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Effect of nitrogen fertilization on net nitrogen mineralization in a grassland soil, northern China

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Abstract

Nitrogen (N) applications can have a significant effect on soil N availability. The effect of 3 years of N fertilization on soil net N mineralization during the growing season (May–September) was studied in 2005 and 2006 in grassland of northern China. The experimental design was a randomized complete block with four replications of five rates of N addition as urea (0, 2, 4, 8 and 16 g N m^{-2} $year^{-1}$). Results indicated that net N mineralization rate varied seasonally and between years, ranging from −0.04 to 0.52 $\mu g\ g^{-1}\ d^{-1}$ in 2005 and from −0.09 to 0.39 $\mu g\ g^{-1}\ d^{-1}$ in 2006. Mean N mineralization and nitrification rates were highest in July, in 2005 and 2006, whereas highest ammonification rates occurred in September. Rainfall was significantly correlated with net nitrification. In comparison with the untreated control, N mineralization increased sharply when N fertilization increased from 2 to 8 g N m^{-2} $year^{-1}$. Mobile soil NO_3^- accumulated late in the growing season for the 16 g N m^{-2} $year^{-1}$ treatment, suggesting the potential for NO_3 and associated cation leaching. These results suggest that N fertilization of 8 g N m^{-2} $year^{-1}$ (80 kg N ha^{-1}) is suitable for the management of grassland ecosystems of Inner Mongolia.

Keywords: managed grasslands, N fertilization, N mineralization, nitrification, Mongolia

Introduction

Soil nitrogen (N) availability is frequently the most limiting factor for plant productivity in temperate terrestrial ecosystems (Pastor *et al.*, 1984; Turner *et al.*, 1997; Galloway *et al.*, 2008). Along with light and water availability (Knapp and Gilliam, 1985; Gilliam *et al.*, 1987), N commonly limits productivity in grasslands (Gilliam, 1987; Blair, 1997; Knapp *et al.*, 1998), with mineralization of organic N to inorganic forms as the primary process maintaining soil N availability. Thus, an understanding of the dynamics of soil N mineralization is essential for land managers to refine prescriptions for N fertilizer application for grassland ecosystems. Indeed, N fertilizers can play an important role in regulating soil N transformations (Raun *et al.*, 1998; Vourlitis *et al.*, 2007; van der Krift and Berendse, 2001).

A number of studies have been carried out to investigate the effects of N fertilization on soil N transformation and N availability. These have yielded conflicting results, precluding broad generalizations and necessitating site-specific examination of fertilizer ⁄ N mineralization relationships. Some studies reported that N fertilization enhanced soil net N mineralization (Brenner *et al.*, 2005; Dijkstra *et al.*, 2005; Sirulnik *et al.*, 2007), whereas others have indicated that fertilization either had no significant effects (Chappell *et al.*, 1999; Antil *et al.*, 2001; Gilliam *et al.*, 2001; Nohrstedt, 2002), or have demonstrated negative effects (Carpenter-Boggs *et al.*, 2000; Fisk and Fahey, 2001; Soon and Malhi, 2005). Such wide-ranging responses may partly reflect variability in soil nutrient status, types of fertilizers used and periods over which they were applied, or differential responses to varying levels of N fertilization in contrasting ecosystem types (Aggangan *et al.*, 1998).

Temporal variations in soil N transformation may be correlated with seasonal patterns of environmental conditions (Vitousek and Matson, 1985; Amador *et al.*, 2005; Zhang *et al.*, 2008). Some studies have shown that net N mineralization rates are positively correlated with seasonal temperature fluctuation, but less sensitive to soil moisture content (Hatch *et al.*, 1991; Sierra, 1997), whereas others indicate that temporal variations

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in soil N mineralization was poorly correlated with soil moisture and soil temperature (Trindade *et al.*, 2001). Gilliam *et al.* (2001) found that both ambient temperature and soil moisture significantly controlled net N mineralization and nitrification in untreated hardwood-forest soil, but not in the case of N-treated hardwood-forest soil. The effects of the physical environment on soil N mineralization must also be considered if the relationship between N fertilizer and soil N availability/mobility is to be fully understood.

Grassland in northern China is a major vegetation type of Eurasia (Li *et al.*, 1998). Beginning in the late 1970s, overgrazing has greatly altered many of these grasslands (Liu *et al.*, 2006), resulting in land degradation and soil N deficiency (Christensen *et al.*, 2004; Yuan *et al.*, 2006; Huang *et al.*, 2008). To restore these ecosystems and improve their biomass productivity, new management practices, including N fertilization, have been carried out since 2000 (Zhang *et al.*, 2010). Excessive N fertilization in both grassland and agricultural areas has resulted in serious environmental problems, including NO_3^- leaching and release of N_2O, a greenhouse gas (Ju *et al.*, 2009; Guo *et al.*, 2010; Zhang *et al.*, 2010). Often, increased N fertilization fails to result in increased plant productivity (Wang *et al.*, 2001). Therefore, studies are required to determine suitable levels of N fertilization for grassland soils in China, and to optimize biomass production while minimizing potential negative effects of excess N, such as acidification, N and associated cation leaching, and emissions of gaseous N.

