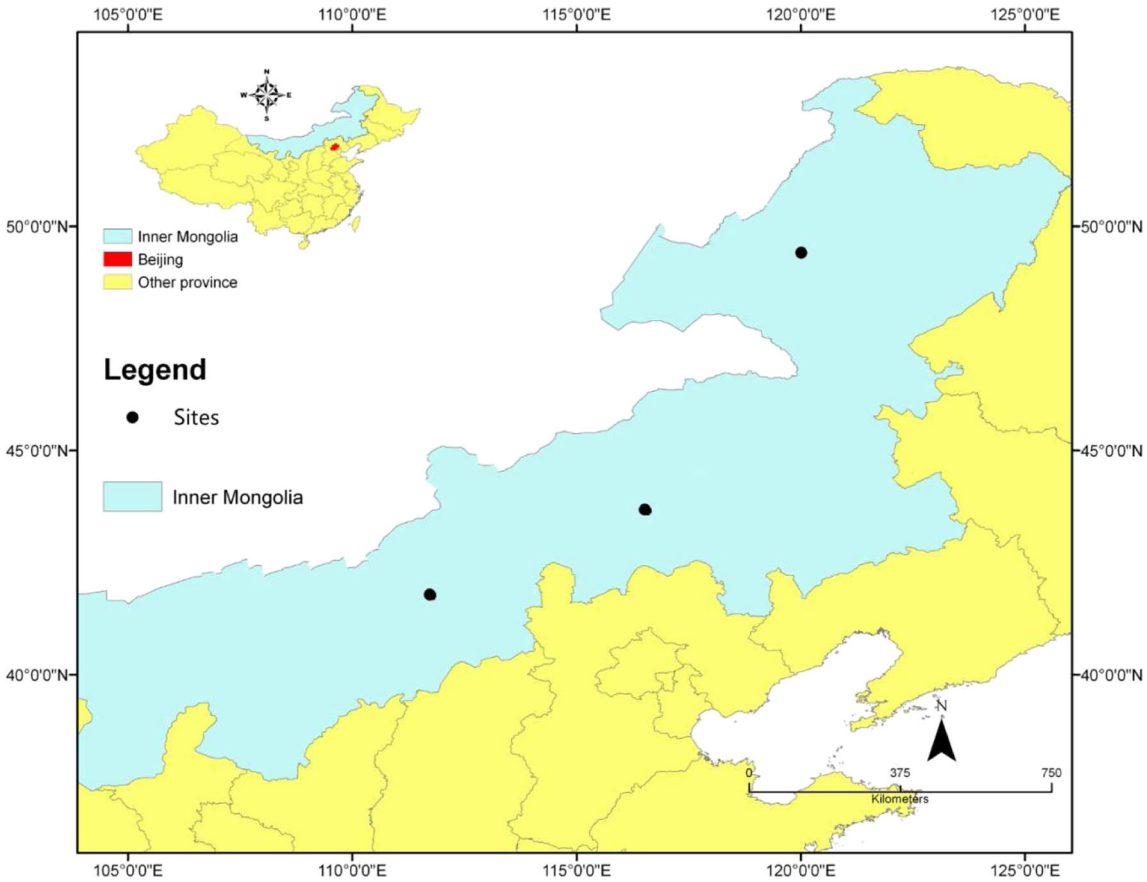

Fig. 1 Location map of the three paired study sites along the Inner Mongolia grassland

The meadow steppe was located in Xieertala Ranch near the Hulunber Grassland Ecosystem Research Station of the Chinese Academy of Agriculture Sciences (49°19′N, 119°55′ E, 628 m a.s.l.). This area is characterized by semiarid climate, with annual mean (1982–2010) precipitation of 400 mm and annual mean temperature of 0 °C (Chen et al. 2012). The soil type is dark chestnut (Chinese classification) or Calcicorthic Aridisol (US soil taxonomy classification system). The native meadow-steppe grassland is dominated by *Stipa baicalensis*, and other prevalent species include *L. chinensis* and *Filifolium sibiricum*. Previously, this region had been grazed at a very low stocking density before the density increased to "moderate to heavy" level four and five decades ago. The enclosure was established in 2006 in order to investigate the effect of grazing on organisms, including aboveground plants and belowground microorganisms and where grazing density was moderate (about 0.34 cattle ha^{-1}).

