Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China

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Abstract

Intact soil cores from three adjacent sites (Site A: grazed, Site B: fenced for 4 years, and Site C: fenced for 24 years) were incubated in the laboratory to examine effects of temperature, soil moisture, and their interactions on net nitrification and N mineralization rates in the Inner Mongolia grassland of Northern China. Incubation temperature significantly influenced net nitrification and N mineralization rates in all the three grassland sites. There were no differences in net nitrification or N mineralization rates at lower temperatures (−10, 0, and 5 °C) whereas significant differences were found at higher temperatures (15, 25, and 35 °C). Soil moisture profoundly impacted net nitrification and N mineralization rates in all the three sites. Interactions of temperature and moisture significantly affected net nitrification and mineralization rates in Site B and C, but not in Site A. Temperature sensitivity of net nitrification and N mineralization varied with soil moisture and grassland site. Our results showed greater net N mineralization rates and lower concentrations of inorganic N in the grazed site than those in the fenced sites, suggesting negative impacts of grazing on soil N pools and net primary productivity.

Keywords: Grassland; Grazing; N; Nitrification; Soil moisture; Temperature

1. Introduction

In response to the elevated atmospheric CO2 concentration resulted from land use change and fossil fuel combustion, the Earth’s surface temperature has been increased by 0.6 °C in the 20th century and is predicted to increase 1.4–5.8 °C by the end of this century (IPCC, 2001). As a consequence of global warming, precipitation pattern is also expected to change. Responses of ecosystem carbon (C) cycling to the above global change factors determine whether terrestrial ecosystems will act as a sink or source of the atmospheric CO2, resulting in negative or positive feedbacks to global climate change. As a main limiting factor for plant growth and net primary productivity, soil nitrogen (N) availability and its responses to global environmental change are critical for the projection of ecosystem and global C budgets (Hungate et al., 2003; Luo et al., 2004). Net N mineralization, the transformation process from organic N to inorganic N, primarily determines soil N availability. A better understanding of the effects of temperature, soil water availability, and their interactions on net N mineralization in soils will facilitate our predictions of soil N dynamics and net primary production in terrestrial ecosystems under global climate change.

Soil microclimate (temperature and moisture) plays a very important role in regulating soil N mineralization and availability. Net N mineralization rate has been found to exponentially increase with temperature under laboratory incubations (Sierra, 1997; Cookson et al., 2002; Dalias et al., 2002). Consequently, elevated temperatures manipulated with different warming facilities in the field are reported to stimulate net N mineralization rate in various biomes across the world (Rustad et al., 2001; Shaw and Harte, 2001; Melillo et al., 2002; Wan et al., 2005). Soil moisture content can also significantly impact nitrification and net N mineralization rates (Sierra, 1997; Paul et al., 2003), with the maximum N mineralization rate occurring when soil moisture is near field water holding capacity (Stanford and Epstein, 1974). With the concurrent variations in temperature and precipitation in the field, it is critical to study interactions of temperature and soil
moisture on net N mineralization. As one of the driving factors of global change, land use could profoundly impact soil N cycling through altering abiotic and biotic characteristics of soil (Templer et al., 2005). However, effects of land use on soil net N mineralization still remain controversial. All increases (McNaughton et al., 1988; Holland and Detling, 1990; Holland et al., 1992; Seagle et al., 1992; Milchunas and Lownroth, 1993; Frank and Groffman, 1998; Frank et al., 2000), decreases (Biondini et al., 1998; Andersson et al., 2002), and no changes (Goodale and Aber, 2001) in net N mineralization have been reported under different land use patterns. In addition, it is not clear whether effects of temperature, soil moisture, and their interactions on net N mineralization change with land use patterns.

This study was conducted to examine effects of temperature, soil moisture, and their interactions on soil net N mineralization in three temperate grassland sites with different land use patterns in Northern China. Undisturbed soil cores were incubated up to 5 weeks under six temperature levels (−10, 0, 5, 15, 25, and 35 °C) and three moisture levels (15, 25, and 35%). The main objectives of this experiment are to examine: (1) effects of temperature, moisture and their interactions on net N mineralization rate and (2) whether there are differences in these effects among the three grassland sites with different land use histories.

2. Materials and methods

2.1. Research site

This study was conducted in a typical steppe ecosystem in Northern China, which is located near the Inner Mongolia Grassland Ecosystem Research Station (IMGERS, 116°42'E, 43°38'N) of the Chinese Academy of Sciences. Average elevation is approximately 1200 m. Mean annual precipitation is about 350 mm, mainly occurring from June to August. Mean annual temperature is 2.0 °C. Mean growing season length is approximately 150 days (Bai et al., 2004).

