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Gross Nitrification and Denitrification in Alpine Grassland Ecosystems on the Tibetan Plateau

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Abstract

Nitrification and denitrification are key microbiological processes in the soil nitrogen cycle and are the main biological sources of N_2O emissions from soils. In this work, we measured gross nitrification and denitrification rates of northern Tibet alpine grassland ecosystems during the growing season and evaluated the influence of soil environmental factors. The results showed that the soil inorganic nitrogen concentration and gross nitrification and denitrification rates of both alpine meadow and alpine steppe varied obviously across the season. During the growing season mean values of gross nitrification and denitrification rates of the alpine meadow site were 3.0 and 2.3 times greater than those of the alpine steppe site, respectively. Both gross nitrification and denitrification rates were not significantly correlated with the determined soil characteristics which include soil microbial biomass, inorganic nitrogen, and soil temperature, except that gross nitrification seemed associated with the microsite where soil moisture was higher. Our results demonstrate that soil moisture can explain partly the higher soil nitrogen (N) transformation rates in alpine meadow sites, but soil N transformation microorganisms and enzyme activities studies covering prolonged observation periods are still needed to clarify the key soil environmental factors that control gross nitrification and denitrification processes in alpine grassland ecosystems.

Introduction

Nitrogen (N) has long been recognized as the most limiting nutrient that regulates plant growth and net primary productivity in terrestrial ecosystems (Jones et al., 2004). This is particularly true in alpine and arctic ecosystems, where the mineralization of nitrogen is slow due to low temperatures (Bardgett et al., 2007). Nitrification and denitrification are two key microbiological processes involved in soil N cycling, which contribute to the regulation of the balance between the forms of soil mineral nitrogen (NO_3^- versus NH_4^+) available to plants and nitrogen conservation at the ecosystem level (Castaldi and Aragosa, 2002; Roux et al., 2003). Moreover, they are the main biological sources of soil nitrous oxide (N_2O), which is a potent greenhouse gas that has high radiative forcing and a long atmospheric lifetime of approximately 120 years (Hsieh et al., 2005; Rosenkranz et al., 2006).

Nitrification is an important microbial process that converts ammonium (NH_4^+) to nitric oxide (NO), nitrite (NO_2^-), and eventually nitrate (NO_3^-) through bacterial oxidation. It is a strictly aerobic process because the oxidation of NH_4^+ to NO_2^- by ammonium oxidizers and the oxidation of NO_2^- to NO_3^- by nitrite oxidizers require O_2 as the terminal electron acceptor and are optimal in aerobic conditions (Khalil et al., 2004; Bateman and Baggs, 2005). During the oxidation of ammonium to nitrite, N_2O can be produced as an intermediate and liberated to the atmosphere (Estavillo et al., 2002; Mørkved et al., 2007). Denitrification is an N-cycling transformation in which bacteria reduce NO_3^- to NO_2^-, N_2O, and eventually N_2. It is the only pathway by which reactive forms of nitrogen in terrestrial and aquatic ecosystems are transformed back into N_2 gas. Denitrification occurs under anaerobic conditions, in which organic carbon is used as an energy source and NO_3^- or NO_2^- is used instead of oxygen as the terminal electron acceptor by heterotrophic soil denitrifiers (Aulakh et al., 2000a, 2000b; Dodla et al., 2008). Soil denitrification is often considered the main N_2O producing process in soils, and many studies have observed increased N_2O emissions with increasing soil moisture content after nitrogen application (Skiba and Ball, 2002; Bateman and Baggs, 2005). Nitrification and denitrification may take place simultaneously in the same soil and often in close vicinity so that a substantial part of the NO_3^- formed by nitrification in an oxic zone can diffuse towards an anaerobic zone where it can be denitrified into N_2 (Khalil et al., 2004).

Factors affecting nitrification in soils are pH, moisture, temperature, C/N ratio of the litter, the presence of plant-produced allelochemicals, and the supply of essential nutrients (Gödde and Conrad, 2000; Zaman and Chang, 2004). In most soils, nitrification is particularly important during the growing season, when ammonium mineralization rates are high. This generalization is based upon measurements of net nitrification rates (Verchot et al., 2001). The net nitrification rate is easier to measure than the gross nitrification rate since it is basically calculated from the net change in the extractable pool of NO_3^- by incubating a soil sample, usually for one week or more, thereby disregarding consumption of nitrate by assimilation and denitrification (Hart and Gunther, 1989; Dalias et al., 2002). Thus changes in the NO_3^- pool do not absolutely reflect the gross nitrification of NH_4^+ to NO_3^-, net nitrification rates can be lower compared with the gross nitrification rates. (Ross et al., 2004; Kiese et al., 2008; Sun et al., 2009). It is generally accepted that gross nitrification rates allow a more mechanistic explanation of the processes concerned than can be derived from net nitrification rates (Stark and Firestone, 1996; Hatch et al., 1998).