2.2 Climate condition, soil sampling, and plant community analysis

Total rainfall of 2011 was 317.4, 226.7, and 241.9 mm in meadow steppe, typical steppe, and desert steppe, respectively, with rainfall amount being lower than long-term mean annual precipitation of the three grassland types (400, 335, and 280 mm). Interestingly, the precipitation in July in meadow steppe accounted for 58.2 % of the total rainfall, while typical steppe and desert steppe represented 34.1 and 36.4 %, showing an extremely humid soil condition during the soil sampling time in meadow steppe. The monthly mean air temperature was similar in the three grassland types, which was highest from June to August and lowest in January.

We sampled soil from grazing enclosure experiment in meadow steppe, typical steppe, and desert steppe to investigate the effect of grazing on aboveground plants, soil properties, and belowground microorganisms in similar enclosing time to reduce the possibility of other factors biasing the results. Soil samples were taken in late-July 2011 when all experimental regions were in peak plant biomass in the growing season. There were several precipitation events (for a total rainfall of 21, 30.4, and 87.5 mm in a week in desert steppe, typical steppe, and meadow steppe, respectively) before soil sampling. At each site, this sampling was performed on triplicate grazed and non-grazed plots of 1×1 m in size. We sampled soil at a depth of 0–10 cm, using a 5-cm-diameter soil core sampler. At each plot, five randomly selected subsamples were taken after the removal of the vegetation litter with a rake. Five subsamples were pooled together and passed through a 2-mm sieve and then subsequently maintained at 4 °C during transport from the field to the laboratory for molecular and chemical analyses.

We analyzed aboveground plant community at the same time as soil sampling. Coverage was measured by visual estimation method, and plant aboveground standing biomass was determined in three 1 × 1-m quadrates at each site. All the plant materials were oven-dried at 65 °C for 48 h and weighed for each plant species. The Shannon-Wiener index estimating diversity of plant community was used to calculated H' with the following equation:

$$H' = -\sum_{i=1}^{S} p_i \mathrm{In} p_i$$

where S is species number and p_i is the proportion of individuals belonging to the ith species in the dataset of interest.

2.3 Soil analyses

Gravimetric moisture content was determined by oven-drying at 105 °C to a constant mass. Soil pH was measured using a pH meter (Oakton, California, USA). The concentrations of NH_4^+-N and NO_3^--N were determined by extraction with 2 M KCl (Abu-Qaoud et al. 1991) on an Alpkem Flow Solution III (OI Analytical, Oregon, USA). Total soil organic carbon content was analyzed using the potassium dichromate heating method, and total nitrogen content was analyzed using the semi-micro Kjeldahl method with a Vario EL III elemental analyzer (Elementar, Germany).

2.4 DNA extraction and quantification of functional genes by real-time PCR

DNA was extracted from the soil using the PowerSoil® DNA Isolation Kit (MO BIO, California, USA) according to the manufacturer's instructions. Functional genes encoding an enzyme involved in nitrogen fixation (*nifH*), archaeal and bacterial ammonia monooxygenase (*AOA*, *AOB*), nitrate reductase (*narG*), nitrite reductase (*nirK*, *nirS*), and nitrous oxide reductase (*nosZ*) were quantified in triplicate by quantitative real-time PCR using an ABI 7500 FAST system (Applied Biosystems, California, USA). For qPCR, 2 μl of template DNA, 12.5 μl of 2× SYBR Green qPCR Master Mix (Takara, Japan), 9.5 μl of ddH_2O, and 1 μl of primer (Table 1) were mixed into a total reaction volume of 25 μl. The reaction efficiencies of qPCRs were 86 % for *nif*H, 88 % for *AOA*, 89 % for *AOB*, 66 % for *narG*, 84 % for *nirK*, 74 % for *nirS*, and 79 % for *nosZ*, and the R^2 values were 0.99 for all runs. The abundances of all functional genes were finally calculated as copy number per gram of dry soil.