Three adjacent experimental sites with different land use histories were selected in 2003. Site A was grazed grassland. Site B (500 m × 600 m) had been fenced for 4 years since 1999, and Site C (400 m × 600 m) had been fenced for 24 years since 1979. Soils in the three experimental sites are all dark chestnut (sandy and silty loam in texture; Li, 1990). Dominant species in the three grassland sites include: Leymus chinensis, Setaria grandis, Achnatherum michnoi, Achnatherum sibiricum, Carex korshinskii, Cleistogenes squarrosa, and Allium condensatum.

2.2. Sampling and incubation methods

Five 10 m × 10 m plots were selected for soil sampling in each experimental site. The distance between plots was 2 m. After plants and litter were removed, 72 paired soil cores were taken from each plot. In each pair, two PVC cylinders (12 cm in length, 5 cm in diameter) were inserted 10 cm into the soil (5 cm apart) to take undisturbed soil cores from 20 to 24 July 2003. One soil core from each pair was taken as the un incubated sample to measure initial NH$_4^+$-N and NO$_3^-$-N concentrations. The other core was incubated in the laboratory (Sierra, 1992). Laboratory incubation was conducted at six temperature levels (−10, 0, 5, 15, 25, and 35 °C) and three moisture levels (15, 25, and 35% g H$_2$O/100 g dry soil) for 7, 14, 21 and 35 days. NH$_4^+$-N and NO$_3^-$-N concentrations in the incubated samples were also analyzed after incubation. Therefore, we prepared 1080 incubated soil cores (3 sites × 6 temperatures × 3 moisture levels × 4 incubation times × 5 replicates) and 1080 unincubated soil cores. Distilled water was added to each incubated soil to obtain the required moisture content based on the gravimetric water content of the adjacent soil core. Water was gently injected into the soil with a syringe. Water loss during incubations was negligible. The PVC cylinders containing soils were covered with 0.01 mm thick plastic film and incubated in six incubation chambers at the set temperatures.

All soil cores (unincubated and incubated) were hand-sorted to remove stones and coarse roots. Soils were mixed thoroughly by hand, sieved with 2 mm mesh, and stored at 4 °C. To analyze the inorganic N, a 10-g aliquot soil sample from each unincubated and incubated soil cores was first taken, and then 50 ml of 2 M KCl solution was added. The mixture of soil and extractant was shaken for 1 h on a reciprocal shaker. After shaking, the soil suspension was filtered (Whatman No. 1 filter paper, 12.5 cm in diameter). Soil solutions were kept frozen prior to analysis for NH$_4^+$-N and NO$_3^-$-N on a FIAStar 5000 Analyzer (Foss Tecator, Denmark).

Two 1 m × 1 m subplots in each plot were clipped at the soil surface to measure aboveground biomass in the middle August of 2003. Plant materials were oven-dried at 65 °C for 48 h and weighed. Soil moisture was measured gravimetrically using a subsample from the unincubated cores. Soil pH (water:soil = 2.5:1) was measured using air-dried soil that passed through 2 mm sieve. Air-dried soil passed through 0.5 mm sieve were used to analyze soil organic C and N. Soil organic C was analyzed using H$_2$SO$_4$-K$_2$Cr$_2$O$_7$ oxidation method (Nelson and Sommers, 1982). Soil organic N was analyzed using Kjeldahl acid-digestion method with an Alpkm autoanalyzer (Kjektect System 1026 Distilling Unit, Sweden). Available P was analyzed colorimetrically using Olsen-P method (Olsen et al., 1954).

2.3. Calculations

Net N mineralization on a dry mass basis was calculated as the changes in inorganic N (NH$_4^+$-N + NO$_3^-$-N) in the initial and incubated samples.