In this study, we investigated the suitability of N fertilizer use in a grassland of Inner Mongolia for which we used five levels of experimental N addition. The objective of this research was to provide preliminary results to help inform recommendations of suitable N fertilizer application rates and management practices for restoration of grasslands of Inner Mongolia. More specifically, we examined the effects of adding varying levels of N fertilizer on above-ground grass growth and net N mineralization and nitrification of grassland soils.

Material and methods

Study site and experimental design

The experiment was carried out at the Duolun Restoration Ecology Research Station of the Chinese Academy of Sciences (CAS), located in Duolun County (41°49′–42°27′N; 115°56′–116°51′E; 1344 m a.s.l.), in the centre of the Inner Mongolia Autonomous Region, China. The dominant species in this typical grassland are *Stipa krylovii* Roshev. and *Artemisia frigida* Willd. The mean annual (1994–2003) soil temperature (0–5 cm depth) is 5.3°C, ranging from minimum in January (−16.7°C) to maximum in July (24.3°C), with mean soil temperature from 14.9 to 24.3°C during the growing season (May–September). The mean annual (1994–2003) precipitation is 401 mm of which 353 mm falls between May and September. Weather data were obtained from the Duolun meteorological station. During the period of field incubation in 2005 and 2006, weather data were recorded by a complete micrometeorological system installed on the eddy towers close to the experimental plots. The soil type is classified by a Chinese classification as 'chestnut soil' and belongs to the Calcisorthic Aridisol in the United States Soil Taxonomy classification (Yuan *et al.*, 2005).

We employed a randomized complete block design, with four replications of five rates of N addition: 0 (N_0 : control), 2 g N m^{-2} year $^{-1}$ (N_2), 4 g N m^{-2} $year^{-1}$ (N_4), 8 g N m^{-2} $year^{-1}$ (N_8) and 16 g N m^{-2} $year^{-1}$ (N_{16}). These amounts were equivalent to N fertilizer rates of 0, 20, 40, 80 and 160 kg N ha^{-1}, respectively. Established in 2003, sample plots were 10 × 15 m, with a minimum buffer zone of 4 m between plots. Nitrogen was applied as urea annually on July 15. Neither phosphorus nor potassium fertilizers were used in this study.

Grass biomass and soil sampling and analysis

Peak green biomass (Table 1) was measured in mid-August each year (2005 and 2006) by clipping in 1 × 1 m quadrats on each plot. Dry weight was determined by oven drying all plant samples at 70°C to constant weight.

Net N mineralization was measured monthly during the growing season (May–September) in 2005 and 2006 using intact soil core incubations (Raison *et al.*, 1987). A pair of PVC tubes (5.0 cm diameter × 15 cm long) was driven 10 cm into the soil in each plot each month. One tube was sampled immediately and measured as initial soil inorganic N (NH_4-N + NO_3-N) concentration. Another one was capped with plastic film; eight 1-mm diameter holes were punctured through the film on each tube, and all tubes left in the field for 30 d. After collection, incubated soil was extracted for the final concentration of inorganic N.

Both initial and incubated soils were sieved (2 mm mesh) to remove large organic materials. Approximately 10 g of sieved fresh soil was analysed and extracted for inorganic N concentration with 50 mL of 2M KCl for 1 h on a variable speed reciprocal shaker (Apparatus Co. Ltd. Changzhou, China). Soil extracts were analysed for NH_4-N and NO_3-N by a Segment Flow Analyser (Scalar SAN^{plus}, Breda, The Netherlands) in the laboratory of Institute of Botany, Chinese Academy of Sciences.

Table 1 Soil properties under different N fertilizer treatments and their aboveground biomass in August (Values are means plus standard errors and $n = 4$).

Treatment	Total organic N (g kg^{-1} dry soil)	Total organic C (g kg^{-1} dry soil)	C/N ratio	pH	Harvested aboveground biomass (g m^{-2})	
					2005	2006
N_0	2.05 (0.16)^{a}	20.93 (1.68)^{a}	10.2 (0.3)^{a}	7.30 (0.07)^{b}	113.4 (4.7)^{a}	151.5 (16.6)^{a}
N_2	2.17 (0.04)^{ab}	21.44 (0.85)^{a}	9.9 (0.3)^{a}	7.27 (0.05)^{b}	131.3 (7.8)^{a}	165.6 (7.6)^{a}
N_4	2.28 (0.15)^{ab}	23.73 (1.24)^{a}	10.5 (0.2)^{a}	6.97 (0.07)^{b}	162.9 (16.9)^{b}	251.4 (38.1)^{a}
N_8	2.31 (0.07)^{ab}	24.37 (0.85)^{a}	10.6 (0.5)^{a}	6.92 (0.25)^{b}	170.4 (0.9)^{b}	393.8 (61.1)^{b}
N_{16}	2.53 (0.12)^{b}	24.65 (1.24)^{a}	9.8 (0.6)^{a}	6.41 (0.24)^{a}	176.8 (9.0)^{b}	516.0 (24.5)^{c}

Letters indicate statistical significance among the treatments at $P < 0.05$ level.

Net N mineralization was calculated as the difference between post- and pre-incubation inorganic N values, net nitrification as the difference between post- and pre-incubation NO_3-N concentrations and net N ammonification as the difference between post- and pre-incubation NH_4-N concentrations. Annual accumulations (during the growing season) of net N mineralization, nitrification and ammonification were determined by summing mineralized, nitrified and ammonified N for all of the incubation periods (Pastor *et al.*, 1984; Subler *et al.*, 1998; Trindade *et al.*, 2001).