Table 1 The PCR primers used for quantitative real-time PCR

Target group	Annealing time and temperature	Elongation time and temperature	Primer	Primer sequence (5′→3′)	Product size (bp)	Reference
ifH	55 °C, 60 s	72 °C, 60 s	nifH-F	AAAGGYGGWATCGGYAARTCCACCAC	458	Rosch and Bothe (2005)
			nifH-R	TTGTTSGCSGCRTACATSGCCATCAT		
AOA	55 °C, 60 s	72 °C, 45 s	amoAF	STAATGGTCTGGCTTAGACG	635	Rotthauwe et al. (1997)
			amoAR	GCGGCCATCCATCTGTATGT		
AOB	57 °C, 45 s	72 °C, 45 s	A189	GGHGACTCCCAYTTCTGG	491	Okano et al. (2004)
			A-2R’	CCTCKGSAAAGCCTTCTTC		
narG	55 °C, 60s	72 °C, 45 s	narGG-F	TCGCCSATYCCGGCSATGTC	173	Bru et al. (2007)
			narGG-R	GAGTTGTACCAGTCRGCSGAYTCSG		
nirK	58 °C, 30 s	72 °C, 30 s	nirK1F	GGMATGGTKCCSTGGCA	515	Braker et al. (1998)
			nirK5R	GCCTCGATCAGRTTRTGG		
nirS	58 °C, 30 s	72 °C, 30s	cd3AF	GTSAACGTSAAGGARACSGG	425	Michotey et al. (2000)
			R3cd	GASTTCGGRTGSGTCTTGA		
nosZ	60 °C, 30 s	72 °C, 30 s	nosZ-F	CGYTGTTCMTCGACAGCCAG	454	Henry et al. (2006)
			nosZ-R	CGSACCTTSTTGCCSTYGCG		

2.5 Statistical analyses

First, we used one-way ANOVA to analyze the effect of grazing on soil properties and abundance of functional genes. Means were contrasted post hoc by Tukey’s studentized range (HSD) for comparing the samples from grazing and non-grazing treatments. Then, two-way ANOVA was performed testing the main and interactive effects of treatment (grazing or non-grazing) and precipitation (meadow steppe, typical steppe, and desert steppe) on soil properties and abundance of functional genes. Statistix, version 8.0 (Analytical Software, Tallahassee, USA) was used for all ANOVA analyses. Second, a model based on stepwise regression was made predicting the relationship between environmental variables (soil, plant, and precipitation data) and functional groups of nitrogen cycling. All these analyses were performed with SAS software, version 8.0 (SAS Institute, North Carolina, USA).

3 Results

3.1 Plant community analysis

Significant treatment, precipitation, and interactions of treatment and precipitation were observed for aboveground standing biomass and the Shannon-Wiener index. A significant effect of treatment was found for plant coverage in three grasslands (Fig. 2). The plant coverage was significantly decreased by 44.2 % under grazing treatment in meadow steppe (F=16.41, p=0.016), but there was no significant difference between grazing treatments in desert steppe and typical steppe (Fig. 2a). Grazing significantly decreased aboveground standing biomass by 43.6, 46.6, and 87.5 % (Fig. 2b), but it markedly increased the Shannon-Wiener index by 0.77, 1.55, and 0.87 times in desert steppe, typical steppe, and meadow steppe, respectively (Fig. 2c).

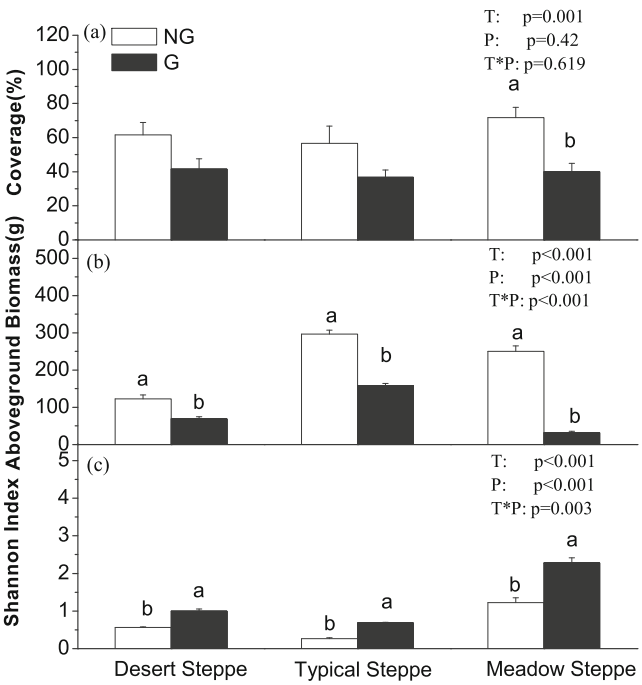

Fig. 2 Plant coverage (**a**), aboveground biomass (**b**), and Shannon index (**c**) in desert steppe, typical steppe, and meadow steppe along low-precipitation gradient. *NG* denotes non-grazing treatment and *G* denotes grazing treatment. Values (mean±SE) followed by *different letters* are significantly different within each set of sample data. *T* treatments, *P* precipitation, *T*P* treatment×precipitation