For a time interval $\Delta t = t_{i+1} - t_i$,

\[ A_{\text{amn}} = c(NH_4^+ - N)_{i+1} - c(NH_4^+ - N)_i \]  
(1)

\[ A_{\text{nit}} = c(NO_3^- - N)_{i+1} - c(NO_3^- - N)_i \]  
(2)

and

\[ A_{\text{in}} = A_{\text{amn}} + A_{\text{nit}} \]  
(3)
where \( t_i \) and \( t_{i+1} \) was the initial and post incubation time; \( A_{\text{amn}} \) was the accumulation of \( \text{NH}_4^+ - \text{N} \); \( c(\text{NH}_4^+ - \text{N})_i \) and \( c(\text{NH}_4^+ - \text{N})_{i+1} \) were the mean concentrations of ammonium-N in the initial and incubated samples, respectively. \( A_{\text{nit}} \) was the accumulation of \( \text{NO}_3^- - \text{N} \); \( c(\text{NO}_3^- - \text{N})_i \) and \( c(\text{NO}_3^- - \text{N})_{i+1} \) were the mean concentrations of \( \text{NO}_3^- - \text{N} \) in the initial and incubated samples, respectively. \( A_{\text{min}} \) was the accumulation of total inorganic N (\( \text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N} \)).

\[
R_{\text{nit}} = \frac{A_{\text{nit}}}{\Delta t} 
\]

and

\[
R_{\text{min}} = \frac{A_{\text{amn}} + A_{\text{nit}}}{\Delta t} 
\]

where \( R_{\text{nit}} \) and \( R_{\text{min}} \) were net nitrification and mineralization rates, respectively.

2.4. Statistical analyses

Data under 7 days incubation were used to examine effects of temperature, soil moisture and their interactions (Table 2) as well as the differences among the three grassland sites (Table 3). Data incubated at 25°C temperature and 25% moisture were used to examine variation of net N mineralization with incubation times (Fig. 4). Statistical analyses (variance and correlation) were conducted using SAS 8.0 (SAS Institute, Cary, NC, USA). Two-way ANOVA was used to examine the effects of temperature, moisture and their interactions on nitrification and net N mineralization rates. One-way ANOVA was used to compare the difference among the three grassland sites and among different incubation times.

3. Results

3.1. Main effects of temperature on net nitrification and N mineralization rates

The main effects of temperature on net nitrification were statistically significant in all the three sites (\( P < 0.0001 \), Table 2). In Site A, there were no differences in net nitrification rates among the four lower temperatures (–10, 0, 5, and 15°C). However, net nitrification rates at 25 and 35°C were significantly greater than those at the four lower temperatures (–10, 0, 5, and 15°C). In addition, net nitrification rate at 35°C was 67% higher than that at 25°C in Site A (Fig. 1, upper panel). In Site B, net nitrification rates at the three higher temperatures (15, 25, and 35°C) were significantly different from each other and from those at the three lower temperatures (–10, 0, and 5°C). Net nitrification rates at the three lower temperatures (–10, 0, and 5°C) were similar in Site B. In Site C, significant differences in net nitrification rates were found among –10, 15, 25, and 35°C incubation temperatures. Net nitrification rates at 0 and 5°C were neither different from that at –10°C nor from that at 15°C incubation temperature.

Temperature also significantly affected net N mineralization rates in the three sites (\( P < 0.0001 \), Table 1). In Site A, net N mineralization rates at the four lower incubation temperatures (–10, 0, 5, and 15°C) were similar and lower than those at the two higher temperatures (25 and 35°C). Net N mineralization rate at 35°C was 61% greater than that at 25°C temperature (Fig. 1, lower panel). In Site B, there were no differences in net N mineralization rates among –10, 0 and 5°C temperatures. Net N mineralization rates at 15, 25 and 35°C temperatures were significantly different from each other and also greater than those at the three lower temperatures (–10, 0, and 5°C).

![Fig. 1. Effect of incubation temperature on N transformation rates incubated for 7 days in the three sites (Mean ± 1SE). A: grazed grassland; B: grassland fenced for 4 years (1999–2003); C: grassland fenced for 24 years (1979–2003).](image)

