

Natural ^{15}N abundance in soils and plants in relation to N cycling in a rangeland in Inner Mongolia

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Abstract

Aims

Natural ^{15}N abundance provides integrated information about nitrogen (N) input, transformation and output, indirectly reflecting N cycling traits within terrestrial ecosystems. However, relationships between natural ^{15}N abundance and N cycling processes are poorly understood in China. Here, our primary objectives were to (i) examine the effects of grazing at varying levels of intensity on $\delta^{15}\text{N}$ of soils and plants in a semi-arid grassland; (ii) detect the relationships between $\delta^{15}\text{N}$ of soils and four major N cycling processes (i.e. mineralization, nitrification, denitrification and ammonia volatilization); and (iii) determine whether $\delta^{15}\text{N}$ of soils can be used as an indicator of N cycling in this semi-arid grassland.

Methods

The field experiment was conducted within the long-term (17-year) grazing enclosures in a semi-arid grassland in Inner Mongolia. Five grazing intensities (0.00, 1.33, 2.67, 4.00 and 5.33 sheep ha^{-1}) were designed. $\delta^{15}\text{N}$ values of topsoils (0–10 cm), surface soils (0–2 cm) and plants were measured in 2006. Differences in $\delta^{15}\text{N}$ of soils and plants between the five grazing intensities were examined. Rates of four soil N cycling processes were measured periodically during the 2005 and 2006 growing seasons. The $\delta^{15}\text{N}$ values of topsoils were linked to the four N cycling processes to investigate their relationships.

Important Findings

The $\delta^{15}\text{N}$ values of topsoils (5.20–5.96‰) were substantially higher than the $\delta^{15}\text{N}$ values of plants (2.51–2.93‰) and surface soils (1.44–2.92‰) regardless of grazing intensities. The ^{15}N -depleted N losses during microbial decomposition of organic matter in concert with the downward movement of residual substrate over time are the possible causes of higher $\delta^{15}\text{N}$ values in topsoils than in surface soils. In addition, the $\delta^{15}\text{N}$ values of topsoils were positively correlated with the $\delta^{15}\text{N}$ values of both plants and surface soils. Grazing, especially the high-intensity grazing (5.33 sheep ha^{-1}), resulted in a significant decrease in $\delta^{15}\text{N}$ of surface soils. However, no statistically significant variations in $\delta^{15}\text{N}$ of topsoils and plants were found in response to grazing. The $\delta^{15}\text{N}$ values of topsoils exhibited significant dependence on the cumulative rates of NH_3 volatilization, net nitrification and denitrification in 2005 but not in 2006.

Keywords: $\delta^{15}\text{N}$ • NH_3 volatilization • mineralization • nitrification • denitrification • grazing intensity • grassland

Received: 17 January 2010 Revised: 24 June 2010 Accepted: 18 July 2010

INTRODUCTION

Natural ^{15}N abundance in soils is mainly determined by long-term changes in rates of N inputs, transformations and losses

(Frank *et al.* 2000). $\delta^{15}\text{N}$ of soils has been developed as a means to identify N sources and transformation processes and is presumed to be an index of N cycling in terrestrial ecosystems (Bai and Houlton 2009; Högberg 1997; Nadelhoffer and Fry 1994;

Robinson 2001). $\delta^{15}\text{N}$ of plants is also an N source indicator (Dawson *et al.* 2002; Frank *et al.* 2000) and reflects net effects of a series of plant-related N processes (Evans 2001; Robinson 2001; Stewart 2001). Recently, more lines of evidences have been provided for natural ^{15}N abundance as a function of the rates and isotopic compositions of N transformations (Evans and Ehleringer 1994a, 1994b; Templer *et al.* 2007), inputs and outputs (Frank *et al.* 2004).

