

# Interactive effects of soil temperature and moisture on soil N mineralization in a *Stipa krylovii* grassland in Inner Mongolia, China

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**Abstract:** Determining soil N mineralization response to soil temperature and moisture changes is challenging in the field due to complicated effects from other factors. In the laboratory, N mineralization is highly dependent on temperature, moisture and sample size. In this study, a laboratory incubation experiment was carefully designed and conducted under controlled conditions to examine the effects of soil temperature and moisture on soil N mineralization using soil samples obtained from the *Stipa krylovii* grassland in Inner Mongolia, China. Five temperature (i.e. 9°C, 14°C, 22°C, 30°C and 40°C) and five moisture levels (i.e. 20%, 40%, 60%, 80% and 100% WHC, where WHC is the soil water holding capacity) were included in a full-factorial design. During the 71-day incubation period, microbial biomass carbon (MBC), ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) and nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) were measured approximately every 18 days; soil basal respiration for  $\text{qCO}_2$  index was measured once every 2 days (once a week near the end of the incubation period). The results showed that the mineral N production and net N mineralization rates were positively correlated with temperature; the strongest correlation was observed for temperatures between 30°C and 40°C. The relationships between moisture levels and both the mineral N production and net N mineralization rates were quadratic. The interaction between soil temperature and moisture was significant on N mineralization, i.e. increasing temperatures (moisture) enhanced the sensitivity of N mineralization to moisture (temperature). Our results also showed a positive correlation between the net nitrification rate and temperature, while the correlation between the  $\text{NH}_4^+\text{-N}$  content and temperature was insignificant. The net nitrification rate was negatively correlated with high  $\text{NH}_4^+\text{-N}$  contents at 80%–100% WHC, suggesting an active denitrification in moist conditions. Moreover,  $\text{qCO}_2$  index was positively correlated with temperature, especially at 80% WHC. With a low net nitrification rate and high soil basal respiration rate, it was likely that the denitrification concealed the microbial gross mineralization activity; therefore, active soil N mineralization occurred in 60%–80% WHC conditions.

**Keywords:** soil N mineralization; soil temperature; soil moisture; *Stipa krylovii* grassland

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Nitrogen (N) is a major limiting factor for terrestrial ecosystem productivity. In the 1990s, anthropogenic N production, including fertilization and fossil fuel combustion, amounted to more than 160 Tg/a, exceeding biological N fixation on land (110 Tg/a) and in the ocean (140 Tg/a) (Gruber and Galloway, 2008). It has been reported that N deposition in China has changed vegetation growth and diversity, soil pH and

organism diversity, and the cycles of other elements over the last 30 years (Liu et al., 2011). Soil N mineralization is the process of transforming undissolved organic N into dissolved inorganic N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ); the process is influenced by soil temperature, soil moisture, vegetation litter, soil organic matter and soil management (Zaman and Chang, 2004; Zhang et al., 2008; Wang et al., 2009; Liu et al., 2010; He et al.,

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2012; Jiao et al., 2012; Qi et al., 2012). Considering that conceptual models of the soil N cycle have been modified in previous studies (Schimel and Bennett, 2004), soil N mineralization has always been the highlight of past studies due to its importance in the N cycle.

Soil N mineralization results from microbial metabolism and responds to factors that affect microbial activity, such as temperature and moisture. Due to the complicated interactions among environmental factors, the effects of soil temperature and moisture on N mineralization have been obscured in field measurements (Fu et al., 2009; Bernal et al., 2012). Thus, field studies have primarily focused on the N mineralization amount or rate to estimate N availability in the ecosystem (Vernimmen et al., 2007; Zhu et al., 2013) rather than the sensitivity of N mineralization to temperature and moisture. Laboratory incubations under controlled conditions can separate the effects of temperature and moisture on N mineralization, however, with diverse soil nutrient contents, controlling factor level settings and sampling frequencies, different studies have provided inconsistent responses. In order to eliminate the effect of soil nutrient changes, many studies used disturbed soil samples. In those previous researches, the net N mineralization rate has been acknowledged to be positively related to temperature during incubation (Zaman et al., 2004; Wu et al., 2007). However, some studies have shown that the net N mineralization rate was positively related to soil moisture (Zhang et al., 2008; Chen et al., 2012), while others have suggested that the N mineralization rate decreased when the moisture level was beyond an optimal condition (Wu et al., 2007; Zou et al., 2010). Even less information is available regarding the combined effects of these two factors on N mineralization processes (Guntinas et al., 2012); therefore, a thorough explanation of the mineralization mechanism is limited.