<table>
<thead>
<tr>
<th>Site properties</th>
<th>Site A</th>
<th>Site B</th>
<th>Site C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant species</td>
<td>( L. ) chinensis</td>
<td>( S. ) grandis</td>
<td>( S. ) grandis</td>
</tr>
<tr>
<td>( S. ) grandis</td>
<td>( A. ) michhoi</td>
<td>( A. ) sibiricum</td>
<td>( A. ) condensatum</td>
</tr>
<tr>
<td>( C. ) korshinskii</td>
<td>( L. ) chinensis</td>
<td>( L. ) chinensis</td>
<td>( C. ) korshinskii</td>
</tr>
<tr>
<td>( C. ) korshinskii</td>
<td>( C. ) squarroso</td>
<td>( A. ) sibiricum</td>
<td>( C. ) condensatum</td>
</tr>
<tr>
<td>Aboveground Biomass (g m(^{-2}))</td>
<td>120.0 ± 17.23(^{a})</td>
<td>215.7 ± 20.49(^{b})</td>
<td>218.7 ± 24.59(^{b})</td>
</tr>
<tr>
<td>pH value</td>
<td>7.19 ± 0.051(^{a})</td>
<td>7.31 ± 0.027(^{a})</td>
<td>7.10 ± 0.048(^{a})</td>
</tr>
<tr>
<td>Soil bulk density (g cm(^{-3}))</td>
<td>1.31 ± 0.005(^{a})</td>
<td>1.15 ± 0.004(^{a})</td>
<td>1.24 ± 0.004(^{b})</td>
</tr>
<tr>
<td>Total organic C (%)</td>
<td>2.03 ± 0.011(^{a})</td>
<td>2.43 ± 0.015(^{b})</td>
<td>2.16 ± 0.013(^{b})</td>
</tr>
<tr>
<td>Total organic N (%)</td>
<td>0.195 ± 0.0009(^{a})</td>
<td>0.285 ± 0.0011(^{b})</td>
<td>0.200 ± 0.0014(^{a})</td>
</tr>
<tr>
<td>C: N ratio</td>
<td>10.46 ± 0.04(^{b})</td>
<td>10.65 ± 0.05(^{b})</td>
<td>10.80 ± 0.04(^{b})</td>
</tr>
<tr>
<td>Available P (mg kg(^{-1}))</td>
<td>5.95 ± 0.57(^{ab})</td>
<td>4.70 ± 0.46(^{a})</td>
<td>6.35 ± 0.49(^{b})</td>
</tr>
</tbody>
</table>

Site A, B, and C are grazed grassland, grassland fenced for 4 years, and grassland fenced for 24 years, respectively; Dominant species were listed according to decreasing order of aboveground biomass of each species. Different superscript letters represent statistical significance at \( P < 0.05 \) among the three grassland sites. \( n = 15 \) for aboveground biomass, soil pH and available P; \( n = 5 \) for soil bulk density; \( n = 360 \) for other parameters.
In Site C, significant differences were found among −10, 15, 25, and 35 °C temperatures. Net N mineralization rates at 0 and 5 °C were similar with that at −10 °C and with that at 15 °C.

The percentage of net nitrification in net N mineralization increased with incubation temperature. For example, net nitrification accounted for 69 and 83% at −10 and 0 °C, 76 and 90% at 5 and 15 °C, 92 and 94% at 25 and 35 °C, respectively, of net N mineralization in Site B.

3.2. Main effect of soil moisture on net nitrification and N mineralization rates

Soil moisture had significant impacts on net nitrification rates in all Site A (P<0.05), Site B (P<0.0001), and Site C (P<0.0001, Table 2). Net nitrification rates increased with increasing soil moisture. In Site A, net nitrification rate was 63% greater at the 35% than 15% moisture level. No difference in net nitrification rate was found between the 15 and 25% moisture levels or between the 25 and 35% moisture levels. In Site B, there were significant differences in net nitrification rates among the three moisture levels. Net nitrification rates were 50 and 125% higher at the 25 and 35% moisture levels than that at the 15% moisture level, respectively. In Site C, net nitrification rate at the 15% moisture level was 34 and 33% lower than those at the 25 and 35% moisture levels. There was no difference in net nitrification rates between the 25 and 35% moisture levels in Site C (Fig. 2, upper panel).

The main effects of soil moisture on net N mineralization rate were statistically significant in all the three grassland sites (P<0.01, Table 2). In Site A, net N mineralization rates were 79 and 64% higher at the 25 and 35% than 15% moisture level, respectively. But there was no difference in net N mineralization rate between the 25 and 35% moisture levels. In Site B, there were significant differences in net mineralization rates among the three moisture levels. Net mineralization rates were 50 and 125% higher at the 25 and 35% moisture levels than that at the 15% moisture level, respectively. However, net N mineralization rate was 63% greater at the 35% than 15% moisture level. No difference in net nitrification rate was found between the 15 and 25% moisture levels. In Site C, net mineralization rate was 80 and 42% higher at the 35% than 15 and 25% moisture levels, respectively (Fig. 2, lower panel).