Natural ^{15}N abundance is regulated not only by internal N cycling via mineralization, nitrification, leaching, gaseous losses and inputs but also by extrinsic factors such as fertilization (Frank and Evans 1997; Frank *et al.* 2004), climate (Cheng *et al.* 2009), soil age (Brenner *et al.* 2001) and grazing (Frank *et al.* 2000). As one of the controlling factors, herbivores play an important role in regulating N cycling in rangelands (Frank *et al.* 2000). Grazers can pose a direct effect on N cycling by dropping feces, removing plant tissues (van Wijnen *et al.* 1999) and trampling (Zacheis *et al.* 2002). Also, grazers can exhibit an indirect effect by altering plant species composition (Olofsson *et al.* 2001), by causing changes in N concentration of plant tissues (Baron *et al.* 2002; Epstein *et al.* 2001) and by affecting plant litter decomposition or soil microbial activities (Stark and Grellmann 2002). Via these direct or indirect effects, grazers may regulate N cycling rates by redistributing N within systems and consequently show a variety of influences on $\delta^{15}\text{N}$ in soil-plant systems. In some cases, two major pathways of gaseous N losses via denitrification and ammonia volatilization can result in an enrichment of ^{15}N in remaining soils in grazed grasslands (Frank *et al.* 2004; Sutherland *et al.* 1993). Positive correlations of soil ^{15}N levels with net N mineralization and net nitrification rates have been detected in ungrazed grasslands but not in grazed grasslands (Frank *et al.* 2000). Virtually most of these studies mainly examined the differences in $\delta^{15}\text{N}$ of soils between grazed and ungrazed sites in relation to one or two major N cycling processes. In comparison, information about the relationships of $\delta^{15}\text{N}$ of plants and soils with a combination of major soil N cycling processes along a grazing intensity gradient, as stated in our study, has been poorly documented.

There are ~400 million ha of grassland in China, accounting for nearly 42% of the total land area. Totally, 22% of the grasslands are in Inner Mongolia of North China. Sheep grazing is the main land use type in these grasslands in Inner Mongolia, where a nomadic land-use system prevailed for thousands of years. However, these grasslands are faced with severe degradation and desertification due to rapid population growth coupled with poor management (Kang *et al.* 2007). Therefore, since the beginning of the 21st century, the Chinese government has made great efforts to restore the temperate grassland by periodic exclusion of grazing. Understanding the quantitative relationship between N status and grazing in these grassland ecosystems is crucial for rational utilization of the resources and for reversing the ongoing degradation. Here our observations will be of importance to better understanding of these relationships. Our primary objectives were to (i) examine the effects of grazing at varying levels of intensity on

$\delta^{15}\text{N}$ of soils and plants in a semi-arid grassland; (ii) detect the relationships between $\delta^{15}\text{N}$ of soils and four major N cycling processes (i.e. mineralization, nitrification, denitrification and ammonia volatilization); and (iii) determine whether $\delta^{15}\text{N}$ of soils can be used as an indicator of N cycling in this semi-arid grassland.

MATERIALS AND METHODS

Site description

This experiment was conducted within the grazing enclosures of the Inner Mongolia Grassland Ecosystem Research Station of the Chinese Academy of Sciences, located in the central part of Inner Mongolia Autonomous Region (43°50' N, 116°34' E and 1100 m above the sea level). Semi-arid continental climate prevails in the area with cold, dry winters and mild, wet summers. The mean annual precipitation at the site is 350 mm, with most rain events occurring in July and August. Mean annual air temperature is -4°C. Mean monthly air temperatures range from 17.9°C in July to -23°C in January as recorded by a nearby meteorological station. Vegetation type is characterized by a typical temperate grassland, dominated by *Kochia prostrata*, *Artemisia frigida* and *Potentilla acaulis* that are non-N₂-fixing species. Legumes are rare in the plant community in this semi-arid grassland. Soils are coarse textured with a mean of 73% sand, 15% silt, and 9% clay across all experimental plots (Barger *et al.* 2004).

Five grazing intensity treatments were maintained for 17 years from 1989 to 2005. There were 0, 4, 8, 12 and 16 Inner Mongolia fine wool sheep grazing rotationally in three replicated 1-ha plots, representing the grazing intensities of 0, 1.33, 2.67, 4.00 and 5.33 sheep ha⁻¹, respectively. Every year, grazing started on 20 May and ended on 5 October. Each plot was rotationally grazed three times per year, each time for 15 days with a rotation interval of 30 days. The total grazing period in each grazed plot was thus 45 days per year. In order to investigate the restoration process, all the grazing intensity treatments were terminated since 2006.