Soil moisture content was determined after drying at 105°C for 24 h in each sampling time. Subsamples of soil collected in June 2005 were air dried, passed through 0.5 mm mesh and used to analyse soil properties (Table 1). Soil total nitrogen (TN) was measured using Kjeldahl acid-digestion method with an Alpkem autoanalyzer (Kjektec System 1026 Distilling Unit, Sweden); soil organic carbon (SOC) was determined using the H_2SO_4–$K_2Cr_2O_7$ oxidation method, and soil pH was determined by mixing soil with deionized water (1:2 w/v).

Statistical analyses

Effects of sampling time and N fertilizer treatments on soil inorganic N concentration and net N transformation rates were tested using repeated measures ANOVA in a general linear model, with sampling time and fertilizer treatments used as main effects. One-way ANOVA was used to test the difference in soil inorganic N concentration, net N mineralization and accumulation of mineral N among fertilizer treatments; Duncan's multiple range test was used to compare differences at $P = 0.05$. Differences in soil inorganic N, soil N transformation and cumulative mineral N between 2005 and 2006 were compared by paired *t*-test. Pearson product-moment correlation was used to determine the relationship between net N mineralization and meteorological variables in each year. Linear stepwise regression was used to determine the relationship between soil temperature, rainfall, soil water content and soil N transformation in 2005 and 2006. All statistical analyses were performed using SPSS 10.0 (Chicago, IL, USA) procedures.

Results

Meteorological variables and soil inorganic N concentrations

Over the growing season, daily soil temperature (0–5 cm) ranged from 11.2 to 22.2°C in 2005, from 12.7 to 21.0°C in 2006 and was highest in July (Figure 1). Rainfall in 2005 (655 mm) was 86% higher than the 10-year mean (353 mm, from 1994 to 2003), decreasing in 2006 to 394 mm. Seasonal patterns for soil moisture among the five fertilizer treatments were similar in both 2005 and 2006 (Figure 2). Mean soil moisture did not vary significantly among the five fertilizer treatments, but did vary significantly with sampling time (Table 2).

Soil NH_4-N concentration varied from 0.88 to 5.98 μg N g^{-1} soil in 2005 and from 2.56 to 14.62 μg N g^{-1} soil in 2006 (Figure 3) and increased gradually during the periods of incubation in 2005 and 2006. In comparison with the control, soil NH_4-N in the four fertilizer treatments increased by 1.9, 4.3, 29.4 and 49.4% in 2005 and 1.9, 8.6, 12.2 and 77.5% in 2006, respectively. Mean NH_4-N concentration in 2006 was 169% higher than in 2005 ($t = -16.57$, $P < 0.0001$). Soil NO_3-N concentration ranged from 0.18 to 7.66 μg g^{-1} in 2005 and from 0.52 to 22.71 μg g^{-1} in 2006 (Figure 3). There were significant differences in NO_3-N concentrations among fertilizer treatments in 2005 ($F_{4,19} = 175.04$, $P < 0.0001$) and in 2006 ($F_{4,19} = 36.29$, $P < 0.0001$). A paired t-test indicated that soil NO_3-N concentration was significantly higher in 2006 than in 2005 ($t = -11.48$, $P < 0.0001$).

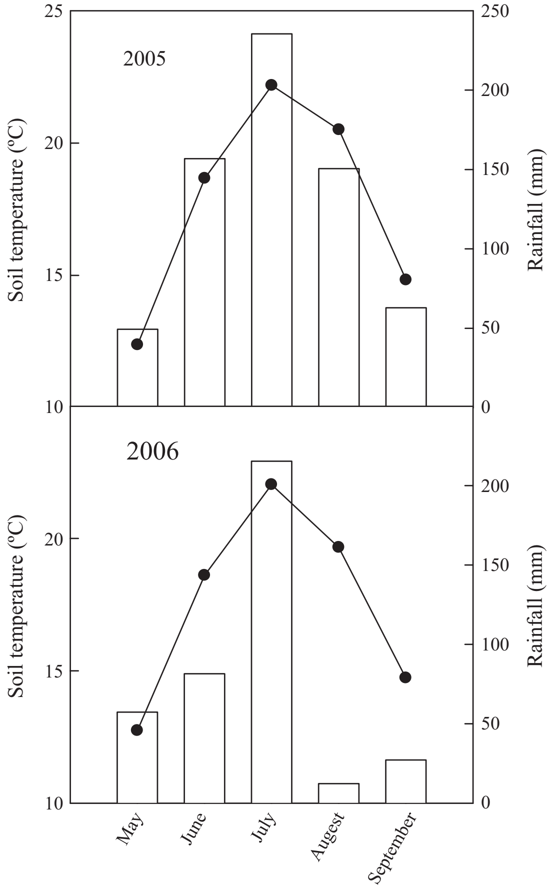

Figure 1 Variations in monthly soil temperatures (0–5 cm) and precipitation during the periods of incubation (May–September) in 2005 and 2006. Lines: mean monthly soil surface temperature during the growing season in 2005 and 2006; white bars: mean monthly precipitation during the growing season in 2005 and 2006.