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Because it occurs under anaerobic conditions, soil denitrification is controlled primarily by soil aeration status, furthermore controlled by soil organic carbon, NO_3^- availability, denitrifier population, temperature, soil pH, texture, etc. (Parry et al., 2000; Van Den Heuvel et al., 2010). Rainfall events, soil texture, soil drainage, and tillage influence denitrification rates by changing the amount of oxygen in the soil. NO_3^- is the source of N for denitrifying bacteria, and available organic carbon serves as the electron donor. Increases in both compounds will increase the soil denitrification rate. Soil pH has a marked effect on denitrification, with lower rates under acid than under neutral to slightly alkaline conditions (Simek et al., 2000). Temperature can influence denitrification both positively and negatively. Denitrification has an optimum temperature, above and below which rates decrease (Hofstra and Bouwman, 2005).

The Tibetan Plateau extends over 2.5 million km^2 with an average altitude of more than 4000 m. It is the youngest and highest plateau in the world. The plateau ecosystem is very vulnerable and sensitive to global climate changes, and the low temperature in this alpine area may restrict the decomposition of litter and soil organic matter (Kato et al., 2004; Luo et al., 2010). The alpine grasslands, which occupy over 60% of the total area, are the most dominant ecosystems on the plateau and represent much of the land area in the Eurasian continent (Wang et al., 2002). However, little is known about nitrification and denitrification rates and the factors affecting these rates in the alpine grassland soils of the plateau. In the present study, two types of alpine grasslands, alpine meadow, which has vegetation coverage of 70% with wet soil conditions, and alpine steppe, which has vegetation coverage of less than 20% with dry soil conditions, were selected to measure the gross nitrification and denitrification rates during the growing season, and to evaluate what are the main soil environmental limiting factors which affect gross nitrification and denitrification processes. A better understanding of this alpine system might contribute to the prediction of variations in the N cycle, following modifications of extremely harsh environmental conditions. We hypothesized that the nitrification and denitrification rates were higher in the alpine meadow than those in the alpine steppe because alpine meadow soils had higher vegetation coverage and water availability. Furthermore, we hypothesized that soil environmental factors, including soil microbial biomass C and N, soil temperature, and moisture were key factors that influence nitrification and denitrification rates.

Materials and Methods

STUDY SITE

Studies were conducted at Shenzha Alpine Steppe and Wetland Ecosystem Observation and Experiment Station (30°57′N, 88°42′E, 4675 m a.s.l.) located in Shenzha County, northern Tibet, China. Northern Tibet is the headwater of many important rivers and high mountain lakes in China, such as the Yangtze, Nu (Salween River), and Lancang (Mekong River). It is also a major livestock production center in Tibet which is one of the China's five key livestock raising provinces. Northern Tibet is located in a cold and semi-arid plateau monsoon climate region. The natural environment is extremely harsh, and the soil is generally quite thin. Out of the total area of northern Tibet, 94.4% is composed of alpine meadows and steppes, with alpine grassland as the dominant vegetation (Gao et al., 2009a). The alpine grassland ecosystem in northern Tibet is rather vulnerable and extremely sensitive to climate change and human activities (Gao et al., 2009b). According to 30-year records from the meteorological station (4671 m a.s.l.) which is about 2 km from the study site, the annual mean air temperature was 0 °C, the mean air temperature during January was −10.1 °C, and the mean air temperature during July was 9.6 °C. There was no absolute frost-free season and the frosty period was up to 279 days. The annual mean time of solar radiation was 2916 hours. The average annual precipitation was 300 mm, most of which occurred from May to September.

The region of the Shenzha Alpine Steppe and the Wetland Ecosystem Observation and Experiment Station is located in a typical alpine grassland ecotone in northern Tibet and different types of land use coexist in the study area. Two types of alpine grassland, alpine meadow and alpine steppe, were selected in this study for the measurement of gross nitrification and denitrification rates. There are nearly 4200 ha of grassland developed into a typical alpine meadow because of the adequate water supply from snow melt and approximately 600 ha of grassland developed into a typical alpine steppe due to drought. The alpine meadow had about 70% vegetation coverage and was dominated by *Kobresia humilis* with scattered *Oxytropis* spp.*, Gentiana squarrosa,* and *Aster tataricus* L. The alpine steppe had less than 20% vegetation coverage, mainly *Stipa purpurea*, *Artemisia capillaris* Thunb, and *Rhodiola rotundaia* assemblages.