Table 2 Main characteristics of the soil in meadow steppe, typical steppe, and desert steppe along low-precipitation gradient

	Desert steppe		Typical steppe		Meadow steppe	
	NG	G	NG	G	NG	G
pH	8.04±0.07b	8.19±0.05a	7.67±0.06a	7.51±0.07b	6.63±0.04a	6.71±0.05a
Moisture content (%)	5.78±0.34a	3.6±0.4b	15.55±0.48a	9.96±0.22b	34.4±1.1a	31.3±2.62a
Soil organic carbon (%)	1.50±0.02a	1.25±0.11b	2.15±0.10a	1.82±0.10b	3.15±0.08a	2.57±0.10b
Total nitrogen (%)	0.11±0.01a	0.10±0.00a	0.17±0.00a	0.13±0.00b	0.27±0.03a	0.26±0.01a
NO_3^--N (mg/kg)	16.93±2.65b	36.0±6.4a	6.46±1.23a	8.06±0.73a	6.87±1.01b	10.33±0.63a
NH_4^+-N (mg/kg)	2.59±0.06a	1.48±0.04b	4.69±0.58b	5.82±0.22a	4.66±0.02a	5.01±0.53a

Values (mean±SE) followed by different letters are significantly different within each set of sample data

G grazing treatment, *NG* non-grazing treatment

3.2 Soil analysis

The moisture content ranged from 3.6 to 34.4 % (Table 2) in the three types of grassland and was significantly lower under grazing treatment in desert steppe (F=55.78, p=0.002) and typical steppe (F=325.87, p=0.001). Soil pH was significantly higher under grazing treatment in desert steppe (F=9.49, p=0.037), but was lower under grazing treatment in typical steppe (F=8.29, p=0.045). Total C content was significantly decreased by 16.7, 15.3, and 18.4 % under grazing treatment in desert steppe (F=14.99, p=0.018), typical steppe (F=16.91, p=0.015), and meadow steppe (F=67.27, p=0.001), while the same phenomenon was only observed in typical steppe (F=736.11, p<0.001) for total N, decreasing from 0.17 to 0.13 %. NO_3^--N concentration was higher under grazing than non-grazing treatment, from 16.93 to 36.0 mg/kg in desert steppe (F=23.0, p=0.009) and 6.87 to 10.33 mg/kg in meadow steppe (F=13.94, p=0.02). Compared with NO_3^--N content, grazing increased NH_4^+-N content by 24.1 % in typical steppe (F=9.97, p=0.034) while it decreased by 42.9 % in desert steppe (F=738.17, p<0.001). It was clearly showed that all measured soil properties demonstrated a significant relationship with precipitation (p< 0.001) and precipitation×treatment (p<0.001), while grazing treatment did not change the characteristics of soil in the three grassland types (Table 3).

Table 3 Results from two-way ANOVA testing the effects of treatment (T), precipitation (P), and T×P on soil properties

Factor	T[a]	P[b]	$T\times P$
Soil properties			
Moisture content	0.14 ns	281.01***	385.27***
pH	0.01 ns	386.75***	372.31***
Soil organic carbon	1.58 ns	58.02***	191.26***
Total nitrogen	0.36 ns	97.74***	64.58***
NO_3^--N	2.82 ns	15.28***	41.81***
NH_4^+-N	0.03 ns	52.44***	73.3***

ns not significant

*p<0.05; **p<0.01; ***p<0.001

[a] T: grazing or non-grazing treatment

[b] P: desert steppe, typical steppe, and meadow steppe

3.3 Abundance of nitrogen-cycling microorganisms

Similar to the soil properties in the three grasslands, the abundance of N-cycling microorganisms measured in both grazing treatments varied significantly with precipitation (p<0.001) and precipitation×treatment effect (p<0.001) (Figs. 3, 4, and 5). *nifH* gene abundance varied from 2.4×10^5 to 2.9×10^6 copies g^{-1} of dry soil and was significantly lower under grazing than non-grazing treatment in desert steppe (F=22.5, p=0.009) and typical steppe (F=11.61, p=