Net N transformations

Soil net N mineralization rates ranged from −0.04 to 0.52 μg N g^{-1} soil d^{-1} during the growing season in 2005 and from −0.08 to 0.39 μg N g^{-1} soil d^{-1} in 2006 (Figure 4), with higher values occurring in July both in 2005 and 2006. In comparison with the control, mean mineralization rate in the four fertilizer treatments increased by 485% in 2005 ($F_{4,19} = 22.52$, $P < 0.0001$) and 451% in 2006 ($F_{4,19} = 35.69$, $P < 0.0001$), respectively (Figure 5). No significant difference in net N mineralization rate was observed between 2005 and 2006 ($t = 0.56$, $P = 0.58$).

Net nitrification rates exhibited seasonal patterns similar to those of net N mineralization (Figure 4), ranging from −0.03 to 0.45 μg N g^{-1} soil d^{-1} in 2005 and from −0.05 to 0.48 μg N g^{-1} soil d^{-1} in 2006. The highest means in both 2005 (0.28 μg N g^{-1} soil d^{-1}) and 2006 (0.30 μg N g^{-1} soil d^{-1}) occurred in July. There were significant differences in net nitrification rates among fertilizer treatments in 2005 ($F_{4,19} = 21.27$, $P < 0.0001$) and in 2006 ($F_{4,19} = 143.69$, $P < 0.0001$).

Net ammonification varied significantly over the periods of incubation, with highest rates among treatments occurring in September in both years (Figure 4). There were significant differences among fertilizer treatments in 2005 ($F_{4,19} = 9.54$, $P < 0.0001$) and in 2006($F_{4,19} = 4.85$, $P = 0.01$), and the mean rate in 2005 (0.05 ± 0.01 μg N g^{-1} soil d^{-1}) was higher than that in 2006 (0.03 ± 0.01 μg N g^{-1} soil d^{-1}).

Repeated measures ANOVA indicated that N fertilizer treatments significantly affected soil N transformations (Table 2). Soil N transformations varied significantly with sampling time; the interaction of fertilizer rates × sampling time was also significant (Table 2).

Net nitrification was positively correlated with soil temperature and rainfall in 2005 and with rainfall in 2006 (Table 3). Relationships between soil temperature and N mineralization in 2005, and rainfall and soil nitrification in both 2005 and 2006 were all significant. Results of linear stepwise regressions suggested that rainfall explained 88% of the variability in soil nitrification in 2005 ($R^2 = 0.88$, $F = 21.97$, $P = 0.018$). In 2006, rainfall and soil moisture explained 90% ($R^2 = 0.90$, $F = 27.19$, $P = 0.014$) and 99% ($R^2 = 0.99$, $F = 112.27$, $P = 0.009$) of variation, respectively.

Accumulation of mineral N

There were significant differences in accumulation of mineral N among the five treatments in 2005 and 2006 (Figure 6); total mineral N in four fertilizer treatments was, respectively, 2.7, 5.3, 6.7 and 6.7 times higher than the control. In 2006, the mean of total mineral N in the four fertilizer treatments was 498% higher than that in control, and the difference among the five treatments was significant ($F_{4,19} = 45.75$, $P < 0.0001$). The difference in cumulative mineral N between 2005 and 2006 was not significant ($T = 0.94$, $P = 0.36$). The difference in cumulative nitrified N among five treatments showed similar patterns with mineral N in both 2005 and 2006. Cumulative net N ammonification of the five treatments (except for N_0) was lower than the cumulative net nitrification both in 2005 and 2006. The difference in cumulative ammonification rate between 2005 and 2006 was significant ($t = 2.17$, $P = 0.04$).

Effects of N fertilization on grass biomass

Grass biomass increased with N additions in both years, but this was significant only in 2006 for the 8 and

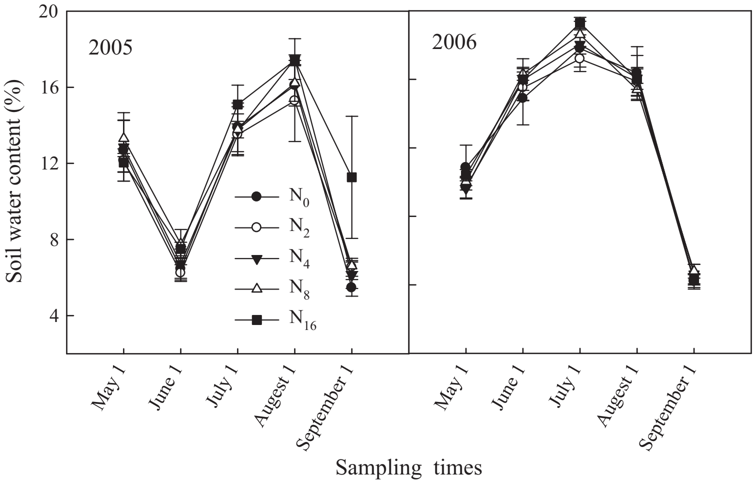

Figure 2 Temporal variations in soil water content for the N fertilizer treatments during the periods of incubation in 2005 and 2006. Values are means plus or minus standard errors (n = 4).

Table 2 Results of *F* tests based on repeated measures ANOVA for soil water content (SWC) (%), soil inorganic N concentration (μg N g^{-1}) before incubation and soil net N mineralization rate (μg N g^{-1} d^{-1}) under different N fertilizer treatments for the growing season.