SOIL SAMPLING AND ANALYSES

Soils were sampled from both grasslands every month during May–September to capture the seasonal dynamics throughout the growing season of 2010. Each sampling time for each grassland, seven intact soil cores were collected using the soil corer (5.6 cm in diameter and 4.1 cm in height) in order to determine gross nitrification and denitrification rates. At the same time, three replicate samples (0–15 cm depth) were collected to measure the inorganic nitrogen content and microbial biomass. Soil samples were kept at 4 °C in cool boxes during transport to the laboratory, then stored in a refrigerator at 4 °C and processed within 10 days of sampling. In addition, the bulk density (BD), pH, soil organic carbon (SOC), total nitrogen, total phosphorus, and total potassium of the soil samples were determined on the first sampling date. Soil moisture and soil temperature were automatically monitored by the HOBO weather stations (Onset Corp., Pocasset, Massachusetts, U.S.A.) every 30 min.

Soil physical and chemical properties were determined using standard analytical methods (Liu, 1996). Soil bulk density (BD) was determined as the moisture-corrected (oven-dried at 105 °C) mass of each sample divided by the measured volume of the excavated soil core. Soil organic carbon was determined using wet oxidation with $K_2Cr_2O_7$. The micro-Kjeldahl digestion method was used to determine total N content. Total P and total K contents were determined using the NaOH and HF-$HClO_4$ digestion methods, respectively. Soil microbial biomass C and N were determined by the chloroform fumigation extraction method (Vance et al., 1987). To determine the inorganic nitrogen (NH_4^+-N and NO_3^--N), fresh soil sample (10 g) was extracted with 2 M KCl (50 mL) and shaken for 30 min. After the extraction, samples were centrifuged at 15000

r/min for 10 min. The supernatants were passed through a 0.22-μm Millipore membrane. The filtrate was analyzed with the potassium chloride-indophenol blue colorimetric method for NH_4^+-N (Liu, 1996) and the ultraviolet spectrophotometry method for NO_3^--N (Song et al., 2007).

MEASUREMENT OF GROSS NITRIFICATION AND DENITRIFICATION

Gross rates of nitrification and denitrification were measured by using the Barometric Process Separation (BaPS) instrument (UMS GmbH Inc., Germany) in laboratory incubations. The BaPS methodology is based on the observation that in a gas tight, isothermal, closed system containing an intact soil core, the main changes in air (CO_2, O_2, and other gas) pressure are due to the microbial processes of nitrification (pressure decrease), denitrification (pressure increase), respiration (pressure neutral, if coefficient of respiration is equal to 1.0), and the non-biological (physicochemical) process of CO_2 dissolution into soil solution. Nitrification is a net gas-consuming process because the oxidation of NH_4^+ to NO_3^- consumes molecular O_2, whereas denitrification is a net gas-producing process because denitrification produces CO_2 as well as gaseous N (N_2, N_2O, NO). Soil respiration is neither a net gas-producing nor a net gas-consuming process, because the amounts of O_2 consumed and CO_2 produced are identical. Therefore, soil respiration itself will not lead to pressure changes within the system. However, CO_2 is produced in the soil and is not absolutely emitted to the atmosphere. Therefore, CO_2 will partly dissolve into the soil solution (aqueous phase) and the resulting pressure decrease is also recorded in the BaPS system. Hence the gross nitrification rates and denitrification rates can be derived by measuring the air pressure changes within the closed system and the O_2 and CO_2 concentrations in the system (Ingwersen et al., 1999; Breuer et al., 2002; Gao et al., 2008; Sun et al., 2009). Seven intact soil cores of each grassland site were put into the BaPS instrument to determine their rates of gross nitrification and denitrification. The BaPS instrument was sealed, kept gas-tight, and incubated for at least 24 hours at the monthly mean value of soil temperature at each site.

STATISTICAL ANALYSES

One-way ANOVA was used to test the differences of soil physical and chemical properties between the two alpine grasslands, and a Least Significant Difference (LSD) test was used to distinguish difference at $p = 0.05$. Two-way ANOVA adapted the alpine grassland type and the sampling time as the main factors for analyzing the following variables: NH_4^+-N, NO_3^--N, inorganic nitrogen (NH_4^+-N and NO_3^--N) concentration, and gross nitrification and denitrification rates. Relationships between the soil environmental factors and the gross nitrification and denitrification rates were tested using Pearson correlation analysis. All analyses were performed using the SPSS 11.5 statistical software package (SPSS Inc., U.S.A.).

Results

SOIL PROPERTIES

Table 1 shows the bulk density (BD), pH, soil organic carbon (SOC), total N, total P, and total K in the two different alpine grasslands. Soil BD, total P, and total K levels differed significantly between the alpine meadow and alpine steppe site, but the differences of soil pH, SOC, and total N were not statistically significant. Monthly soil temperature (range from 7.4 °C to 15.5 °C) and moisture (range from 2.6% to 29.5%) showed seasonal variations across two alpine grassland sites and sampling times (Fig. 1). Over the period of field sampling (May–September, 2010), the average soil temperature was 10.3 °C in the alpine meadow site and 13.6 °C in the alpine steppe site, respectively. The mean soil moisture was 20.0% in alpine meadow site and 6.9% in alpine steppe site, respectively.