To address the aforementioned issues, we conducted an incubation experiment using homogenized soil samples obtained from a *Stipa krylovii* grassland in Inner Mongolia, China, for 5 controlled temperature and 5 moisture levels. The soil mineral N production, net N mineralization rate and net nitrification rate were measured during 5 sampling days. For obtaining

evidence of microbial activity variations, soil basal respiration and microbial biomass carbon were also measured. The goal of this study was to examine how soil N mineralization responds to the interactive effects of soil temperature and moisture, understand the mechanism of N transformation in a temperate grassland ecosystem, and provide fundamental research for regional climate change and N cycle of the terrestrial ecosystem.

## 1 Materials and methods

### 1.1 Study area

The study area is located in Duolun county, Inner Mongolia, China (42.02°N, 116.17°E; 1,341 m asl). The annual mean temperature is 2.1°C, with a monthly mean temperature ranging from a minimum of -17.5°C in January to a maximum of 18.9°C in July. The mean annual precipitation is 385.5 mm; most rainfall events occur from May to September (Niu et al., 2009). The typical soil in this region is chestnut; the soil is composed of 62.75% sand, 20.30% silt and 16.95% clay (Liu et al., 2009). Moreover, the soil pH was 6.8, total organic carbon (TOC) was 2.61%, total nitrogen (TN) was 0.306%, total phosphorus (TP) was 0.539% and the C/N ratio was 8.53. The site is dominated by *Stipa krylovii*, *Artemisia frigida* and *Allium bidentatum* (Huang et al., 2008).

### 1.2 Experimental design

#### 1.2.1 Soil sampling

Surface soil samples (0–20 cm) were collected from 4 randomly selected locations (the distance between any 2 locations was greater than 10 m) in the experimental site on 22 July 2012. After mixing and sieving to <2 mm to remove roots and stones, the fresh soil samples were kept at 4°C for further experiments. Additional samples were air dried and ground to <0.25 mm to measure the TOC, TN and TP contents.

#### 1.2.2 Laboratory incubation

We used a full-factor design of 25 treatments, including 5 temperature (i.e. 9°C, 14°C, 22°C, 30°C and 40°C) and 5 moisture (20%, 40%, 60%, 80% and 100% WHC, where WHC is the soil water holding capacity) levels. Moreover, 500 soil samples (5 temperatures×5 moistures×4 replicates×5 sampling duplicates) were prepared by weighing 50 g of oven-

dried soil into a 250-mL glass flask. The moisture level was determined using two groups of soil samples. One group was oven dried at 105°C to a constant weight to determine the gravimetric water content ( $n=4$ ); the other group was inundated for 24 h before being percolated to a constant weight to determine the WHC ( $n=4$ ). In this study, 20%, 40%, 60%, 80% and 100% WHCs were equivalent to 9%, 17%, 23%, 29% and 33% gravimetric water contents, respectively. After adding deionized water to fix the moisture level, the flasks were plugged using rubber plugs with small ventilation holes and subsequently placed in the incubators.

Incubators were opened and fanners were set to blow toward them for 30 min every week to ensure the O<sub>2</sub> efficiency. The samples were weighed and water was added once during every 7–10 days to maintain the moisture levels.

### 1.2.3 Measurements

Five soil sample duplicates were used to conduct measurements on 14 August, 1 September, 19 September, 6 October and 23 October 2012, respectively. The sampling days were labeled as day 2, day 20, day 38, day 55 and day 72, respectively. Microbial biomass carbon (MBC) was measured using the fumigation extraction method; ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) and nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N) were measured by spectrophotometry. On day 2, day 38 and day 72, additional soil samples were air dried and ground through a 0.25-mm sieve to measure the TOC with the potassium dichromate-oxidation method. Moreover, TN and TP were analyzed using a flow injection analyzer after acid digestion.

The soil basal respiration rate (R) was measured during the incubation period. The soil samples remained in the incubators for 5 days to stabilize. Then, on 13 August, the first measurement was conducted and labeled as 1 day of incubation. Moreover, R was measured using a portable IRGA analyzer (LICOR 6262, Li-cor, Lincoln, Nebraska, USA). After correcting for temperature and pressure, R (μg C/(g soil-d)) was determined to be the slope of the respiration linear function.