		Fertilizer		**Time**		**Time × Fertilizer**	
		F	***P***	***F***	***P.***	***F***	***P***
2005	SWC%	1.31	0.31	119.53	0	0.88	0.59
	NO_3-N	175.04	<0.001	15.47	<0.001	12.61	<0.001
	NH_4-N	9.02	0.001	14.94	<0.001	1.87	<0.001
	Inorganic N	86.65	<0.001	11.69	<0.001	4.92	<0.001
	Mineralization	22.52	<0.001	102	<0.001	3.50	0.001
	Nitrification	21.27	<0.001	12.02	<0.001	4.69	<0.001
	Ammonification	9.54	<0.001	104.31	<0.001	6.35	<0.001
2006	SWC%	0.16	0.95	441.48	0	1.41	0.19
	NO_3-N	36.29	<0.001	2185.28	<0.001	24.69	<0.001
	NH_4-N	38.54	<0.001	87.18	<0.001	3.86	<0.001
	Inorganic N	88.96	<0.001	371.87	<0.001	9.53	<0.001
	Mineralization	35.69	<0.001	15.92	<0.001	2.66	0.007
	Nitrification	143.69	<0.001	59.61	<0.001	3.66	0.001
	Ammonification	4.87	0.01	58.24	<0.001	7.53	<0.001

16 g N m^{-2} year^{-1} treatments (Table 1). When the effects of N fertilizers were assessed as the ratio of grass biomass for each N treatment to that of the control, the significantly positive linear relationship up to 8 g N m^{-2} year^{-1} overestimated biomass found for the 16 g N m^{-2} year^{-1} treatment (Table 1, Figure 7).

Discussion

Effects of N fertilization on net N mineralization

The range of daily rates of net N mineralization for the top 10 cm of soil (–0.08 to 0.53 μg N g^{-1} soil d^{-1}) was similar to values reported for Inner Mongolian grassland soils by Xu *et al.* (2007) and Zhang *et al.* (2008). The relationship between N fertilizer and net N mineralization/nitrification indicated a direct response up to 8 g N m^{-2} year^{-1}, with further, smaller increases beyond that level of N addition (Figure 5). This suggests that N fertilizer treatments in excess of 8 g N m^{-2} year^{-1} (80 kg N ha^{-1}) may lead to N-saturated soils, wherein N supply exceeds biotic demand for N (Gilliam *et al.*, 2005). The pattern of increases in extractable NO_3^- in August and September of both 2005 and 2006 for the N_{16} treatment (Figure 3) is consistent with this notion. The possibility of N saturation owing to N additions represents a potentially

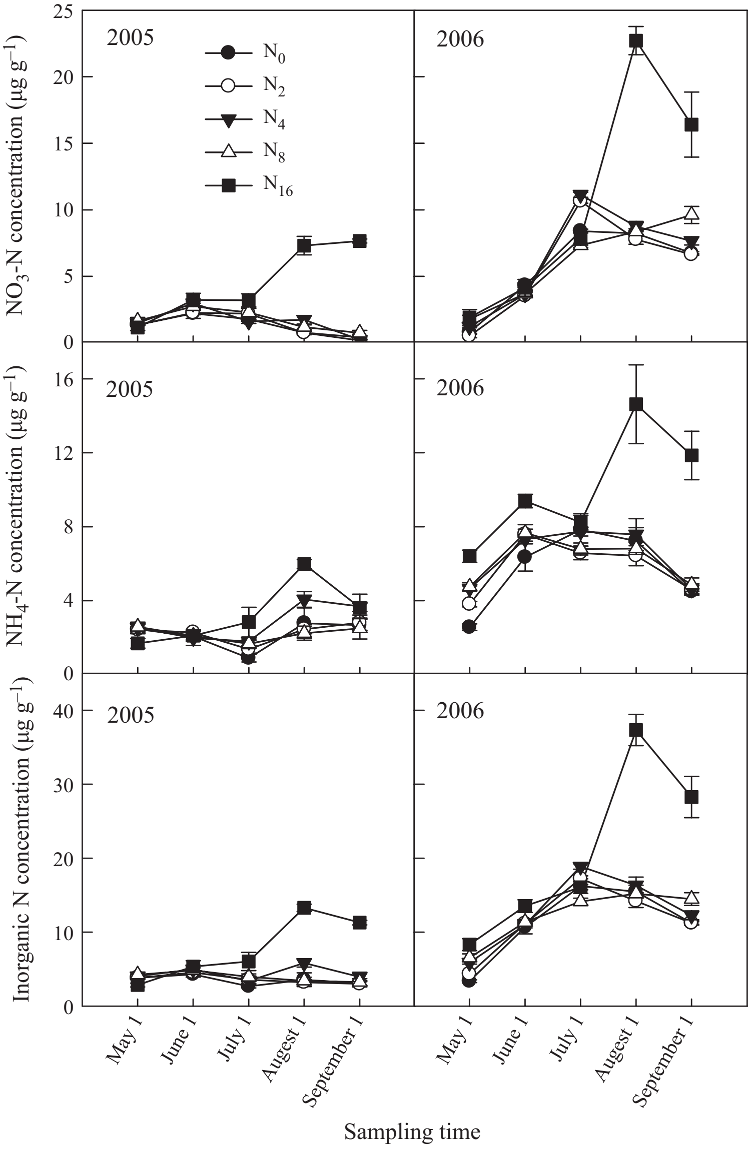

Figure 3 Temporal variations in soil NO_3-N, NH_4-N and soil inorganic N concentrations for the N fertilizer treatments during the periods of incubation in 2005 and 2006. Values are means plus or minus standard errors ($n = 4$). Nitrogen was applied as urea on July 15 each year.

serious threat to long-term sustainability and productivity of soils because increases in highly mobile NO_3^- from N saturation can (i) facilitate leaching losses of essential base cations, for example calcium (Ca) and magnesium (Mg), and (ii) create nutrient imbalances in plant tissue (Gilliam *et al.*, 2001, 2005). Indeed, increases in extractable NO_3^- for the N_{16} treatment are coincidental with the end of the growing season, a time when plant uptake declines rapidly, allowing for more leaching losses from the rooting zone.