Table 2
Results of general factorial ANOVA on the soil net nitrification and N mineralization rates incubated for 7 days in three grassland sites (n=90)

<table>
<thead>
<tr>
<th>Source</th>
<th>Nitrification rate</th>
<th>Mineralization rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F value</td>
<td>P value</td>
</tr>
<tr>
<td>Site A</td>
<td>Temperature</td>
<td>21.32</td>
</tr>
<tr>
<td></td>
<td>Moisture</td>
<td>4.53</td>
</tr>
<tr>
<td></td>
<td>T×M</td>
<td>1.73</td>
</tr>
<tr>
<td>Site B</td>
<td>Temperature</td>
<td>99.50</td>
</tr>
<tr>
<td></td>
<td>Moisture</td>
<td>23.55</td>
</tr>
<tr>
<td></td>
<td>T×M</td>
<td>7.65</td>
</tr>
<tr>
<td>Site C</td>
<td>Temperature</td>
<td>54.23</td>
</tr>
<tr>
<td></td>
<td>Moisture</td>
<td>10.06</td>
</tr>
<tr>
<td></td>
<td>T×M</td>
<td>2.28</td>
</tr>
</tbody>
</table>

The percentage of net nitrification in net N mineralization showed different trends with increasing soil moisture among the three sites. In Site A, net nitrification accounted for 81, 87 and 80% of net N mineralization at the moisture levels of the 15, 25 and 35%, respectively. By contrast, the percentages increased with moisture in Site B (78, 92, and 97%) and C (80, 95, and 106%).

3.3. Interactive effects temperature and soil moisture on net nitrification and N mineralization rates

Temperature and soil moisture significantly interacted with each other to affect net nitrification and N mineralization rates in Site B (P<0.0001) and C (P<0.05), but only marginally impacted net nitrification and N mineralization rates in Site A (P<0.10, Fig. 3, Table 2). At the 15% moisture level, average net nitrification rate was 3.8 times greater at the three higher (15, 25, and 35 °C) than the three lower temperatures (−10, 0, and 5 °C). When incubated under the 25 and 35% moisture levels, average net nitrification rate was 12.5, and 11.0 times greater at the three higher (15, 25, and 35 °C) than the three lower incubation temperatures (−10, 0, and 5 °C), respectively. At the lower temperature range (−10 to 5 °C), average net nitrification rates were not different among the three moisture levels. However, average net nitrification rates under the higher temperatures (15, 25 and 35 °C) increased by 68 and 151% when incubation moisture increased from 15% to 25 and 35%, respectively.

There was similar pattern of the interactive effects of temperature and moisture on net N mineralization rate to that
of net nitrification rate in the fenced sites. For example, at the 15% moisture level, average net N mineralization rate was 2.2 and 7.7 times greater at the three higher (15, 25, and 35 °C) than lower temperatures (−10, 0, and 5 °C) in Site B and C, respectively. When incubated under the 25 and 35% moisture levels, average net N mineralization rate was 9.5 and 12.9 times in Site B and 2.1 and 28.6 times in Site C greater at the three higher than lower temperatures, respectively. At the lower temperature range (−10 to 5 °C), average net N mineralization rates were not different among the three moisture levels in Site B. However, average net N mineralization rates under the higher temperatures (15, 25 and 35 °C) increased by 52 and 120% when incubation moisture increased from 15% to 25 and 35%, respectively.

3.4. Changes in accumulation of inorganic N and net nitrification and N mineralization rates with incubation time

The accumulations of NO\textsubscript{3}\textsuperscript{-}-N and total inorganic N increased with increasing incubation time (Fig. 4, left panels).

Fig. 3. Interactive effects of temperature and moisture on nitrification and net N mineralization rates incubated for 7 days in the three sites (Mean ± 1SE). See Fig. 1 for site abbreviations.

Fig. 4. Changes in the accumulation of inorganic N (left panels) and N transformation rates (right panels) with incubation time under 25 °C temperature and 25% moisture level in the three sites (Mean ± 1SE). \( A\text{\textsubscript{Nit}} \) and \( A\text{\textsubscript{inN}} \) are accumulations of NO\textsubscript{3}\textsuperscript{-}-N and total inorganic N (NH\textsubscript{4}\textsuperscript{+}-N + NO\textsubscript{3}\textsuperscript{-}-N). See Fig. 1 for site abbreviations.
However, the effects of incubation time on the accumulation of inorganic N varied with different sites. Incubation time significantly affected the accumulation of NO$_3^-$-N and total inorganic N in Site B ($P<0.001$) and in Site C ($P<0.01$), but not in Site A ($P>0.05$).