Measurements and calculations of soil N cycling rates

Soil N cycling rates were measured periodically during the 2005 and 2006 growing seasons, approximately every 15 days during the warm and wet season in July and August or 30 days in other months. Seven randomly selected 2 × 2 m quadrates were demarcated and enclosed within each plot at the first sampling date in 2005 and used later for repeated sampling. All the seven quadrats were used to measure denitrification rates, and five of them were used to measure rates of mineralization, nitrification and ammonia volatilization.

Rates of net N mineralization and net nitrification were measured using the closed-top PVC (PolyVinyl Chloride) *in situ* incubation method (Raison *et al.* 1987) as described in detail in our previous study (Xu *et al.* 2007). Denitrification rates were measured using the acetylene inhibition technique (Yoshinari *et al.* 1977), by incubating minimally disturbed soil cores in

a closed system under field conditions as described by Ryden *et al.* (1987). NH_3 volatilization rates were determined using the method of H_2SO_4 solution trapping as described by Billings *et al.* (2002). Briefly, a beveled PVC tube (30 cm in length and 25 cm in diameter) was driven into the soil to a depth of 10 cm within each quadrat. A weigh boat containing 20 ml of 2% H_2SO_4 (v/v) was placed on a tripod at the bottom of the installed chamber. The top of each chamber was covered with aluminum foil secured with a rubber band. The acid solution was collected after the 24-h incubation and analysed for NH_4^+ concentration using an auto-analyser. The extra unincubated H_2SO_4 solution was used to analyse the concentration of background NH_4^+ . Daily rates of ammonia volatilization were calculated from the differences in NH_4^+ concentrations between the 24-h incubation and background samples.

Cumulative amounts at an annual time scale were calculated from the sum of magnitudes over all the intervals throughout each growing season. The amount at each sampling interval was calculated by multiplying mean daily rate by days of this interval. Mean daily rates of denitrification and ammonia volatilization at each interval were estimated as the mean values of two measurements spanning this interval, by using the method reported by Frank and Groffman (1998).

Measurements of $\delta^{15}\text{N}$

Soil and plant samples for $\delta^{15}\text{N}$ determination were collected at the seasonal peak of aboveground biomass in August 2006. Topsoil samples were taken from the extra unincubated soils for mineralization measurement. Surface soil samples were collected with a nipper from the 0- to 2-cm soil layer which is the main decomposition layer of surface litter. The recently expanded leaves of the most widely distributed species (*K. prostrata*) were collected. When sampling, we were careful not to include patches with legumes or cryptobiotic crust since these N_2 fixers could affect soil N sources and $\delta^{15}\text{N}$, even though they were rare in the experimental sites. Each sample was a composite obtained from the five quadrats or from nearby patches within each plot. The analytical method of ^{15}N is similar to that reported by Sah *et al.* (2006). In brief, fresh soil samples were air dried, and fresh plant samples were oven dried at 70°C for 48 h. All these

dry soil and plant samples were ground into fine powders with a mortar and pestle. The $\delta^{15}\text{N}$ of soil and plant samples was determined using a stable isotope mass spectrometer (Thermal Finnigan MAT DELTA^{plus}, Bremen, Germany).

Measurements of environmental factors

Unincubated soils for mineralization measurement were also used to measure pH (water:soil = 2.5:1), organic C content ($\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$ oxidation method) and organic N content (Kjeldahl digestion method). Soil bulk density was determined by the core method. Peak aboveground biomass was determined by the harvest method.

Statistical analysis

One-way analysis of variance was performed for comparison of the differences in $\delta^{15}\text{N}$ values between the five grazing intensities. Duncan's multiple range test was used to determine the significance of the differences. Linear contrasts at a significant level of 0.05 were used to compare group means. Pearson correlation and stepwise multiple linear regression analyses were conducted to detect relationships between soil $\delta^{15}\text{N}$ and N cycling processes. All statistical analyses were performed using SPSS version 13.0 software package.