Additions of N fertilizer could profoundly affect soil N transformation directly or indirectly through the alteration of abiotic and biotic characteristics of soil and soil organic matter quality. Nitrogen fertilizer may affect N mineralization directly by increasing soil nutrition (Delin and Linden, 2002; Güleryüz *et al.*, 2008; Kadono *et al.*, 2008), by building up readily mineralizable soil organic N and by stimulating microbial activity (Aggangan *et al.*, 1998).

Differences in plant biomass among the fertilizer treatments (Table 1) may explain the reason that N fertilization affected soil N mineralization indirectly. Following N fertilization in grasslands, plant production can regulate relationships between species dominance and N availability (Huang *et al.*, 2008). It can also alter rates of litter decomposition (Aerts *et al.*, 2006; Liu *et al.*, 2006). All of these can influence soil microbial activity, mediate conversion of soil organic N to

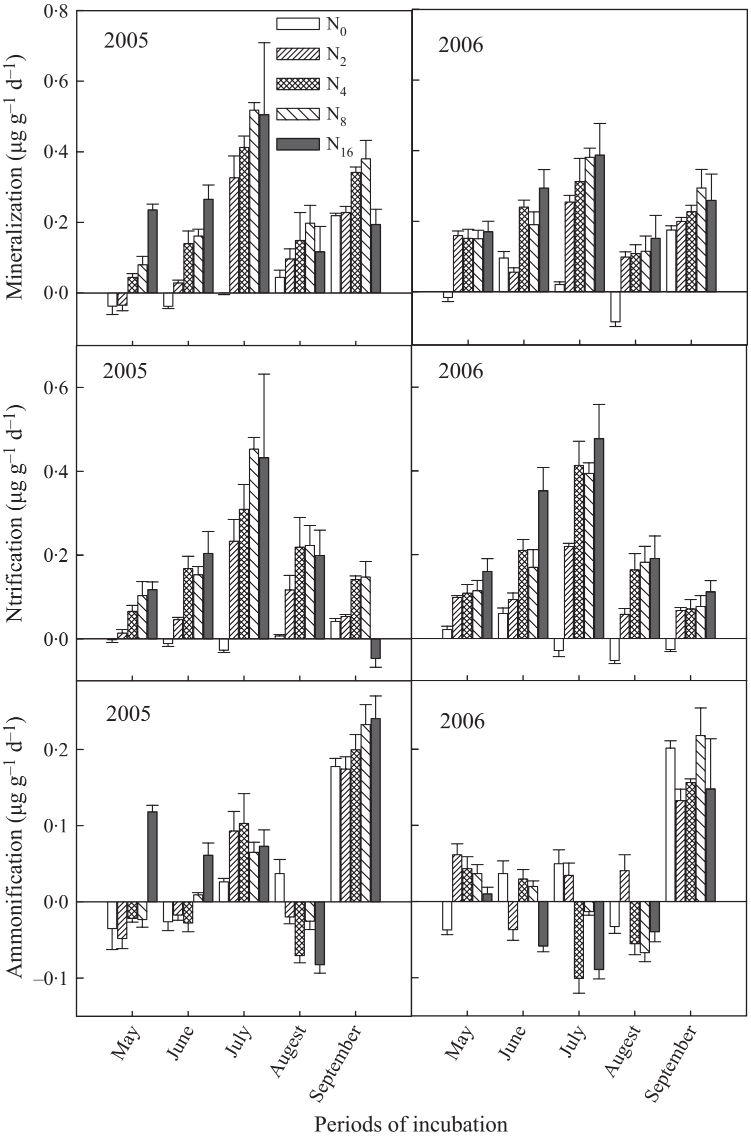

Figure 4 Temporal variations in soil net N mineralization and nitrification rates for the N fertilizer treatments during the periods of incubation in 2005 and 2006. Values are means plus standard errors (n = 4). Nitrogen was applied as urea on July 15 each year.

inorganic forms (NO_3^- and NH_4^+) and affect soil N cycling (Huang *et al.*, 1998; Liu *et al.*, 2000; Weintraub and Schimel, 2003; Sun *et al.*, 2004).

The pattern and amount of soil net N mineralization varied among the four fertilizer treatments (Figure 4). This was likely because the effects of N fertilizer on soil N cycling can vary between N-poor and N-rich sites (Chappell *et al.*, 1999). In the case of the lower rate fertilizer treatment, a larger part of applied N may be immobilized in the soil, whereas at more N-rich sites, more of the applied N may remain in the pools that are actively recycled (Chappell *et al.*, 1999). Another reason may have been that much more plant biomass and litter was produced in the higher fertilizer treatments (Table 1) and maybe supplied as labile soil organic matter, which may be readily mineralized (Chappell *et al.*, 1999).