Incubation time had also significant impacts on net nitrification and N mineralization rates in Site A and B. However, no effects of incubation time were found on net nitrification or N mineralization rates in Site C. Contrary to the trends of accumulations of NO$_3^-$-N and total inorganic N, N transformation rates decreased with increasing incubation time (Fig. 4, right panels). Both net nitrification and N mineralization rates were significant greater under 7 days than 14, 21 and 35 days incubations in Site A and B, respectively.

We compared the total amount of accumulated inorganic N under different temperature and moisture regimes among the three sites. There was a general increase in the accumulation of inorganic N with incubation time under different incubation temperatures and moistures in all the three sites (Fig. 5). Some negative inorganic N accumulation, or N immobilization, occurred when incubation temperature was low ($<5^\circ C$). Significant differences, depending on incubation temperature and moisture, in the total amount of accumulated inorganic N were found among the three sites under 7 and 14 days incubations, but no general patterns were found. Under 35 days incubation, the total amount of inorganic N increased dramatically from the grazed (A) to the fenced (C) sites at higher moisture levels (25 and 35%) and temperatures (25 and 35 °C). For example, at the highest moisture (35%) and temperature (35 °C) levels, the total amount of inorganic N was 1.7 and 1.9 times higher in Site B and Site C than Site A, respectively.

![Fig. 5. Accumulations of total inorganic N (NH$_4^+$-N + NO$_3^-$-N) under different incubation temperatures and moistures in the three grassland sites (Mean ± 1SE). See Fig. 1 for site abbreviations.](image-url)
3.5. Inorganic N concentrations and net N transformation rates among the three sites

Initial NH$_4^+$-N, NO$_3^-$-N and inorganic N concentrations were significantly ($P < 0.001$) different among Site A, B, and C (Table 3). NO$_3^-$-N concentrations were 108 and 128% higher in Site B and C than those in Site A, respectively. NH$_4^+$-N concentrations were 14 and 51% higher in Site B and C than that in Site A, respectively. Total inorganic N concentration in Site A was 52 and 46% lower than those in Site B and C, respectively.

After incubation, the concentrations of NO$_3^-$-N and inorganic N in Site A were significantly ($P < 0.001$) lower than those in Site B and C. There were no differences in NH$_4^+$-N concentrations between Site A and C or between Site B and C. However, NH$_4^+$-N concentration was 20% higher in Site A than in Site B (Table 3).

No differences in net nitrification rate were found among the three sites (Table 3). Net N mineralization rate tended to decrease with declining disturbance from Site A (grazed) to Site B (fenced 4 years) and Site C (fenced for 24 years). Net N mineralization rate in Site C was 20% lower than that in Site A, but there were no differences in net N mineralization rates between Site A and B or between Site B and C.

3.6. Effects of soil moisture on the temperature responses of net N mineralization among the three grassland sites

Like many chemical and biological processes, net nitrification and N mineralization rates also showed exponential increases with temperature (Figs. 1 and 3) and thus their temperature responses can be described by the temperature sensitivity ($Q_{10}$). $Q_{10}$ of net nitrification and N mineralization was calculated as $Q_{10} = \exp(10 \times b)$, where $b$ is one of the constants in the exponential function between net N mineralization rate and temperature ($R_{\text{min}} = a \times \exp(b \times t)$). $Q_{10}$ of nitrification was greater at the 25% moisture level (Site A: 1.99; Site B: 2.30) than those at the 15% (Site A: 1.70; Site B: 1.79) and 35% (Site A: 1.74; Site B: 2.06) moisture levels. Similarly, $Q_{10}$ of net N mineralization was also greatest at the 25% moisture level in Site A (1.91). The above results imply interacting roles of soil moisture and temperature in regulating net nitrification and N mineralization rates in the temperate grassland in Northern China. There were also site differences in $Q_{10}$ of net nitrification and N mineralization. For example, $Q_{10}$ of net N mineralization changed from 1.70 in Site C to 1.91 in Site A and 2.24 in Site B, respectively, when incubated at the 25% moisture level.

4. Discussion

4.1. Temperature and moisture effect on net N mineralization

Soil N transformations involve biological processes that are temperature dependent (Dalias et al., 2002). Observations in our study showed significantly positive correlations between net N mineralization and incubation temperatures (Figs. 1 and 3), with temperature sensitivity ($Q_{10}$) varying from 1.54 to 2.24. The temperature responses and sensitivity of net N mineralization observed in our study were comparable to those of previous studies (Sierra, 1997; Dalias et al., 2002).