### 1.2.4 Statistical analysis

The following formulas were applied in this study:

$$\text{Soil net N mineralization rate} = [(\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})_{\text{latter sampling day}} - (\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})_{\text{former sampling day}}] / \text{interval between two sampling days};$$

$$\text{Soil net nitrification rate} = [\text{NO}_3^-\text{-N}_{\text{latter sampling day}} - \text{NO}_3^-\text{-N}_{\text{former sampling day}}] / \text{interval between two sampling days};$$

$$\text{Metabolic quotient } q\text{CO}_2 = R/\text{MBC}.$$

One-way ANOVA was used to test the temperature and moisture effects on the soil mineral N production and net N mineralization rate. Using a Dunnett-T3 test, we assessed the significance of the differences between temperature or moisture levels. Two-way ANOVA was used to test the interactive effects of temperature and moisture on the soil mineral N production, net N mineralization rate and net nitrification rate. For identifying any highly correlated variables, Spearman correlation analysis was used to analyze the relationships between temperature and moisture and the soil mineral N production, net N mineralization rate, and net nitrification rate. The statistical analyses were conducted using SPSS 13.0; graphs were created using Sigmaplot 12.5.

## 2 Results

### 2.1 Soil characteristics during incubation

Compared to the soil characteristics measured in the field, TOC, TN and TP after the 71-day incubation period decreased by 12.5%, 14.1% and 13.5%, respectively. The soil nutrition contents decreased due to the absence of external nutrition additions (Table 1). Moreover, the soil nutrition contents were higher on day 72 than on days 2 and 38, likely because some organic matter, such as fine roots, passed through the 2-mm sieve and released nutrition *via* microbial decomposition near the end of the incubation period. The C/N ratio increased during incubation; however, the difference was insignificant. Due to changes in the soil environment, additional findings are discussed by separating the incubation period into early, middle and late incubation periods.

**Table 1** Dynamics of TOC, TN, TP and C/N ratio in the surface soil samples during incubation ( $n=100$ )

Incubation day	TOC (%)	TN (%)	TP (%)	C/N ratio
Day 2	2.108	0.244	0.442	8.66
Day 38	2.070	0.238	0.431	8.69
Day 72	2.283	0.263	0.466	8.70

## 2.2 Effects of temperature and moisture on soil mineral N production

The mean soil mineral N productions from the 5 sampling days were 63.62, 70.10, 97.57, 151.49 and 146.28 mg/kg, respectively. The mineral N production dynamics followed an S-like curve, suggesting that the soil environment was favorable for N production in the early incubation period; microbes proliferated quickly after a short adaptation and metabolized vigorously until the middle and late incubation periods when the metabolite production was largest. Due to the concurrent existence of N mineralization and immobilization in the soil (Zaman and Chang, 2004), N production gradually increased before decreasing in the late incubation period, probably due to the immobilization of microbes under stress. The mean soil mineral N productions were 83.40, 72.54, 91.00, 116.40 and 162.88 mg/kg for temperatures of 9°C, 14°C, 22°C, 30°C and 40°C, respectively. Moreover, N production was positively correlated with temperature ( $P<0.01$ ); however, the difference in the soil mineral N production between the different temperature levels was insignificant. The mean soil mineral N productions were 118.18, 181.42, 193.96, 20.86 and 12.24 mg/kg for 20%, 40%, 60%, 80% and 100% WHC, respectively. The maximum mean soil mineral N production occurred for WHCs of 40%–60%. Despite the soil mineral N production differences for 40% WHC versus 60% WHC and for 80% WHC versus 100% WHC being insignificant, differences among other moisture levels were significant. Temperature positively affected soil mineral N production

( $P<0.05$ ) on all sampling days except for day 20; the production was negatively correlated with moisture ( $P<0.001$ ) on all five sampling days because the production decreased substantially at 80%–100% WHC (Fig. 1).

## 2.3 Effects of temperature and moisture on soil net N mineralization rate and net nitrification rate

The mean soil net N mineralization rate of the 4 sampling intervals was positively correlated with temperature ( $P<0.001$ ); the mineralization rates for temperatures at 9°C–22°C were significantly lower than that at 40°C ( $P<0.05$ ). Because the mineralization rates at 80%–100% WHC were lower than those at 20%–60% WHC ( $P<0.01$ ), the net N mineralization rate was negatively correlated with moisture ( $P<0.001$ ). Moreover, the soil net N mineralization rates for the 25 treatments differed largely from day 2 to day 20, with a maximum at 30°C and 60% WHC and a minimum at 40°C and 100% WHC. Negative values indicated that the microbes exploited inorganic N as an N source for proliferation (Fig. 2a).