RESULTS

Soil properties

The bulk densities of soils in the four grazing treatments were significantly higher than those of ungrazed soils ($P < 0.05$, Table 1), whereas there were no significant differences between the bulk densities of soils in the four grazing treatments ($P > 0.05$). In addition, no significant differences were found in other properties including aboveground biomass, organic N content of *K. prostrata*, soil organic C and N content and soil pH in all the five grazing intensities ($P > 0.05$).

$\delta^{15}\text{N}$ values of soils and plants

The $\delta^{15}\text{N}$ values of topsoils ranged from 5.20 to 5.96‰ in the five grazing intensities and were significantly higher than those of plants (2.51–2.93‰, $P < 0.001$) and surface soils (1.44–2.92‰, $P < 0.001$, Fig. 1). Significant positive

Table 1: peak aboveground biomass and physical and chemical properties of soils and plants at different grazing intensities in August 2006^a

Grazing intensities (sheep ha ⁻¹)	0.00	1.33	2.67	4.00	5.33
Aboveground plant biomass (g m ⁻²)	132.54 ± 15.11	112.65 ± 9.24	109.03 ± 3.02	113.98 ± 15.77	105.57 ± 8.09
Organic N of <i>Kochia prostrata</i> (g N kg ⁻¹)	11.61 ± 0.49	11.36 ± 0.52	10.43 ± 0.54	9.86 ± 0.78	11.45 ± 1.66
Soil bulk density (g cm ⁻³)	1.23 ± 0.05 ^b	1.35 ± 0.02 ^a	1.36 ± 0.04 ^a	1.33 ± 0.04 ^a	1.40 ± 0.03 ^a
pH value	7.08 ± 0.01	7.11 ± 0.04	7.03 ± 0.04	7.04 ± 0.06	7.03 ± 0.04
Soil organic C (g C kg ⁻¹)	17.19 ± 0.71	15.92 ± 1.20	14.91 ± 3.52	14.92 ± 4.52	13.73 ± 2.50
Soil total N (g N kg ⁻¹)	0.82 ± 0.01	0.76 ± 0.09	0.73 ± 0.10	0.73 ± 0.19	0.69 ± 0.13

^a Numbers within rows followed by different letters in superscript are statistically significant at $P < 0.05$ level. Means ± standard error, three replicates.

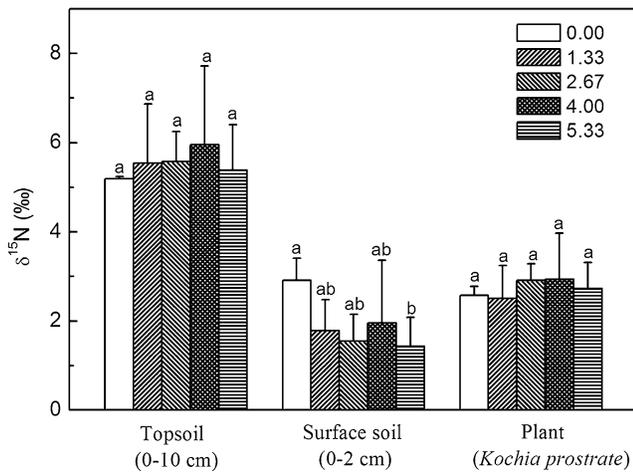


Figure 1: patterns of $\delta^{15}\text{N}$ (‰) of plants, topsoils and surface soils at different grazing intensities. Each bar is the mean of three replicated plots. Error bars represent ± 1 standard error; 0.00, 1.33, 2.67, 4.00 and 5.33 (sheep ha^{-1}) denote that 0, 4, 8, 12 and 16 sheep grazed rotationally in three replicated 1-ha plots, respectively.

correlations between $\delta^{15}\text{N}$ values of topsoils and those of plants and surface soils were found across the 15 plots ($P < 0.05$, Fig. 2).