MICROBIAL BIOMASS C, N

Monthly soil microbial biomass C ranged from 27.3 g m^{-2} to 45.0 g m^{-2} across two alpine grassland sites during the growing season of 2010 (Fig. 2, Part a). The highest microbial biomass C, obtained in July in the alpine meadow site and obtained in August in the alpine steppe site, was 45.0 and 36.4 g m^{-2}, respectively. At both sites, the dynamics of soil microbial biomass N (ranged from 3.6 to 9.5 g m^{-2}) exhibited a similar ''low-high-low'' pattern during the growing season (Fig. 2, Part b). The amounts of microbial biomass N increased from May and reached maximum in July for the alpine meadow site (7.8 g m^{-2}) and in August for the alpine steppe site (9.5 g m^{-2}), and subsequently decreased quickly in September. The mean value of microbial biomass C of the alpine meadow site was 20.4% higher than that of the alpine steppe site, but the mean value of microbial biomass N of the alpine meadow site was 6.1% lower than that of the alpine steppe site.

INORGANIC NITROGEN CONCENTRATION

During the five-month study, NH_4^+-N, NO_3^--N, and inorganic N (NH_4^+-N and NO_3^--N) concentrations varied across the growing season (Fig. 3). The amount of NO_3^--N increased from May and

TABLE 1

Soil physical and chemical properties in the alpine meadow and alpine steppe at northern Tibet.

	BD (g·cm^{-3})	pH	SOC (kg·m^{-2})	Total N (kg·m^{-2})	Total P (kg·m^{-2})	Total K (kg·m^{-2})
Alpine steppe	1.76a (0.04)	8.78a (0.07)	2.93a (0.56)	0.27a (0.05)	0.14a (0.01)	8.24a (0.10)
Alpine meadow	1.15b (0.18)	8.75a (0.01)	2.26a (0.13)	0.18a (0.01)	0.08b (0.00)	4.72b (0.03)

Mean values followed by different letters (a and b) across different grassland types are different from each other at $P < 0.05$ level. Numbers in parentheses are ± standard errors of means ($n = 3$)

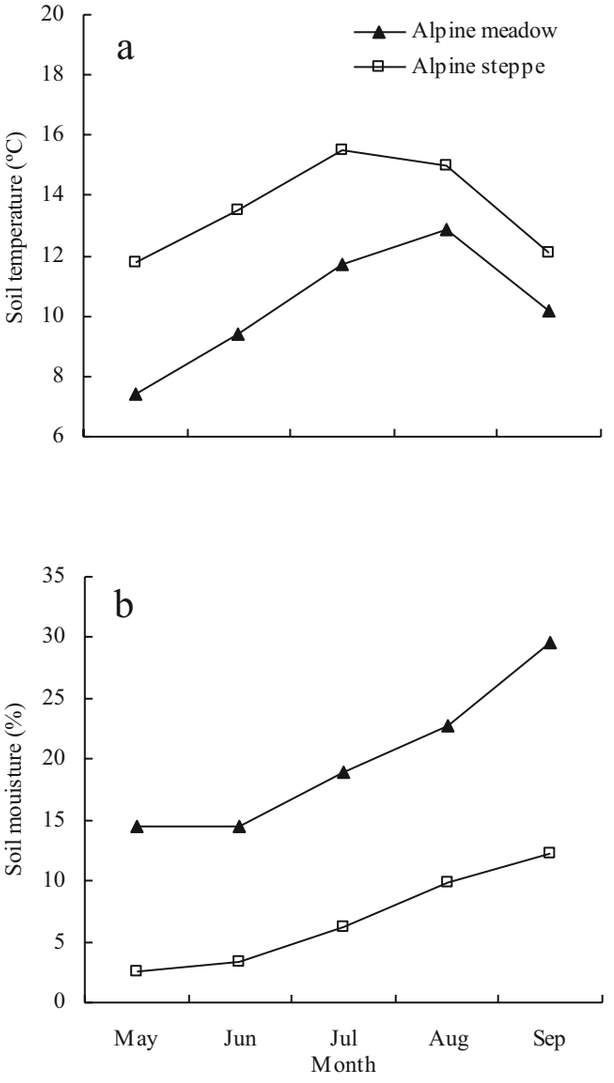

FIGURE 1. Temporal variations in soil (a) temperature and (b) moisture in an alpine meadow and alpine steppe during the growing season.