Furthermore, the net N mineralization rates from day 20 to day 38 and from day 38 to day 55 were positively correlated with temperature ( $P<0.01$ ); most values were positive. From day 20 to day 38, the mineralization rates at 40°C were significantly higher than those at other temperatures ( $P<0.01$ ), suggesting that microbial metabolism increased as the temperature increased; the amount of N released by decomposition finally exceeded the microbial N requirement and inorganic N accumulated in the middle incubation

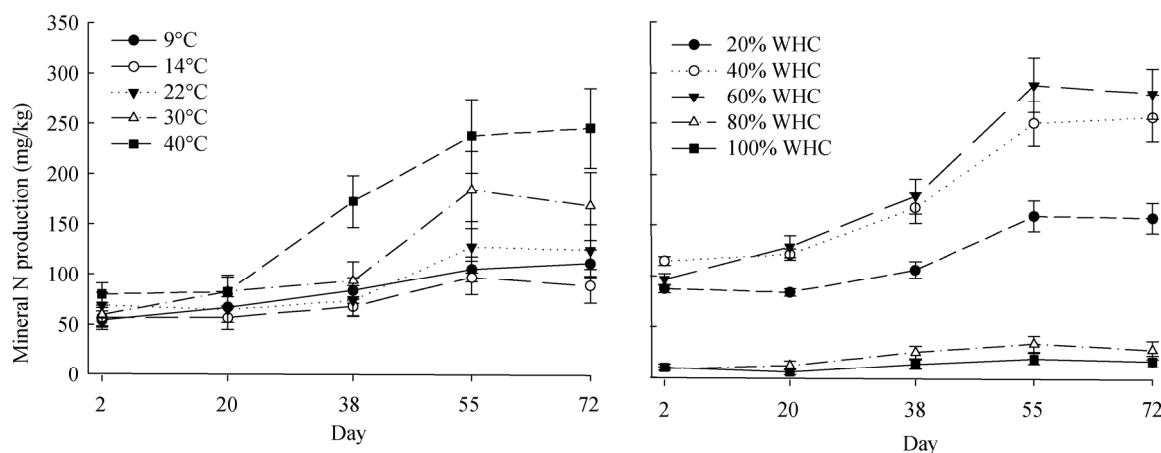
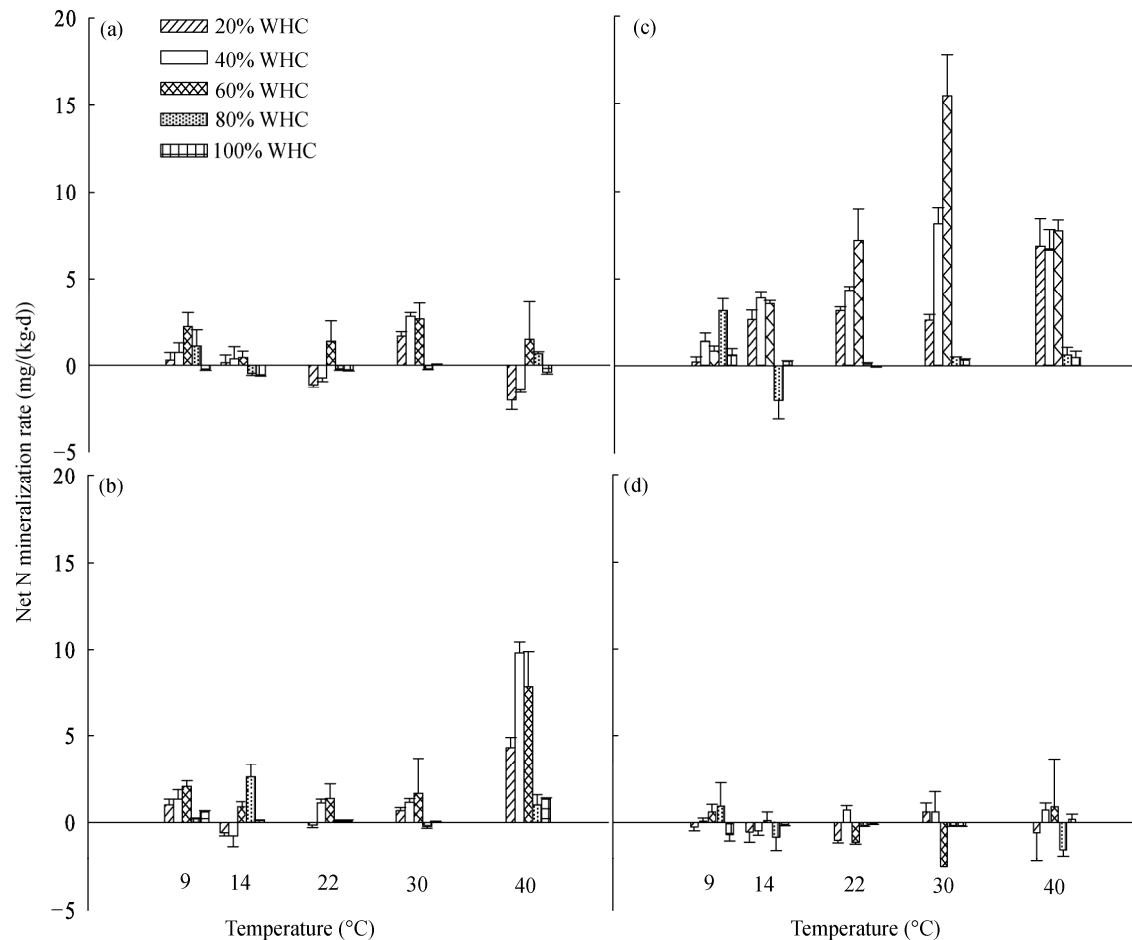