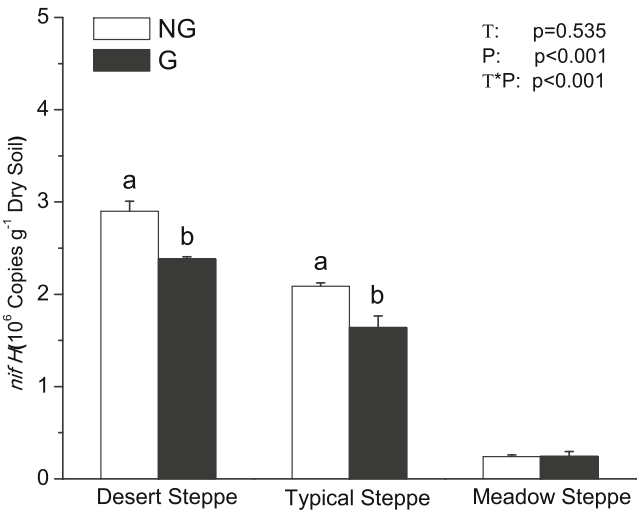

Fig. 3 Abundance of *nifH* gene (copies per gram of dry soil) in desert steppe, typical steppe, and meadow steppe along low-precipitation gradient. *NG* denotes non-grazing treatment and *G* denotes grazing treatment. Values (mean±SE) followed by *different letters* are significantly different within each set of sample data. *T* treatments, *P* precipitation, *T*P* treatment×precipitation

0.027), but there was no significant difference in meadow steppe (Fig. 3).

The abundance of nitrifier groups was quantified by analysis of the ammonia monooxygenase subunit A (*amoA*) gene (Fig. 4). *AOB* gene abundance was outnumbered by *AOA* gene by 1 to 2 orders of magnitude in all samples. The abundance of *AOB* gene was significantly lower under grazing treatment, from 4.2×10^5 to 2.3×10^5 copies g^{-1} in desert steppe (F=11.97, p=0.026) and 5.6×10^5 to 2.8×10^5 copies g^{-1} in typical steppe (F=40.54, p=0.003), but there was no significant effect between grazing treatment in meadow steppe (Fig. 4b). The abundance of *AOA* gene measured in grazed soil was significantly lower in typical steppe (F=12.36, p=0.025), but was not significantly different in both desert steppe and meadow steppe (Fig. 4a).

Four functional genes associated with denitrification were quantified in this study (Fig. 5). The abundance of *narG* gene under grazing treatment (1.5×10^7 copies g^{-1}) increased to double compared to that under non-grazing treatment (8.3×10^6 copies g^{-1}), but there was no significant difference in meadow steppe (Fig. 5a). The abundance of *nirK* gene in meadow steppe was two times of magnitude greater than in the other two sites, but there was no significant difference between the grazing and non-grazing treatments (Fig. 5b). As

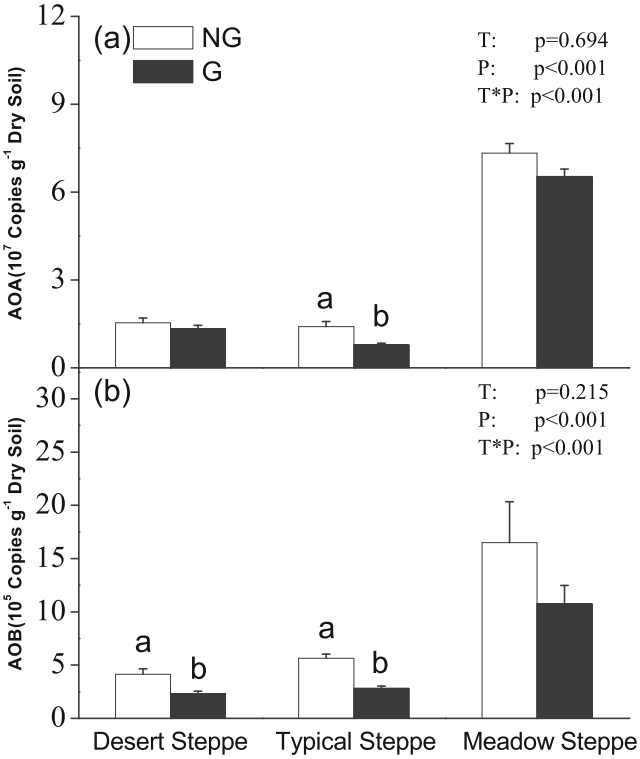

Fig. 4 Abundance of *AOA* (**a**) and *AOB* (**b**) genes (copies per gram of dry soil) in desert steppe, typical steppe, and meadow steppe along low-precipitation gradient. *NG* denotes non-grazing treatment and *G* denotes grazing treatment. Values (mean±SE) followed by *different letters* are significantly different within each set of sample data. *T* treatments, *P* precipitation, *T***P* treatment×precipitation