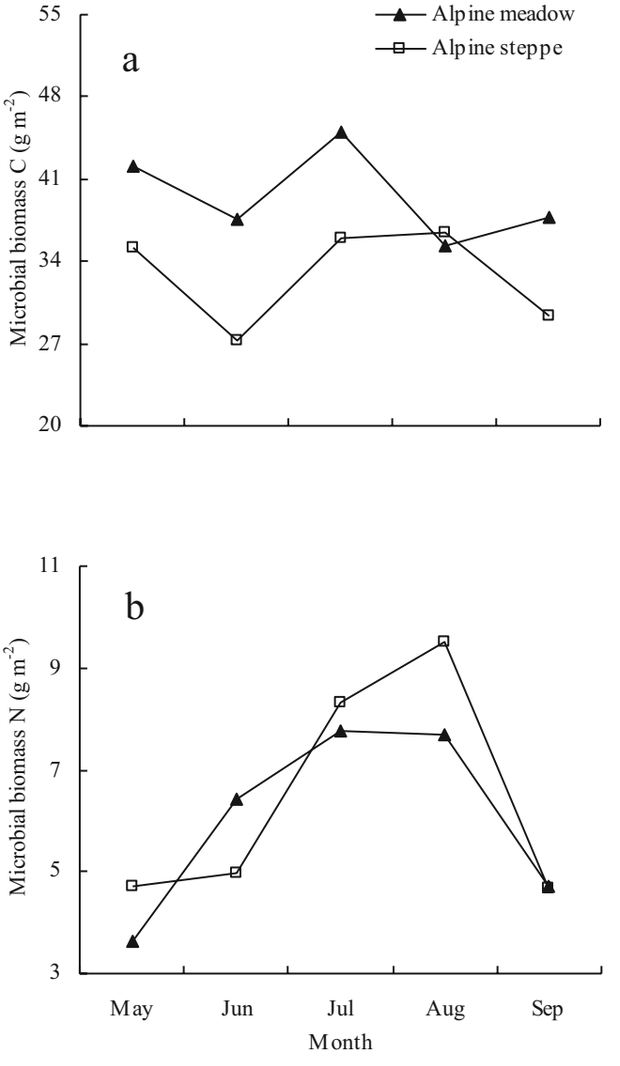

FIGURE 2. Temporal variations in soil (a) microbial biomass C and (b) microbial biomass N concentrations in an alpine meadow and alpine steppe during the growing season.

reached maximum concentration in July in the alpine meadow (0.6 g m^{-2}) and alpine steppe (0.8 g m^{-2}) (Fig. 3, Part a). In contrast, the NH_4^+-N concentration at the two sites showed a different pattern. In the alpine steppe site, the NH_4^+-N concentration increased slightly and peaked (0.6 g m^{-2}) in June and subsequently decreased to the minimum (0.1 g m^{-2}) in August, whereas the highest value (0.2 g m^{-2}) in the alpine meadow site was observed in July (Fig. 3, part b). Soil inorganic N pools ranged from 0.2 to 0.8 g m^{-2} for the alpine meadow and 0.4 to 1.1 g m^{-2} for the alpine steppe during the five sampling times, and also showed similar seasonal trends to NO_3^--N concentrations (Fig. 3, part c). Results from two-way ANOVA demonstrate that alpine grassland type, sampling time, and their interaction were all statistically significant as the effect for NH_4^+-N, NO_3^--N, and inorganic nitrogen concentrations (Table 2).

GROSS NITRIFICATION AND DENITRIFICATION

Monthly soil gross nitrification rates also showed seasonal variations ranging from 0.2 mg m^{-2} h^{-1} to 4.6 mg m^{-2} h^{-1} across all alpine grassland plots and sampling times (Fig. 4). The highest gross nitrification rate was 2.0 mg m^{-2} h^{-1} for the alpine steppe site in June and 4.6 mg m^{-2} h^{-1} for the alpine meadow site in July. Soil denitrification rate also showed seasonal variations during the growing season for the two alpine grassland sites. The denitrification exhibited a single peak value at 3.1 mg m^{-2} h^{-1} for the alpine meadow site in August and 1.7 mg m^{-2} h^{-1} for the alpine steppe site in July. The gross nitrification and denitrification rates were much higher in the alpine meadow site than in the alpine steppe site. The mean values of gross nitrification and denitrification rates during the growing season of the alpine meadow site were 3.0 and 2.3 times greater than those of the alpine steppe site, respectively. Statistical analyses showed that soil gross nitrification and denitrifi-