Fig. 1 Dynamics of soil mineral N production during incubation in different temperature levels (a) and different moisture levels (b).  $n=4$ .



**Fig. 2** Soil net mineralization rate in different temperature and moisture levels during incubation (a) from day 2 to day 20, (b) from day 20 to day 38, (c) from day 38 to day 55, (d) from day 55 to day 72.  $n=4$ .

period (Figs. 2b and c). While microbial activity partially increased with increasing moisture content, the net N mineralization rates were significantly lower at 80%–100% WHC ( $P<0.01$ ) from day 38 to day 55. The net N mineralization rates were generally low from day 55 to day 72; the differences were insignificant between both different temperature and moisture levels. The negative values suggested that substrate depletion caused environmental stress and forced the microbes to immobilize inorganic N (Fig. 2d).

In soil mineralization processes, released  $\text{NH}_4^+$  was typically oxidized into  $\text{NO}_2^-$  and then  $\text{NO}_3^-$ , leading to relatively low  $\text{NH}_4^+$  contents but large  $\text{NO}_3^-$  contents in the soil (Zou et al., 2010). The net nitrification rate was highly correlated with the net N mineralization rate in the 4 sampling intervals ( $R^2>0.90$ ,  $P<0.05$ ). From day 2 to day 20, the correlations between the net nitrification rate and different environmental factors

were insignificant; however, the nitrification rate was much higher at 60% WHC than at 20% WHC and 80%–100% WHC ( $P<0.05$ ). Moreover, the extremely low (even negative)  $\text{NH}_4^+$ -N contents at 20%–40% WHC were possibly related to the conversion of  $\text{NH}_4^+$  to  $\text{NH}_3$  and the immobilization of microbes (Liu et al., 2007). During the middle incubation period, the soil net nitrification rate exhibited a positive feedback to rising temperature. From day 38 to day 55, the net nitrification rates were significantly higher at 20%–60% WHC than at 80%–100% WHC ( $P<0.01$ ); the net nitrification rates were generally outcompeted by the net N mineralization rates, demonstrating that active metabolism increased the accumulation of inorganic N in the middle incubation period. As a result of the environmental stress in the late incubation period, the nitrification response to temperature or moisture was suppressed. Moreover,  $\text{NH}_4^+$ -N content was relatively

high at 80%–100% WHC despite the low net nitrification rates, suggesting that the high moisture condition inhibited aerobic respiration and nitrification such that  $\text{NH}_4^+$  was not converted to  $\text{NO}_3^-$  and that denitrification might participate in converting N into a gaseous state (Wang et al., 2004; Levin et al., 2010).