Effects of grazing intensity

No statistically significant differences between $\delta^{15}\text{N}$ values of topsoils or plants in different grazing intensities were found at the level of $P < 0.05$ (Fig. 1). Nevertheless, the four grazing treatments, especially the moderate-intensity grazing (4.00 sheep ha^{-1}), tended to have higher $\delta^{15}\text{N}$ values. In contrast, there were significant differences between $\delta^{15}\text{N}$ values of surface soils in different grazing intensities ($P < 0.05$, Fig. 1). The four grazing treatments generally resulted in decreases of 1.13–1.48‰ in $\delta^{15}\text{N}$ of surface soils, and the treatment with the highest grazing intensity (5.33 sheep ha^{-1}) led to a significant reduction compared with the no-grazing treatment ($P < 0.05$).

Relationships between $\delta^{15}\text{N}$ and N cycling processes

As for $\delta^{15}\text{N}$ of topsoils in relation to N cycling processes in 2005, strong positive correlations were detected between the $\delta^{15}\text{N}$ values of topsoils and the cumulative NH_3 volatilization rates ($r^2 = 0.39$, $P < 0.01$) and denitrification rates ($r^2 = 0.31$, $P < 0.05$, Fig. 3). Whereas the $\delta^{15}\text{N}$ values of topsoils were not correlated with either the cumulative net N mineralization or the nitrification rates ($P > 0.05$, Fig. 4). The $\delta^{15}\text{N}$ values of topsoils were negatively correlated with the concentrations of soil nitrate N ($r^2 = 0.44$, $P < 0.01$) and total inorganic N ($r^2 = 0.32$, $P < 0.05$, figure not shown) by the end of the 2005 growing season (October). When stepwise multiple regressions were applied, the cumulative NH_3 volatilization and nitrification rates were significant variables ($P < 0.05$),

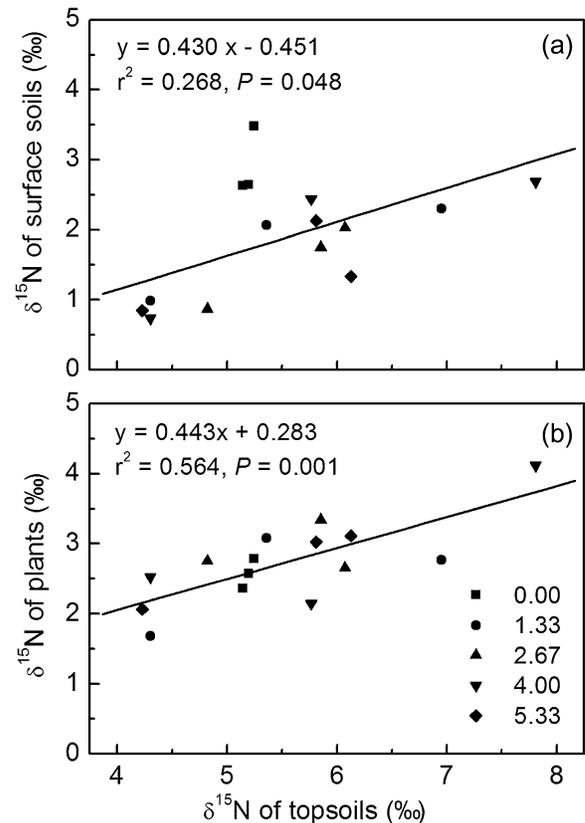


Figure 2: correlations of the $\delta^{15}\text{N}$ values (‰) of topsoils with those of plants and surface soils. The regression line was calculated using the values across the 15 plots. See Fig. 1 for the grazing intensity abbreviations.

and the combination of the two processes explained 59.5% of the variations in $\delta^{15}\text{N}$ of topsoils.