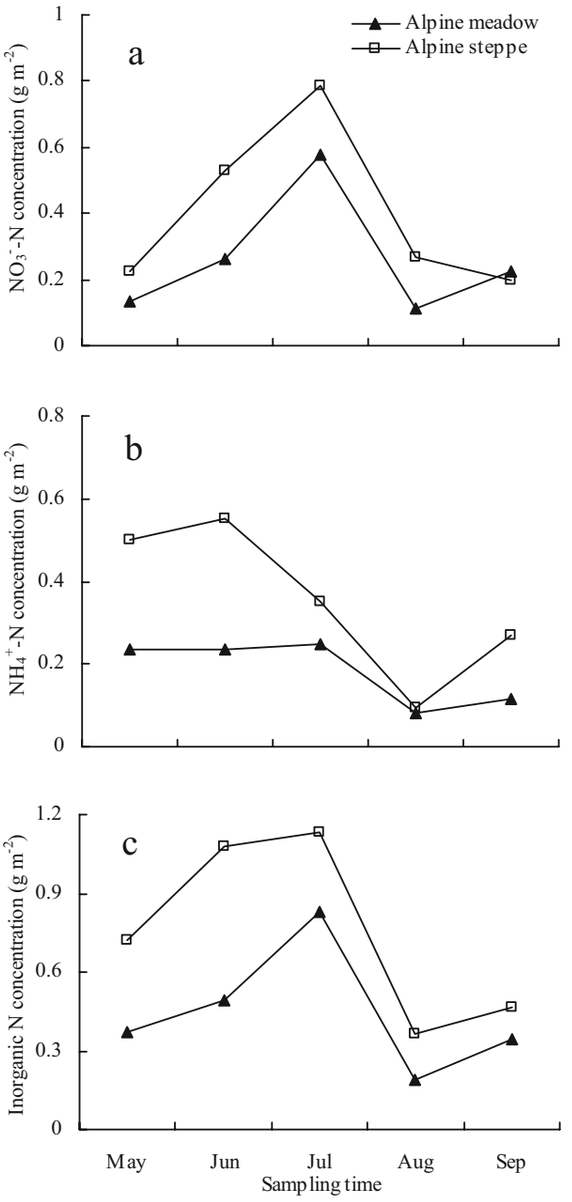

FIGURE 3. Temporal variations in soil (a) NO_3^--N, (b) NH_4^+-N and (c) inorganic nitrogen (NO_3^--N and NH_4^+-N) concentrations in an alpine meadow and alpine steppe during the growing season.

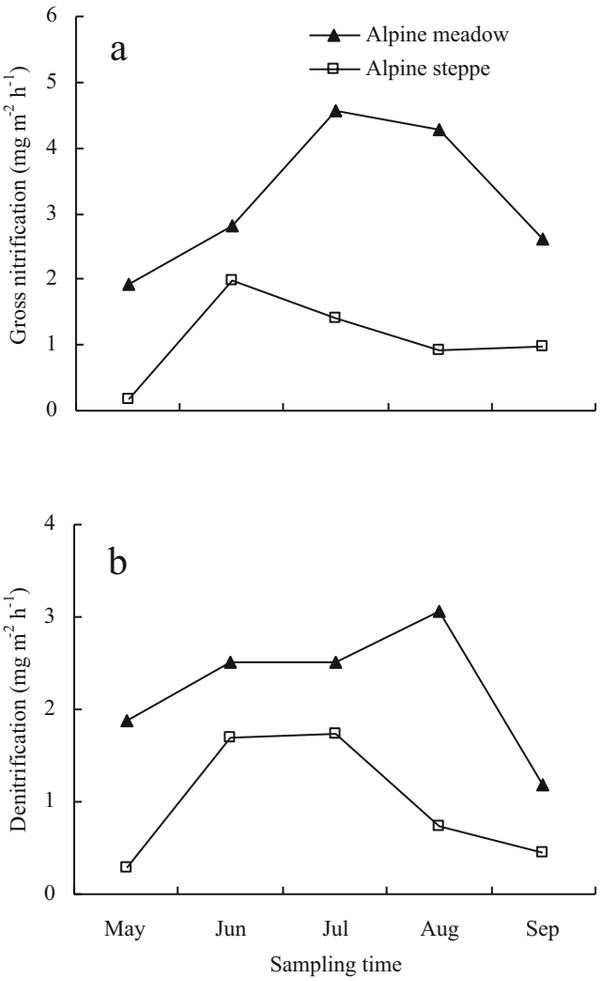

FIGURE 4. Temporal variations of (a) gross nitrification rates and (b) denitrification rates in soil from an alpine meadow and alpine steppe during the growing season.

TABLE 2

Two-way ANOVA for the soil NH_4^+-N, NO_3^--N, and inorganic nitrogen concentrations and gross nitrification, denitrification rates with significant effects in italics.

Main effects	NO_3^--N (g m^{-2})			NH_4^+-N (g m^{-2})			Inorganic nitrogen (g m^{-2})			Gross nitrification rate (mg m^{-2} h^{-1})			Denitrification rate (mg m^{-2} h^{-1})		
	d.f.	*F*-ratio	*P*-value	d.f.	*F*-ratio	*P*-value	d.f.	*F*-ratio	*P*-value	d.f.	*F*-ratio	*P*-value	d.f.	*F*-ratio	*P*-value
AGT	*1*	*21.52*	*<0.001*	*1*	*32.77*	*<0.001*	*1*	*52.72*	*<0.001*	*1*	*19.84*	*0.01*	*1*	*16.28*	*0.02*
ST	*4*	*41.79*	*<0.001*	*4*	*14.66*	*<0.001*	*4*	*36.22*	*<0.001*	4	1.98	0.26	4	3.09	0.15
AGT × ST	*4*	*2.93*	*0.03*	*4*	*3.42*	*0.02*	*4*	*3.64*	*0.01*						
Residual	50			50			50			4			4		
Total	59			59			59			10			10		

AGT: Alpine grassland type, ST: Sampling time.

cation rates were significantly different between the two alpine grasslands, but were not significantly different among the five sampling times (Table 2).