#### 2.4 Interactive effects of temperature and moisture on soil N mineralization

The study found that the mean soil mineral N productions, net N mineralization rates and nitrification rates for the five sampling days all responded to the interactive effects of temperature and moisture ( $P < 0.001$ ; Table 2). The interactive effects of temperature and moisture were significant on the soil mineral N production and net nitrification rate during the entire incubation period ( $P < 0.001$ ). Moreover, during the early and middle incubation periods, temperature and moisture exhibited interactive effects on the net N mineralization rate ( $P < 0.01$ ), except from day 55 to day 72 when the interactive effects were insignificant ( $P > 0.1$ ).

The range of net N mineralization rates among different moisture levels at 9°C and 14°C were 0.78 and 1.09 mg/(kg·d), which increased to 4.68 mg/(kg·d) at 40°C, indicating that low temperatures inhibited the response of the soil net N mineralization rate to moisture. Moreover, increasing temperatures could increase exoenzyme activity and enhance the sensitivity of microbial metabolism to moisture. The soil net N mineralization rate was highest at 60% WHC; however, the rate decreased significantly at 80%–100% WHC. The net N mineralization rate was observed to increase with increasing temperature at 20%–60% WHC; the mineralization rates for this range of WHCs were 2.79, 3.46 and 3.99 mg/(kg·d), respectively, indicating that increased moisture content stimulated the sensitivity of the net N mineralization rate to temper-

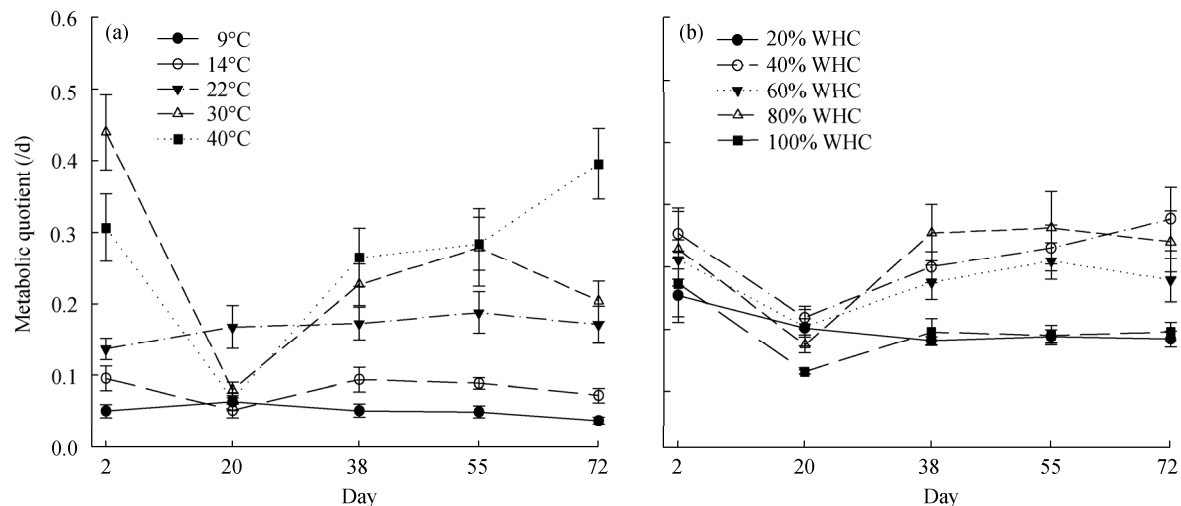
ature. In summary, increased temperatures (moisture) enhanced the sensitivity of the N mineralization to moisture (temperature). The abnormality that occurred at 80%–100% WHC was likely caused by the alteration of microbial activity or community composition.