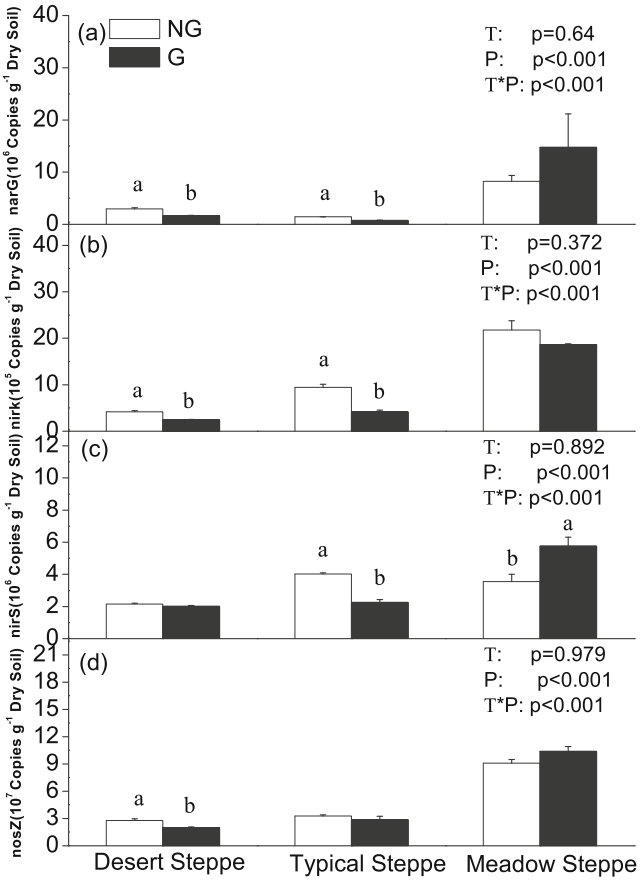

Fig. 5 Abundance of *narG* (**a**), *nirK* (**b**), *nirS* (**c**), and *nosZ* (**d**) genes (copies per gram of dry soil) in desert steppe, typical steppe, and meadow steppe along low-precipitation gradient. *NG* denotes non-grazing treatment and *G* denotes grazing treatment. Values (mean±SE) followed by *different letters* are significantly different within each set of sample data. *T* treatments, *P* precipitation, *T***P* treatment×precipitation

another gene encoding nitrite reductase, *nirS* gene has higher abundance under grazing than in non-grazing treatment, from 3.6×10^6 to 5.8×10^6 copies g^{-1} in meadow steppe (F=9.28, p=0.038), but markedly decreased from 4.0×10^6 to 2.3×10^6 copies g^{-1} in typical steppe (F=102.81, p<0.001) (Fig. 5c). For *nosZ* gene, grazing significantly decreased the abundance of *nosZ* gene by 27.8 % in desert steppe (F=18.31, p=0.013), but did not significantly influence the abundance of *nosZ* gene in both meadow steppe and typical steppe (Fig. 5d).

3.4 Environmental variables controlling functional groups associated with nitrogen cycling

In 2011, soil moisture was positively related with the abundance of *AOA*, *narG*, *nirS*, and *nosZ* genes at meadow steppe, typical steppe, and desert steppe (Table 4), suggesting that soil moisture was a primary factor controlling population sizes of N-cycling groups. Soil pH, concentration of NO_3^--N, and precipitation together explained 98.16 % of the variation in the abundance of *nifH* gene. Soil moisture, pH, and mean

Table 4 The environmental variables that are significantly correlated with microbial functional groups

Model	*p* value	R^2
nifH=1.6E6+9.4E5×pH−3.1E4×NO_3^--N−2.1E4×MAP	$p<0.0001$	0.9816
AOA=5.9E8+3.2E6×MC−4.4E7×pH−8.4E5×MAP	$p<0.0001$	0.9406
AOB=−8.6E5+7.5E5×SOC	$p<0.0001$	0.751
narG=1.4E7+9.7E6×MC−1.2E7×SOC	$p<0.0001$	0.7529
nirK=−8.9E5+1.1E7×TN	$p<0.0001$	0.9568
nirS=4.1E6+1.8E5×MC−1.9E6×SOC	$p=0.0002$	0.745
nosZ=1.7E7+2.7E6×MC−7.3E4×Shannon	$p=0.0004$	0.9646

p value and R^2 were determined between the size of functional groups and environmental variables