Discussion

Until now, investigations of the seasonality of nitrification and denitrification rates in ecosystems are often limited to point measurements during dry and wet season conditions (Zak and Grigal, 1991; Breuer et al., 2002). In this study, we explored the temporal variability of gross nitrification rate and denitrification rate during the growing season in two different types of alpine grassland in northern Tibet. The monthly soil gross nitrification and denitrification rates showed strong seasonal variations at both sites. This is in agreement with previous reports on the significant seasonal variation of soil gross nitrification and denitrification rates in different geographic regions (Zak and Grigal, 1991; Kiese et al., 2008; Zhang et al., 2008; Rosenkranz et al., 2010). The highest gross nitrification rate was observed in July for the alpine meadow site and in June for the alpine steppe site. And the highest denitrification rates of the alpine meadow site and the alpine steppe site were observed in August and July, respectively. As discussed by Zhang et al. (2008), during the growing season plant growth is stimulated by rising soil temperature and increasing fluxes of photosynthetically active radiation; this increases the demand for inorganic N. The period of maximum plant uptake was preceded by peaks in the rates of soil microbial production of inorganic N, and variations in seasonal patterns of N transformation matched the variation in plant growth.

The mean values of gross nitrification and denitrification rates during the growing season of the alpine meadow site were 3.2 and 2.2 mg m^{-2} h^{-1} and those of the alpine steppe site were 1.1 and 1.0 mg m^{-2} h^{-1}, respectively. Mean values of gross nitrification and denitrification rates of the alpine meadow site were 3.0 and 2.3 times greater than those of the alpine steppe site, respectively. These results support our hypothesis that the nitrification and denitrification rates were higher in the alpine meadow than those in the alpine steppe. Compared with an alpine meadow ecosystem on the eastern Tibetan Plateau reported previously (Sun et al., 2009), the gross nitrification rate of the alpine meadow in the current study falls well within the same (1.4–4.0 mg m^{-2} h^{-1}) (Gao et al., 2008; Sun et al., 2009). With regard to alpine steppe, to date there is no available data about the soil N transformation in the alpine steppe in Tibetan Plateau and no comparison can be made. Alpine ecosystems are characterized by the low activity of microbial processes due to the extreme conditions of the highlands. The short summer and low temperature retard the mineralization of organic compounds and hinder the nitrogen transformation (Schürmann et al., 2002; Kizilova et al., 2006). Compared with other alpine grassland ecosystems, the low gross nitrification and denitrification rates in northern Tibet are similar to those of the alpine pasture of the Austrian Alps (Hackl et al., 2000), the *Festuca varia* grasslands, the *Geranium gymnocaulon* and *Hedysarum caucasicum* meadows of the northern Caucasus (Makarov et al., 2003), and the alpine mountain–meadow of the Teberda Reserve (Kizilova et al., 2006).

During the growing season the variations of gross nitrification and denitrification rates of alpine grassland ecosystems in northern Tibet were not significantly correlated with either microbial biomass C or microbial biomass N (Table 3). This is consistent with some studies that also found no significant relationship between gross nitrification and denitrification rates and microbial biomass (Francez et al., 2000; Zaman and Chang, 2004). But in contrast some other studies microbial biomass C and N were found in some variable fields to be better indicators of N transformation in soils disturbed by human activities (Zaman et al., 1999; Ullah and Zinati, 2006). This disagreement may be accounted for by the different environmental (e.g. temperature and moisture) and soil conditions (undisturbed versus mixed) used in the field and in laboratory experiments (Zaman et al., 1999). Several studies have reported significant relationships between nitrification, denitrification rates and extractable NH_4^+-N, NO_3^--N (Gödde and Conrad, 2000; Castaldi and Aragosa, 2002). These variables were not highly correlated in this study (Table 3) and the similar results were also found in semi-arid grasslands of Yellowstone National Park, in which gross nitrification was not significantly correlated with both NH_4^+-N and NO_3^--N (Verchot et al., 2002). These results indicated that soil microbial biomass and substrate availability were not limiting gross nitrification and denitrification in alpine grassland ecosystems.