As for $\delta^{15}\text{N}$ of topsoils in relation to N cycling processes in 2006, no strong correlations were detected between the $\delta^{15}\text{N}$ values of topsoils and the rates of N cycling ($P > 0.05$). Only the cumulative NH_3 volatilization rates showed a marginal negative correlation with the $\delta^{15}\text{N}$ values of topsoils ($r^2 = 0.26$, $P = 0.053$). When stepwise multiple regressions were conducted, none of the N cycling rates was a significant variable.

DISCUSSION

Variations in $\delta^{15}\text{N}$

The range of 5.20–5.96‰ for the $\delta^{15}\text{N}$ of topsoils in this grassland was within the broader ranges observed in other grasslands, e.g. the ranges from 1.4 (ungrazed grassland) to 7.9‰ (grazed grassland) in soils of Yellowstone national park (Frank and Evans 1997) and from 1.0 to 8.1‰ in soils spanning 3–3000 Ky in a California annual grassland (Brenner *et al.* 2001). The range of 2.51–2.93‰ for the $\delta^{15}\text{N}$ of plants in the present study was also within the range of –2.4 to 6.8‰ for the $\delta^{15}\text{N}$ of plants in Inner Mongolian in an earlier

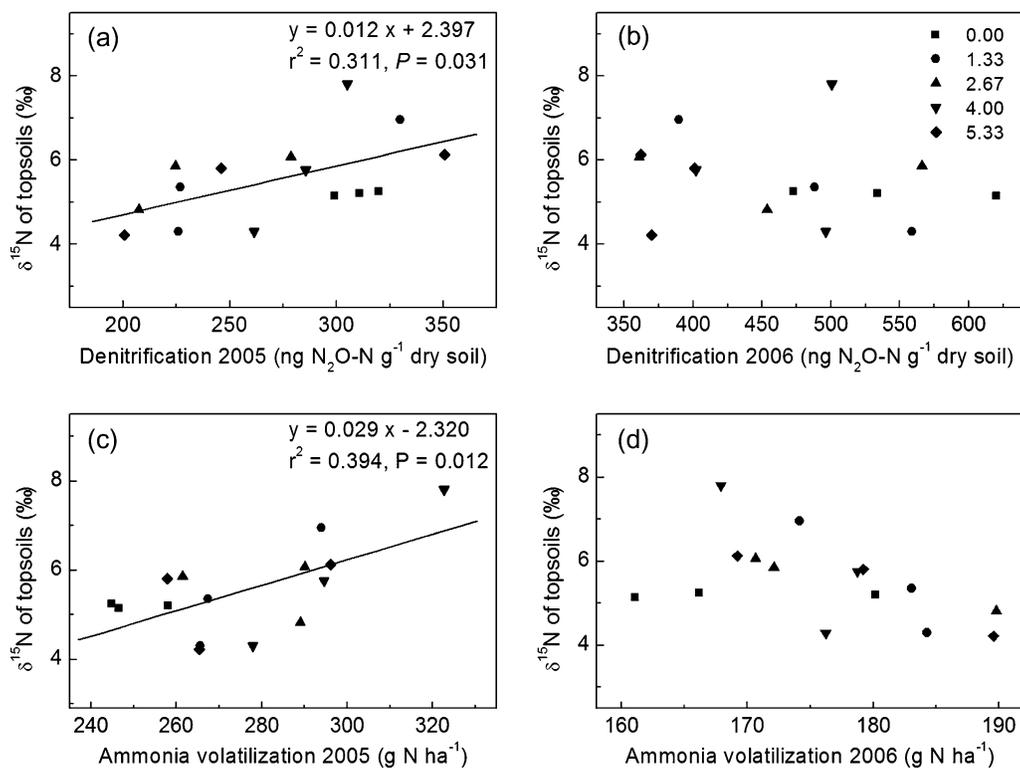


Figure 3: correlations of the $\delta^{15}\text{N}$ values (‰) of topsoils in 2006 with the cumulative denitrification rates ($\text{ng N}_2\text{O-N g}^{-1}$ dry soil) and NH_3 volatilization rates (g N ha^{-1}) in 2005 and 2006. The regression line was calculated using the values across the 15 plots. See Fig. 1 for the grazing intensity abbreviations.