No differences in net nitrification or N mineralization rates among the lower temperatures (−10, 0, and 5 °C, Fig. 1) could be primarily attributed to the low-temperature constraint on microbial activity. However, the rapid increases in net nitrification and N mineralization rates at the higher temperatures (15–35 °C) were in agreement with the results of the previous studies (Stanford and Smith, 1972; Nicolardot et al., 1994; 2001; Stark and Firestone, 1996). The greater percentages of net nitrification in net N mineralization at the higher than lower temperatures suggested that mesophilic nitrifiers (such as fungi and actinomycetes) were more active at the temperature range of 25–35 °C (Macduff and White, 1985; Nicolardot et al., 1994; 2001; Stark and Firestone, 1996).

Water availability controls soil microbial activity and thus the rates of net N mineralization (Nicolardot et al., 1994; 2001; Stark and Firestone, 1996). Maximal net N mineralization occurs when soil moisture is close to field water holding capacity. Stanford and Epstein (1974) examined the relationships between N mineralization and soil moisture for nine different soil types. Maximum N mineralization rates occurred at soil matric potential between 0.3 and 0.1 Mpa depending on soil types, which is approximately 10–35% gravimetric moisture content. In our study, greater rates and temperature sensitivity of net nitrification and N mineralization were observed at the moisture levels of 25 and 35% than 15%.

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Table 3
Inorganic N concentrations (mg kg⁻¹) before and after incubation, and net N mineralization rate (R, mg kg⁻¹ d⁻¹) under 7 days incubation in the three grassland sites (Mean ± 1SE, n = 90)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Site A</th>
<th>Site B</th>
<th>Site C</th>
</tr>
</thead>
<tbody>
<tr>
<td>c(NO$_3^-$-N)</td>
<td>5.93 ± 0.210$^a$</td>
<td>12.32 ± 0.350$^b$</td>
<td>13.50 ± 0.430$^c$</td>
</tr>
<tr>
<td>c(NH$_4^+$-N)</td>
<td>1.84 ± 0.057$^a$</td>
<td>2.10 ± 0.093$^b$</td>
<td>2.78 ± 0.062$^c$</td>
</tr>
<tr>
<td>c(NH$_4^+$-N + NO$_3^-$-N)</td>
<td>7.77 ± 0.240$^a$</td>
<td>14.42 ± 0.362$^b$</td>
<td>16.28 ± 0.463$^c$</td>
</tr>
<tr>
<td>c(NO$<em>3^-$-N)$</em>{10}$</td>
<td>14.61 ± 1.127$^b$</td>
<td>21.24 ± 1.202$^b$</td>
<td>21.47 ± 1.003$^c$</td>
</tr>
<tr>
<td>c(NH$<em>4^+$-N)$</em>{10}$</td>
<td>3.60 ± 0.179$^a$</td>
<td>3.01 ± 0.160$^b$</td>
<td>3.16 ± 0.151$^b$</td>
</tr>
<tr>
<td>c(NH$_4^+$-N + NO$<em>3^-$-N)$</em>{10}$</td>
<td>18.22 ± 1.183$^b$</td>
<td>24.25 ± 1.215$^c$</td>
<td>24.63 ± 1.011$^c$</td>
</tr>
<tr>
<td>$K_{\text{min}}$</td>
<td>1.24 ± 0.161$^c$</td>
<td>1.27 ± 0.169$^b$</td>
<td>1.14 ± 0.146$^c$</td>
</tr>
<tr>
<td>$K_{\text{max}}$</td>
<td>1.49 ± 0.170$^f$</td>
<td>1.40 ± 0.174$^{b,c}$</td>
<td>1.19 ± 0.149$^b$</td>
</tr>
</tbody>
</table>

Site A: grazed grassland; Site B: grassland fenced for 4 years (1999–2003); Site C: grassland fenced for 24 years (1979–2003); $R_{\text{min}}$: nitrification rate; $R_{\text{max}}$: net N mineralization rate. Different superscript letters represent statistical significance at $P < 0.05$ among the three grassland sites.
moisture level. Our results suggested that the 15% moisture level limited net N mineralization in our experimental sites.

4.2. Interactive effect of temperature and moisture on net N mineralization

As the controlling factors over soil microbes, temperature and moisture could interactively affect net soil N transformation processes (Knoepp and Swank, 2002). However, the interactive effects of temperature and moisture were observed to vary with different N transformation processes and grassland sites. No difference in net N mineralization rate among the three moisture levels at low temperatures (−10, 0, and 5 °C), increasing net N mineralization rate with moisture at higher temperatures (15, 25, and 35 °C), and the greater enhancement in net N mineralization rate by increasing temperature at higher (25 and 35%) than lower (15%) moisture level suggest shifts in the limitations of low temperature and/or soil moisture on microbial activities and net N mineralization in the temperate grassland in North China. The interactive effects of temperature and moisture on N transformation processes observed in our study and previous studies (Frazer et al., 1990; Mazzarino et al., 1991) facilitate the projection of the responses of net N mineralization and availability in natural ecosystems where there are concurrent variations in temperature and precipitation.