It is well known that nitrification and denitrification rates increase with rising temperature until an optimal temperature is reached (Gödde and Conrad, 2000). Nitrification and denitrification are microbially governed processes; hence the increase in soil temperature is likely to stimulate soil N transformation by promoting microbial populations and activity (Saad and Conrad, 1993; Sierra, 2002). However, in alpine grassland ecosystems, both gross nitrification and denitrification rates were not significantly correlated with soil temperature (Table 3). It is reported that the optimum temperature for gross nitrification rate ranges from 25 to 35 °C, although nitrification may still occur below 5 °C or above 40 °C (Stark, 1996; Sierra and Marban, 2000; Zaman and Chang, 2004). However, the monthly soil temperature only ranged from 7.4 to 15.5 °C across all alpine grassland sites and sampling times during the growing season, due to the high altitude of study sites (about 4700 m). The soil temperatures in our alpine grassland ecosystems were well below the likely optima for gross nitrification and denitrification, and this might explain the absence of a statistically signifi-

TABLE 3

Coefficients of determination (r^2) for correlations between gross nitrification, denitrification rates and soil characteristics (*$P < 0.05$).

	MBC	MBN	NH_4^+-N	NO_3^--N	IN	ST	SM
Gross nitrification	0.21	0.06	0.18	0.003	0.03	0.04	0.46*
Denitrification	0.15	0.08	0.08	0.02	0.003	0.03	0.18

MBC: microbial biomass C; MBN: microbial biomass N; IN: inorganic N; ST: soil temperature; SM: soil moisture.

cant relationship between soil temperature and rates of both gross nitrification and denitrification.

Soil moisture content also, however, regulates microbial processes and ecological interactions involved in nutrient cycling, therefore affecting soil nitrification and denitrification rates. Soil moisture content influences substrate diffusion and exerts diffusional constraints on soil O_2, which is of fundamental importance in determining if the aerobic process of nitrification, or the anaerobic process of denitrification, will prevail. (Castaldi and Aragosa, 2002, Zaman and Chang, 2004). In this study gross nitrification rates increased significantly with increasing soil moisture in alpine grassland ecosystems ($r = 0.68$, $p = 0.03$), but denitrification rates did not significantly increase with soil moisture ($r = 0.42$, $p = 0.23$). Some previous observations found that nitrification rates in some ecosystems were higher at higher soil moisture (Kiese et al., 2008; Chen et al., 2011). By contrast, in a lowland rain forest in central Kalimantan of Indonesia a negative effect of soil moisture on nitrification throughout the measured moisture range was observed (Vernimmen et al., 2007). In alpine grassland ecosystems, the soil temperatures were considerably low at both alpine meadow and alpine steppe sites at all times. It may be that temperature controls nitrification and denitrification, but that fluxes were universally limited. However, the gross nitrification and denitrification rates of the alpine meadow site were much higher than the alpine steppe site despite the soil temperatures being lower in the alpine meadow site (Fig. 4). This is maybe because the soils of the alpine meadow site were much wetter than those of the alpine steppe site during the growing season (Fig. 1).

The grassland ecosystem's type can profoundly impact soil N transformations by altering the physical and chemical properties of the soil environment and therefore the structure and functioning of the soil microbial communities (Jones et al., 2004; Zhang et al., 2008; St. Clair et al., 2009). In the present study comparing the alpine meadow with the alpine steppe, the differences in soil bulk density and total P and K were statistically significant, but the differences in soil pH, SOC, and total N were not (Table 1). However, mean values of gross nitrification and denitrification rates of the alpine meadow site were much greater than those of the alpine steppe site. The hypothesis was that soil environmental factors, including soil microbial biomass C and N, soil temperature, and moisture were key factors that influence nitrification and denitrification rates in alpine grassland ecosystems. This hypothesis turned out only to be partly right because the variations of gross nitrification and denitrification rates were not significantly correlated with the determined soil characteristics, except that gross nitrification rates were significantly correlated with soil moisture. The correlation between gross nitrification and soil moisture is probably due to increasing soil N transformation by microorganisms and enzyme activities with rising soil moisture. But to support this hypothesis it would be necessary to determine the quantities of nitrifier and denitrifier, to determine the enzyme activities of nitrification and denitrification enzyme of two alpine grassland sites, and to test their relationship to soil environmental factors.

Conclusion

When observing variations of soil N transformation in two different alpine grasslands, alpine meadow and alpine steppe, clearly seasonal variations of gross nitrification and denitrification rates were detected. And the gross nitrification and denitrification rates were much higher in the alpine meadow site than in the alpine steppe site during the growing season. Soil microbial biomass, inorganic N, and soil temperature were not the key factors that control soil gross nitrification and denitrification, but soil moisture plays a critical role in soil gross nitrification of alpine grassland ecosystems in northern Tibet. Based on the present study, the increased soil moisture therefore appears to be one of the most important factors explaining the high soil N transformation rates in the alpine meadow ecosystem. Nevertheless, how the grassland ecosystem's type affects gross nitrification and denitrification rates and what are the crucial factors which regulate the soil N transformation in alpine grassland ecosystem remain to be clarified, thus, more detailed studies regarding the soil N transformation bacteria (nitrifier and denitrifier, etc.) and enzyme (nitrification and denitrification enzyme activity, etc.) covering prolonged observation periods for the difference in gross nitrification and denitrification are necessary to further elucidate the underlying mechanisms.

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