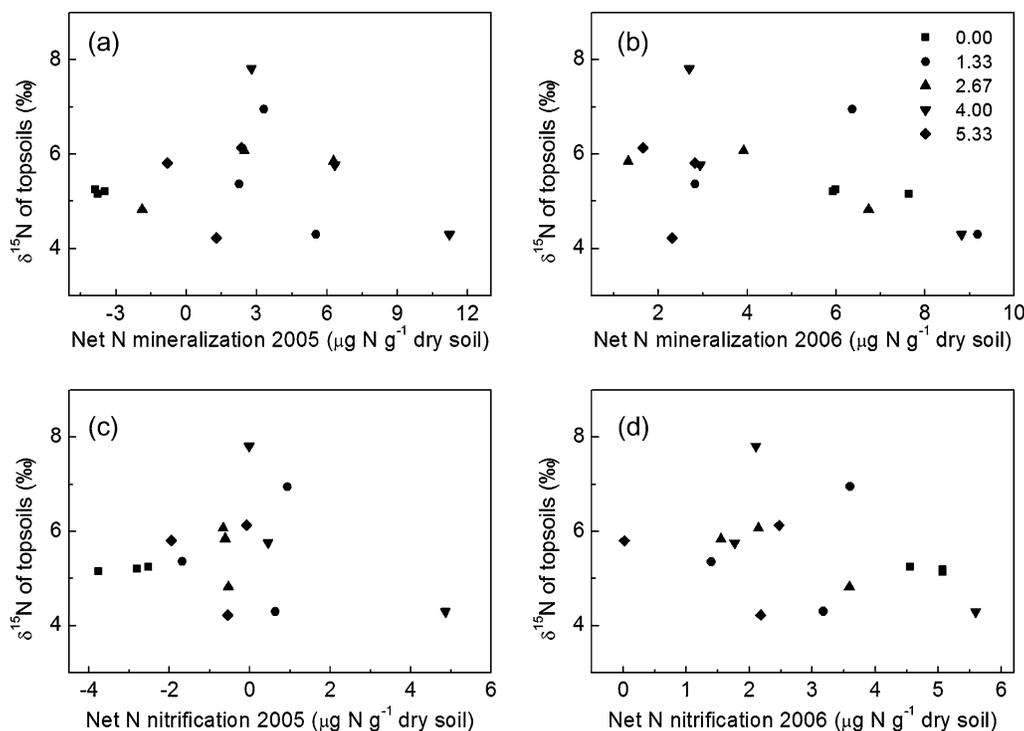


Figure 4: correlations of the $\delta^{15}\text{N}$ values (‰) of topsoils in 2006 with the cumulative net N mineralization and nitrification rates ($\mu\text{g N g}^{-1}$ dry soil) in 2005 and 2006. See Fig. 1 for the grazing intensity abbreviations.

study (Handley *et al.*, 1999). These narrower ranges for the $\delta^{15}\text{N}$ might be associated with the relatively uniform distributions of soil and vegetation as well as the controlling factors at this experimental site.

Topsoils had higher $\delta^{15}\text{N}$ values than surface soils, consistent with most previous reports that $\delta^{15}\text{N}$ of soils increased with increasing soil depth (Adams and Grierson 2001; Högberg 1997; Ledgard *et al.* 1984). This phenomenon could be explained by the ^{15}N -depleted N losses during microbial decomposition of organic matter in concert with the downward movement of residual substrate over time (Evans and Ehleringer 1994b).

^{15}N abundance in non- N_2 -fixing plants can be used to determine the source of plant N, the soil depth at which N is taken up and the form of N used (Nadelhoffer *et al.* 1996). No significant differences were found between the $\delta^{15}\text{N}$ values of plants in the five grazing intensities in this study, reflecting that the major N source and the form of N utilized by these plants did not differ between different grazing intensities.