#### 2.5 Relationship between the metabolic quotient $q\text{CO}_2$ and soil N mineralization

Generally,  $q\text{CO}_2$  is an index used to evaluate soil microbial metabolism efficiency, which is often called the basal respiration rate per microbial biomass. The index is relevant to soil substrate availability and microbe community composition; large values signify the need for a large portion of C in assimilates for microbial maintenance respiration rather than cell growth and reproduction (Zhou et al., 2009). On all 5 sampling days,  $q\text{CO}_2$  was positively correlated with temperature and quadratically related to moisture content; the lowest  $q\text{CO}_2$  values occurred at 20% WHC and 100% WHC. Moreover,  $q\text{CO}_2$  was marginally positively related to temperature ( $P = 0.07$ ) and negatively related to moisture ( $P < 0.001$ ) on day 2, when  $q\text{CO}_2$  was stimulated by increasing temperature ( $P < 0.001$ ); however, no significant correlation with moisture was observed on the other sampling days (Fig. 3). Furthermore, the  $q\text{CO}_2$  values for 9°C–22°C were as low as 0.03, 0.04 and 0.05/d, implying low microbial activity, slow metabolism and minor changes in the incubation environment (Li and Qu, 2002); therefore, the net N mineralization rate was low and mineralization production steadily increased. For temperatures of 30°C–40°C,  $q\text{CO}_2$  increased to 0.36 and 0.33/d; large changes were previously shown for the mineral N production and net N mineralization rate within this temperature range. Additionally, on day 20,  $q\text{CO}_2$  at 30°C–40°C decreased significantly because of the blooming microbial biomass in the early incubation

**Table 2** Two-way ANOVA for soil mineral N production, net N mineralization rate and nitrification rate

Source	Mineral N production			Net N mineralization rate			Net nitrification rate		
	df	F	P	df	F	P	df	F	P
Temperature (T)	4	476.22	<0.001	4	48.35	<0.001	4	56.00	<0.001
Moiture (M)	4	2,615.37	<0.001	4	107.91	<0.001	4	162.65	<0.001
T×M	16	73.13	<0.001	16	12.59	<0.001	16	17.09	<0.001



**Fig. 3** Dynamics of microbial metabolic quotient  $qCO_2$  during incubation (a) in different temperature levels and (b) in different moisture levels.  $n=4$ .

period;  $qCO_2$  at 30°C–40°C increased again in the following incubation period and exceeded the values found for temperatures of 9°C–22°C. This finding indicates that increased maintenance respiration, decreased microbial biomass and the alteration of the microbial community composition occurred in high temperature conditions (Alvarez et al., 1995; Dilly and Munch, 1998). The mean  $qCO_2$  was lowest at 20% WHC and 100% WHC due to the low substrate mobility at 20% WHC and the limitation of  $O_2$  efficiency at 100% WHC. Moreover, the soil basal respiration rate was inhibited and microbes might utilize inorganic N as an N source for low energy consumption (Gao et al., 2008), which caused small mineral N productions and low net N mineralization rates. The soil basal respiration was highest at 80% WHC, while the net N mineralization rate was very low due to denitrification;  $qCO_2$  was lower at 60% WHC than at 40% WHC and 80% WHC. The soil mineral N production and net N mineralization rate were highest at 60% WHC.

### 3 Discussion

#### 3.1 Effect of temperature on soil N mineralization

The optimal temperature for soil microbial activity is approximately 25°C–35°C. Increased temperatures under favorable moisture conditions can contribute to maintain microbial respiration, which leads to increased soil N mineralization (Chapin et al., 2002). Previous studies on Umbrisols under Atlantic oak for-

est, grassland and cropland and bog soil under alpine wetland soil found that N mineralization peaked at 35°C (Gao et al., 2008; Guntinas et al., 2012). Other studies found an optimal temperature of 25°C for mountain brown soil under deciduous broadleaved forest and peat soil under alpine wetland soil (Zhou et al., 2001; Gao et al., 2008). The diversity in the optimal temperature for N mineralization can be attributed to soil N availability and seasonal microbial community dynamics (Knoepp and Swank, 2002; Shibata et al., 2011). We found that the mineral N production and net N mineralization rate both increased with increasing temperatures and peaked at 30°C–40°C (the difference between the two temperatures was insignificant), suggesting that the optimal temperature for the net N mineralization rate in the *Stipa krylovii* grassland was 30°C–40°C.

As mentioned in previous studies,  $NH_4^+$  should accumulate at low temperatures and nitrification should increase with increasing temperatures (Liu et al., 2007; Zou et al., 2010). We found that the net nitrification rate was positively correlated with temperature, while the  $NH_4^+$ -N content did not exhibit any significant correlation with temperature, which was likely because the soil in the experiment site was chestnut and the pH was 6.8; therefore, negatively charged soil particles can bind with  $NH_4^+$  or  $NH_4^+$  can be converted to  $NH_3$  (Sun et al., 2013). Nitrification has a relatively high energy requirement and uses  $NH_4^+$  as a substrate. Increasing temperatures increased the exoenzyme activity and vigorous microbial metabolism induced

immobilization of  $\text{NH}_4^+$  (Chapin et al., 2002; Schimel and Bennett, 2004).