SOC soil organic C, *MC* moisture content, *MAP* mean annual precipitation, *TN* total N, *Shannon* Shannon diversity index

annual precipitation explained 94.06 % of the variation of *AOA* gene abundance, while soil organic C explained 75.1 % of that of *AOB* gene abundance. Soil moisture and organic C together accounted for 75.29 % of the total variance of *narG* gene abundance. Total N explained 95.68 % of the variation of *nirK* group, while soil moisture and organic C explained 74.5 % of the variation of the *nirS* group. Soil moisture and Shannon diversity index represented 96.46 % of the variations of *nosZ* gene abundance.

4 Discussion

4.1 Changes in the abundance of functional genes associated with N-cycling change along a low-precipitation gradient

A series of researches focus on the change of ecosystem process along this precipitation gradient such as primary production (Bai et al. 2008), stable C and N (Ma et al. 2012), and soil microbial communities (Chen et al. 2014) in Inner Mongolia, and all these studies showed the precipitation or soil moisture was the primary environmental factor controlling the ecosystem process. In our study, we found that the plant variables (aboveground standing biomass and Shannon-Wiener index) kept a significant relationship with precipitation in meadow steppe, typical steppe, and desert steppe (Fig. 2b, c). The result was consistent with most studies in arid and semiarid grasslands (Bai 1999; Bai et al. 2000; Liu et al. 2007), indicating the importance of precipitation in water-limited grassland ecosystem.

In N-fixation process, we found that *nifH* gene abundance significantly increased with the increase of soil pH (Table. 4), indicating a close linkage between N-fixation group and soil pH. This result was different from that obtained in Australia (Lindsay et al. 2010) and Europe (Meyer et al. 2013) where *nifH* gene abundance was mainly controlled by total N in nitrogen-rich grassland (Meyer et al. 2013). In our result, soil pH can explain 93.15 % of the variance of *nifH* gene abundance (Table 4), suggesting that soil pH was the main ecological factor for *nifH* gene in Inner Mongolia, which is most likely associated with that in other ecosystem. Acidic soil would inhibit dinitrogenase reductase and decreased the gene abundance of N-fixer groups (Pereira e Silva et al. 2013). Our research supports the results of Zhang et al. (2013) that the abundance of N-fixer groups was directly controlled by soil pH in Inner Mongolia grassland.

In nitrification and denitrification process, the abundance of nitrifier and denitrifier groups all increased with the increase of rainfall amount (Figs. 4 and 5) and soil moisture was the main ecological factor changing *AOA*, *narG*, *nirS*, and *nosZ* gene abundance except *AOB* and *nirK* genes (Table 4), indicating that precipitation regulates the soil moisture which changes the abundances of nitrifier and denitrifier groups in arid and semiarid grasslands. This finding was also consistent with other ecosystem such as forest ecosystem (Szukics et al. 2010). Szukics et al. (2010) investigated the response of nitrifier and denitrifier to a change in moisture and found that *AOA* abundance rapidly responded to water content while *nirK* gene abundance increased remarkably after short-term incubation under wet soil condition, suggesting the sensitivity of nitrifier and denitrifier groups to soil moisture. However, our result was inconsistent with recent research works in Europe, revealing plant diversity (Lange et al. 2014). pH and substrate (Yao et al. 2013) were the most important factors controlling the microbial functional groups. This difference with those researches in Europe might be due to the difference in precipitation in the two research regions, with the mean annual precipitation in Europe being abundant, ranging from 501 mm to approximately 2,000 mm per year, implying that microbial functional groups might be more responsive to other abiotic and biotic factors than moisture, while in Inner Mongolia, the precipitation (280~400 mm per year) which limits the growth of plants, microbial community, and ecosystem functioning (Bai et al. 2004, 2008), suggesting moisture as the primary factor in arid and semiarid grasslands. Compared with other size of functional groups significantly relating with moisture, our result indicated that the abundance of *AOB* gene