Controlling factors for $\delta^{15}\text{N}$

Some studies showed that grazing could lead to an increase in $\delta^{15}\text{N}$ (Frank and Evans 1997; Frank *et al.* 2000, 2004; Sutherland *et al.* 1993), while no apparent effects of grazing on $\delta^{15}\text{N}$ have also been reported (Austin and Sala 1999; Neilson *et al.* 1998). We found that $\delta^{15}\text{N}$ of topsoils changed little even over a 17-year grazing at varying levels of intensity. One often used explanation is that $\delta^{15}\text{N}$ of soil total N, dominated by the isotopic signature of stable N, is a stable index and is unlikely to change over decades (Johannisson and Högberg 1994).

In contrast, grazing, especially that with the highest grazing intensity, had a negative influence on $\delta^{15}\text{N}$ of surface soils. This indicates a negative feedback on discrimination against ^{15}N of surface soil, particularly under high grazing pressure. An interpretation of this observation is difficult because a single effect of grazing on $\delta^{15}\text{N}$, via dropping feces, trampling and feeding plants, is often positive (Frank and Evans 1997; Frank *et al.* 2000, 2004; Schulze *et al.* 1998). However, a study in Namibia (Schulze *et al.* 1991) revealed an interesting phenomenon that $\delta^{15}\text{N}$ increased in the region of main grazing activity, while $\delta^{15}\text{N}$ decreased again in the less grazed but more arid part. Therefore, we guess that the interaction between aridity and grazing might have played an important role in determining $\delta^{15}\text{N}$ of surface soils in this semi-arid grassland.

The $\delta^{15}\text{N}$ values of topsoils were highly correlated with the cumulative rates of ammonia volatilization, nitrification and denitrification in 2005 but not in 2006. This result suggests that gaseous N losses and the proportion of NO_3^- consumed might be the major factors controlling $\delta^{15}\text{N}$ of topsoils in this semi-arid grassland under certain conditions, as has been reported by Bai and Houlton (2009). In addition, it implies that $\delta^{15}\text{N}$ of topsoils can be used as an indicator of N losses via ammonia volatilization, nitrification and denitrification under some conditions.

Nevertheless, other ways of N loss via leaching and N_2O emission by nitrification, and N input via N deposition and N_2 fixation might also be responsible for the pattern of $\delta^{15}\text{N}$ of topsoils. Additionally, differences in extrinsic controlling factors such as urine and dung patches (Frank and Evans 1997; Frank *et al.* 2004) between 2005 and 2006 might also play a critical role hereof. However, in this study, only a 2-year observation was conducted, and the effects of other factors are also beyond the scope of this analysis. It is certain that we failed to measure all the related factors in sufficient detail due to the limitations in time and condition. This highlights the needs for a further study to ascertain these interpretations suggested. We will attempt to make up for these limitations while simultaneously enhance research capacity in future studies.

CONCLUSIONS

The $\delta^{15}\text{N}$ values of topsoils were substantially higher than those of plants and surface soils regardless of grazing intensities. Changes in grazing intensity did not lead to significant variations in $\delta^{15}\text{N}$ of topsoils and plants, but resulted in a significant decrease in $\delta^{15}\text{N}$ of surface soils. The $\delta^{15}\text{N}$ values of topsoils were significantly correlated with the cumulative rates of NH_3 volatilization, net nitrification and denitrification in 2005 but not in 2006. Our findings suggest that $\delta^{15}\text{N}$ of topsoils might be used as an indicator of N cycling rates concerning gaseous N losses via ammonia volatilization and N_2O emission under certain conditions. However, further studies are needed to ascertain these mechanisms suggested.

Funding

State Key Basic Research and Development Program of China (2010CB833502); Chinese Academy of Sciences (KZCX2-XB2-01).

ACKNOWLEDGEMENTS

We thank the staff of the Inner Mongolian Grassland Ecosystem Research Station, the Chinese Academy of Sciences for providing the meteorological data, Dr Shiping Wang for the maintenance of the field experiment plot and Mr Yan Zhiqiang for his assistance in the field. We would like to express our gratitude for the valuable and constructive comments from the anonymous reviewers, and we are also highly appreciative of the editors' care and much patience in handling our manuscript.

Conflict of interest statement. None declared.

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