4.3. Effect of incubation time on inorganic N accumulation

The observations in our study showed that the accumulations of nitrate and inorganic N increased whereas net nitrification and N mineralization rates decreased with prolonged incubation time. Other studies also reported increasing accumulation of inorganic N with incubation time (Cookson et al., 2002; Knoepp and Swank, 2002; De Neve et al., 2003; Amador et al., 2005). The increasing accumulation of inorganic N with incubation time is clearly attributable to the lack of plant uptake and nitrate leaching in laboratory incubation experiments (Alexander, 1964; Sierra, 1997).

Soil net N mineralization has long and well been studied in different ecosystems both in the field and in the laboratory. However, there are great variations in the incubation time used in different studies (Sierra, 1997; Knoepp and Swank, 2002; Cookson, et al., 2002; De Neve et al., 2003; Amador et al., 2005). For example, incubation times as short as 14 days (Verchot et al., 2001) and as long as 550 days (Dallas et al., 2002) have been reported. Given the rapid turnover of soil microbes, various incubation times could lead to substantial differences in the rates of microbial N immobilization and net N mineralization as shown in our study and previous study (Pansu et al., 2005). The inconsistencies in the incubation time make it difficult to compare the effects of temperature, moisture and their potential interactions on net N mineralization across different ecosystems.

4.4. Variations of soil N pools and net N mineralization among the three grassland sites

Lower soil organic C and N, C:N ratio, and concentrations of ammonium, nitrate, and inorganic N in the grazed site than the two fenced sites suggest negative effects of grazing disturbance on soil N pools. Our results are consistent with those of previous studies (Goodale and Aber, 2001; Wright et al., 2004). For example, Lamb (1980) found that total inorganic N content was significantly higher in the oldest community than in the youngest community. The reduced soil N pools under grazing disturbance could be caused by: (1) enhanced N loss through leaching and gaseous emission from denitrification, (2) increased biomass N removal by animals and less N release from plant litter (Biondini et al., 1998), and (3) changes in N transformation rate due to altered soil physical (i.e., bulk density and air permeability, Van Veen and Kuikman, 1990; Ladd et al., 1996) and chemical characteristics (i.e., C concentration and C:N ratio, Alexander, 1964; Garcia and Rice, 1994; Frank et al., 2000; Prescott et al., 2000).

The grazed site had higher net N mineralization rate than the site fenced for 24 years whereas there were no differences in net nitrification rate among the three sites. Other in situ incubation studies showed increases in net N mineralization rate under grazing disturbance (McNaughton et al., 1988; Holland et al., 1992; Seagle et al., 1992; Milchunas and Lowenroth, 1993). However, Goodale and Aber (2001) found higher net N mineralization rates in the long-term undisturbed sites than the historically disturbed sites. The inconsistent results might have been caused by the changes in the temperature and water regimes in laboratory comparing with in the field. As shown in our observations (Fig. 4), varying incubation times in different studies may cause substantial differences in net N mineralization rate and contribute to the inconsistency.

In the Inner Mongolia grassland region, there is great synchrony between plant growth and high temperature and moisture. The lower concentration of inorganic N in the grazed site, especially when moisture and temperature are high, suggest that plant growth may be limited by soil N availability. Greater net N mineralization rates in the grazed site comparing to the fenced sites may lead to greater N loss through leaching in the grazed site because of less N uptake by plants. Stimulated N loss by grazing may result in even lower N availability, negatively feeding back to plant growth and biomass production (Table 1).

In conclusion, soil temperature and moisture had significant effects on net nitrification and N mineralization in the Inner Mongolia grassland. The interactive effects of temperature and moisture on net nitrification and N mineralization were statistically significant in the two fenced sites, but not in the grazed site. Grazing seems to reduce concentrations of soil inorganic N, increase net N mineralization rate, and have no effect on net nitrification rates. The changes in the temperature sensitivity of nitrification and net N mineralization with soil moisture among the three grassland sites will complicate...
the projection of soil N availability and dynamics under global climate change.

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References