### 3.2 Effect of moisture on soil N mineralization

As another factor affecting microbial metabolism, soil moisture was highest at 60% WHC and lowest at 80%–100% WHC for both the mineral N production and net N mineralization rate. We found that the positive feedback of N mineralization to moisture exhibited a threshold; the mineral N production and net N mineralization rate decreased beyond the optimal moisture content, which resulted from inhibited exoenzyme activity and increasing denitrification. This finding was consistent with other studies in the natural condition (Wang et al., 2004; Fu et al., 2009). Moreover, the net N mineralization rate was low at 20% WHC and 80%–100% WHC because the cellular desiccation of microbes occurred under low moisture conditions (Chen et al., 2011), and inhibited air exchange caused the inactivity of aerobic microbes and increased denitrification under high moisture conditions (Tingey et al., 2006; Zou et al., 2010).

Net nitrification rate was large at 60% WHC throughout the incubation period; however,  $\text{qCO}_2$  was lower at 40% WHC and 80% WHC. Regardless of the low net nitrification rate, the soil basal respiration rate was high at 80% WHC, suggesting that denitrification likely concealed microbial mineralization activity, and the alteration of the microbial community caused by denitrifiers might contribute to the high  $\text{qCO}_2$  at 80% WHC. In summary, 60% WHC was considered to be the optimal moisture level for the net N mineralization rate in the *Stipa krylovii* grassland, while soil N mineralization process remained active under 60%–80% WHC conditions.

### 3.3 Interactive effects of temperature and moisture on soil N mineralization

Significant responses in N mineralization to the interaction of temperature and moisture were found in some studies, both in the field experiments and laboratory incubations (Wang et al., 2004; Bell et al., 2008; Shibata et al., 2011). In this study, net N mineralization rates were synonymous with previous researches ( $P < 0.001$ ), and soil mineral N production and net nitrification rate both respond to the interactive effects of temperature and moisture. The effect of one factor is strengthened or weakened by the other when inter-

active effects occur between two regulating factors (Kirschbaum, 1995). Soil moisture could influence the role of temperature on microbes by changing the energy balance, substrate availability and  $\text{O}_2$  concentration (Fan et al., 2008; Liu et al., 2009), while temperature could regulate the exoenzyme activity and soil oxidation to influence the microbial response to moisture (Xie et al., 2006). We found that the soil net N mineralization rates were low at 9°C–22°C and for all moisture levels, indicating that at low temperatures, microbial activity was slow and N mineralization was suppressed. At 20%–60% WHC, the range of the net N mineralization rates among different temperature levels increased with moisture levels, showing that the sensitivity of the net N mineralization rate to temperature increased with increasing moisture levels; however, the range decreased at 80%–100% WHC due to the large decrease in the net N mineralization rate.

Furthermore, soil N transformation includes the processes of absorption (utilization by plants and microbes) and loss (e.g.  $\text{NH}_3$  and  $\text{N}_2\text{O}$ ). Using the mineral N production and net N mineralization rate may underestimate the response of gross N mineralization to temperature and moisture (Zaman and Chang, 2004; Shibata et al., 2011). Moreover, the sampling frequency has been reported to influence the results (Liu et al., 2007; Liu et al., 2010). In this study, the sampling frequency was once every 18 days, which was sufficient to measure the soil N mineralization dynamics. However, higher sampling frequency is expected for drawing the N mineralization potential curve, especially in the early incubation (Yang et al., 2010).

## 4 Conclusions

Significant changes in soil N mineralization under the influence of hydrothermal factors have been shown in the *Stipa krylovii* grassland in Inner Mongolia, China. The mineral N productions and net N mineralization rates were positively correlated with temperature. The relationships between moisture and the mineral N production and net N mineralization rate were both quadratic. Moreover, the interactive effects of temperature and moisture on N mineralization were found to be significant. Active denitrification existed at 80%–100% WHC due to the high  $\text{NH}_4^+$ -N contents



and the low net nitrification rates. Considering that  $qCO_2$  was relatively large at 80% WHC, it was likely that the denitrification concealed the microbial gross mineralization activity; therefore, active soil N mineralization occurred under 60%–80% WHC conditions. Measurements of gaseous N loss and microbial community composition should be performed in future studies. Moreover, long-term laboratory incubations with more frequent sampling events should be conducted to construct a potential N mineralization model.

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