was positively related with soil organic C (Table 4). Mineralization of nitrogen collaborating with pool of soil organic C supplied NH_4^+-N, a kind of substrate used for nitrification by ammonia-oxidizing bacteria (Forbes et al. 2009). Soil organic C in our research explained the largest statistically proportion of variation in *AOB* gene abundance among abiotic and biotic factors, suggesting the important role of soil organic carbon in the process of nitrification. The copper nitrite reductase and cytochrome cd_1 nitrite reductase respectively encoded by *nirK* and *nirS* genes are involved in the reduction of NO_2^- to NO (Zumft 1997). Our result showed that the abundance of the *nirS* groups significantly increased with increasing soil moisture, while the *nirS* groups appeared to be more related with total nitrogen (Table 4), indicating the difference in phylogenetic diversity between *nirK* and *nirS* denitrifiers (Philippot et al. 2009).

4.2 Effect of grazing on nitrogen fixer, nitrifier, and denitrifier groups along a low-precipitation gradient

For all functional groups, our finding showed that grazing significantly decreased the abundance of *nifH*, *AOA*, *AOB*, *narG*, *nirK/S*, and *nosZ* genes in typical steppe and desert steppe, but did not affect them in meadow steppe (Figs. 3, 4, and 5). This result was different from that obtained in the studies made by Mirza et al. (2014) and Chroňáková et al. (2009) in Brazil and Europe, respectively, where the precipitation or soil moisture condition was higher than that in our site. In these recent reports, the abundance of functional genes involved in N cycling was significantly higher in grazed soil than in non-grazed soil, indicating the effect of variation in C and N supply induced by grazing on sizes of microbial population. In meadow steppe, our result demonstrated that there was no significant difference in the abundance of functional genes between grazing and livestock exclusion. The mechanism behind this pattern remained unclear, and we assumed that the different response of functional groups to grazing might be explained by the following two reasons: (1) some researchers suggested that the abundance of functional genes was more responsive to soil characteristics than to present grazing pasture (Wakelin et al. 2008, 2009; Lindsay et al. 2010). Wakelin et al. (2009) reported that the abundance of functional genes was not significantly different under pasture treatment but varied with variation in soil properties such as pH or nutrient content. As mentioned above, the abundance of *nifH* genes was mainly affected by soil pH while other genes associated with nitrification and denitrification processes were closely related to moisture. In this case, soil pH and moisture were not significantly different in grazing and non-grazing treatments, which might attribute to the observed similarity in size of N-cycling groups. (2) In our result, the experimental region in meadow-steppe grassland received much higher amount of precipitation (87.5 mm) in a week before soil sampling compared to desert steppe (21 mm) and typical steppe (30.4 mm), so that soil moisture (31.3 and 34.4 % in grazed and non-grazed soil) was similar to that in Europe with moisture ranging from about 30 to 60 % (Chroňáková et al. 2009). We speculated that grazing imposing no significant impact on the abundance of functional genes might due to a buffering effect induced by moisture on grazing. Furthermore, our finding was consistent with a study in meadow steppe that grazing did not significantly change gene abundance of nitrifier and denitrifier groups (Zhong et al. 2014). In this research, the abundance of *AOA* and *narG* genes was not obviously different between grazing and livestock exclusion in July while a significant difference was observed in May and September, confirming that moisture condition controls the effect of grazing on soil N-cycling microbial functional potentials. In typical steppe and desert steppe, our finding shows that the abundance of functional genes was lower in grazing treatment than in non-grazing treatment. In Inner Mongolia, although studies which focus on the effect of grazing on the abundance of functional genes are rare, several researches reported the effect of grazing on C, N, and P content (Wen et al. 2013) and plant communities (Wang et al. 2014), and all these studies showed the negative effect of grazing on ecosystem process in typical steppe and desert steppe. Our result had a similar trend that grazing decreased the abundance of functional genes, indicating the vulnerability of functional groups to grazing under low-moisture condition.

5 Conclusions

In summary, by investigating the effect of grazing on N-cycling gene abundances in three different grassland types in Inner Mongolia, our result showed that soil pH controlled the nitrogen fixation process and soil moisture was the dominant factor controlling the gene abundance of nitrification and denitrification process along the precipitation gradient. Grazing had no effect on the gene abundance of N-cycling process in meadow steppe but decreased it in desert and typical grasslands. This suggests that grazing may not necessarily be associated with a reduction in microbial functional potentials in meadow grassland because of the relatively good soil moisture condition but it will decrease the soil microbial functional potentials in a more arid environment in northern China grasslands.

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