Seasonal patterns of soil net N mineralization

Strong seasonal patterns were observed in net N mineralization and nitrification during the study, with rates generally increasing from May through June to July as soil warmed, reaching a maximum in July, and then declining (Figure 4). These patterns were consistent with previous results in which higher soil net N mineralization and nitrification rates occurred during the warmest month (Xu *et al.*, 2007; Zhang *et al.*, 2008).

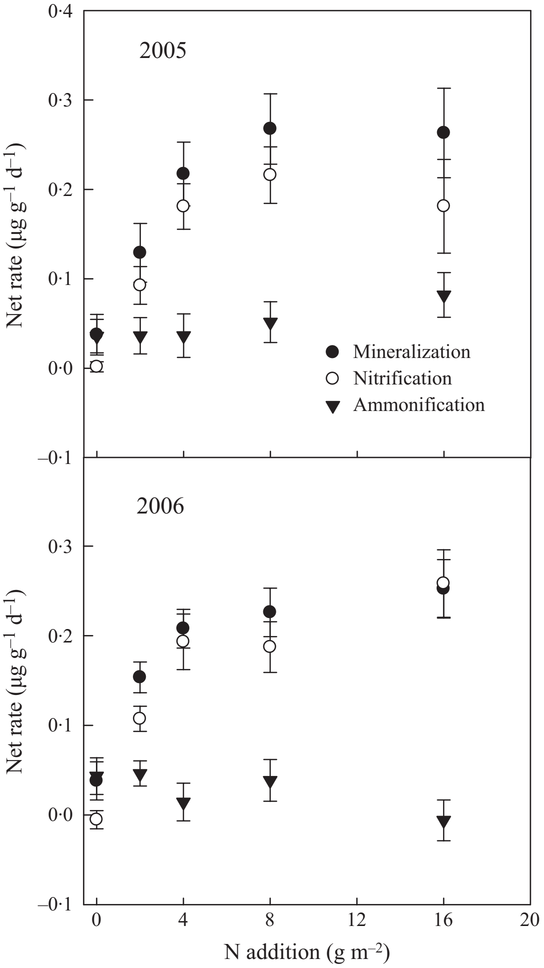

Figure 5 Differences in soil N mineralization, nitrification and ammonification rates among five fertilizer treatments. Significance of soil net N mineralization rates in 2005 ($F_{4,19}$ = 22.52, $P < 0.0001$) and in 2006 ($F_{4,19}$ = 35.69, $P < 0.0001$), nitrification rates in 2005 ($F_{4,19}$ = 21.27, $P < 0.0001$) and in 2006 ($F_{4,19}$ = 143.69, $P < 0.0001$) and net N ammonification rates in 2005 ($F_{4,19}$ = 9.54, $P < 0.0001$) and in 2006($F_{4,19}$ = 4.85, $P < 0.01$) among N fertilizer treatments were measured by one-way ANOVA.

Seasonal variations in soil temperature and precipitation may directly stimulate soil N transformation (Sleutel *et al.*, 2008), or by regulation of soil microbial activity (Sierra, 1997; Gilliam *et al.*, 2001; Bell *et al.*, 2008). In this study, seasonal patterns of soil net N mineralization and nitrification rates in May, June and July were consistent with the variation in soil temperature and rainfall. The rise in temperature and adequate precipitation during this time (May–July) should increase microbial activity and enable more inorganic N to be released (Table 1). Maximum rates of N mineralization occurred during the warmest period in 2005 and 2006, mainly in the form of nitrification (Table 3). Significant and positive correlations between net nitrification and soil temperature and precipitation over the two growing seasons support this contention. Monthly rates of soil N transformations were also dependent on small-scale variation in both soil microclimate and species cover, which can affect microbial activity and regulate soil N mineralization (Steltzer and Bowman, 1998; Eviner *et al.*, 2006).

Prevailing conditions in August at this Inner Mongolian grassland site, including suitable soil moisture content (Figure 2) and input of high C:N ratio substrates from root turnover and senescing of above-ground plant biomass, might reduce soil N mineralization (Figure 4) because labile C with high C ⁄N ratio could stimulate microbial growth and assimilate mineral N into microbial biomass (Luizão *et al.*,1992; van der Krift and Berendse, 2001; Xu *et al.*, 2007). In September, mean net nitrification was lower over the incubation periods, while soil net N mineralization was higher than in May and August, and mainly in the form of ammonification (Table 3). One reason may be that nitrification can be greatly reduced at temperatures below 5°C (10-year means for soil temperature were <5°C at this site), and the rate declined in September (Anderson and Boswell, 1964).

Effects of N fertilization on grass biomass

Grass above-ground biomass responded positively to all N fertilizer treatments and in both years, although the response was far more pronounced in 2006 than in 2005 (Figure 7). For both years, there was a linear relationship between our measure of fertilizer-enhanced grass yield (biomass ratio of treatment:control) and N fertilizer added up to the N_8 treatment, but not beyond. In fact, yield for the N_{16} treatment was consistently 25% less than that which would have been predicted from the linear model (based on 0–8 g N m^{-2} $year^{-1}$) for each year. This suggests that N fertilizer additions beyond 8 g N m^{-2} $year^{-1}$ (80 kg ha^{-1} $year^{-1}$) may not provide an adequate return on investment when attempting to increase grass yield in Inner Mongolian grasslands. Dry-matter yield as a function of added N declined almost fourfold from N_2 to N_{16} treatments, averaged over both years, from approximately 75 to 21 kg dry matter kg^{-1} N added, respectively.

Some of these patterns may be related to other factors, for example weather and the effect of residual N fertilizer, as has been demonstrated for grasslands outside China (Kowalenko and Bittman, 2000; Vellinga *et al.*, 2010). However, the proportional drop in yield

Table 3 Correlation coefficients (*r*) and significance levels (*P*) of mean net N mineralization rate in the five treatments with climatic factors (*n* = 5), respectively.

		Mineralization (μg g^{-1} d^{-1})		Nitrification (μg g^{-1} d^{-1})		Ammonification (μg g^{-1} d^{-1})	
		2005	**2006**	**2005**	**2006**	**2005**	**2006**
Soil temperature (°C)	*r*	0.46	0.27	0.88	0.78	−0.23	−0.61
	P	0.43	0.66	0.05	0.12	0.72	0.27
Rainfall (mm)	*r*	0.50	0.70	0.94	0.95	−0.24	−0.39
	P	0.39	0.19	0.02	0.01	0.70	0.51
Soil water content (%)	*r*	−0.04	−0.07	0.50	0.79	−0.53	−0.94
	P	0.95	0.92	0.39	0.12	0.36	0.02

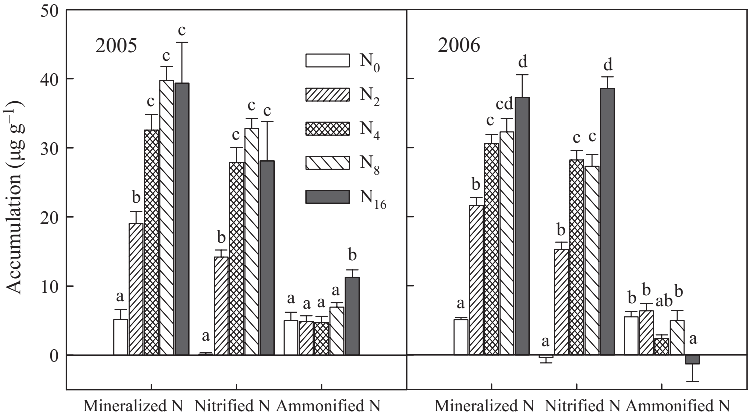

Figure 6 Accumulation of net N mineralization, nitrification and ammonification under different fertilizer treatments for the growing seasons in 2005 and 2006. Values are means ± standard errors.

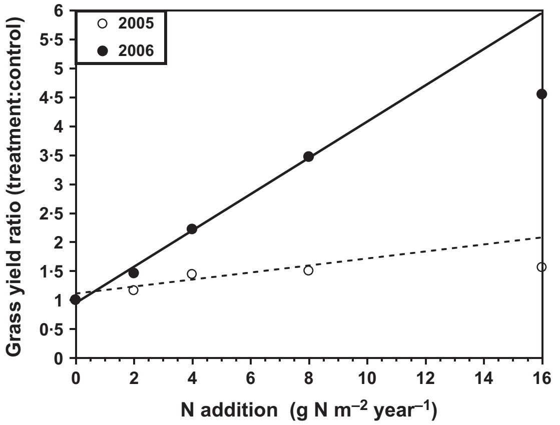

Figure 7 Relationships between grass yield ratio (calculated as ratio of grass biomass of control to grass biomass for a given N treatment) and N additions for 2005 (open symbols, dashed line) and 2006 (closed symbols, solid line). Lines shown are linear regressions for 2005 and 2006, $y = 1.05 + 0.06x$, $r^2 = 0.86$, $P < 0.077$ and $y = 0.93 + 0.32x$, $r^2 = 0.995$, $P < 0.002$, respectively.

found in Figure 6 is not likely related to abiotic factors, considering the close proximity of the sample plots. On the other hand, the pronounced variation in yield/N response between 2005 and 2006 suggests that residual effects, as reported in Canadian and European grasslands (Kowalenko and Bittman, 2000; Schröder *et al.*, 2010; Vellinga *et al.*, 2010), may have occurred.

Conclusions

As expected, we found distinct temporal patterns in soil N mineralization during the growing season, with highest rates in July and similar seasonal patterns in N mineralization/nitrification among fertilizer treatments that appeared to be primarily regulated by ambient temperature and moisture. Net N mineralization was greatly dominated by nitrification, suggesting a prevalence of populations of nitrifying bacteria in these grassland soils. Overall rates of soil N transformations increased significantly in response to experimental additions of N in Inner Mongolia grassland, particularly up to 8 g N m^{-2} year^{-1}, but also beyond this level, ultimately leading to substantial accumulations of mobile NO_3^- late in the growing season in the 16 g N m^{-2} $year^{-1}$ treatment, a time when the potential for leaching of NO_3^- and nutrient cations, such as Ca and Mg, is particularly high. More importantly, the application of N fertilizer beyond 8 g N m^{-2} $year^{-1}$ did not result in proportional increases in grass yield, and the actual grass yield at 16 g N m^{-2} $year^{-1}$ was approximately 25% less than that predicted from linear models up to 8 g N m^{-2} $year^{-1}$ for both years. Accordingly, grassland managers might consider 8 g N m^{-2} $year^{-1}$ as a target level when applying N fertilizer to improve yield of managed grasslands of Inner Mongolia.

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