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## Responses of soil nitrogen mineralization to temperature and moisture in alpine ecosystems on the Tibetan Plateau

Yuqiang Tian <sup>a,\*</sup>, Hua Ouyang <sup>b</sup>, Qiong Gao <sup>a</sup>, Xingliang Xu <sup>b</sup>, Minghua Song <sup>b</sup>, Xia Xu <sup>a</sup>

<sup>a</sup>State key laboratory of earth surface process and resource ecology, Beijing Normal University, Beijing 100875, China.

<sup>b</sup>Institute of Geographical Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China.

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### Abstract

The responses of soil net nitrogen (N) mineralization to temperature and moisture were investigated in four alpine ecosystems of forest, shrub, meadow and steppe by laboratory incubation method with undisturbed soil cores on the Tibetan Plateau. The results indicated the soil net N mineralization varies greatly between alpine ecosystems. The soil net N mineralization rate in three incubating moisture of forest ecosystem rose markedly, and that of meadow ecosystem rose gently from temperature of 5 °C to 35 °C, while that of shrub and steppe ecosystems increased from temperature of 5 °C to 25 °C and reduced from temperature of 25 °C to 35 °C. At the same incubating temperature, the soil net N mineralization of four alpine ecosystems increased in the middle moisture and decreased in the low or high moisture.

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*Keywords:* Response; Nitrogen mineralization; Temperature; Moisture; Alpine ecosystems; Tibetan Plateau

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### 1. Introduction

The soil available N, the important limiting factor in many ecosystems, greatly affected the plant primary production process [1, 2, 3, 4]. The soil N availability mostly was controlled by N mineralization process, the process of organic N mineralized into inorganic N [1, 5]. To date, most studies on N mineralization were separately carried out in agriculture, forest, or grasslands ecosystem and seldom simultaneously carried out in different alpine ecosystems [6]. Studies show soil total N is rich in the meadow ecosystems on the Tibetan Plateau, available N directly used by plants is poor [4]. So the works on N mineralization process in the N-limitation ecosystems on the Tibetan Plateau is urgently needed to be done, which would deepen our knowledge on the N cycling mechanisms in this special geographic-zone, and also guide us for the future management of alpine ecosystems. The soil temperature and moisture were two most important environmental factors affecting N mineralization [4, 7, 8, 9]. This may arise from a fact N mineralization was driven by microbes, and the population, quantity and activity of

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\* Corresponding author. Tel.: 010-58801848.

E-mail address: [tyq276811@163.com](mailto:tyq276811@163.com); [tyq@bnu.edu.cn](mailto:tyq@bnu.edu.cn).

microbes were all highly related to soil temperature and moisture. The laboratory incubation method, precisely control the temperature and moisture, was widely used in soil N mineralization study [8, 9]. It could accurately quantify the response of N mineralization to temperature and moisture, and provide us parameters for the process-based ecosystem model building.

The objective of this paper was to test: a) whether the response of soil net N mineralization to temperature and moisture vary in certain alpine ecosystem; b) whether the net N mineralization varies under different temperature and moisture gradients between alpine ecosystems.

## 2. Materials and methods

### 2.1 Study sites

The Tibetan Plateau lying in the southwest of China, averaged 4000 meters high, covers about more than 26.8% of whole nation areas [10, 11]. The zonal vegetations of forest, shrub, meadow, steppe, and desert respectively distributes on the Tibetan Plateau from southeast to northwest [12], almost including all the vegetations of north hemisphere from south to north. The vegetation zones are so narrow that these vegetations are very sensitive to environmental change and a minor change of temperature and moisture would lead to a significant change in zonal vegetation distribution and their compositions and functions [12].

**Table 1.** The experiment sites in alpine ecosystems on the Tibetan Plateau

Ecosystem	Dominant plant	E °	N °	Altitude(m)	AP(mm)	AT(°C)
forest	<i>Abies georgei var.smithii</i>	94.55	29.56	3774	705	0.6
shrub	<i>Lonicera</i>	91.6	29.8	3808	437	5
meadow	<i>Kobresia pygmaea</i>	91.68	32.07	4724	450	-2.8
steppe	<i>Stipa pures</i>	92.47	34.25	4559	288	-3.4

Note: AP: annual precipitation; AT: annual temperature.

Based on the objective of comparative study on N mineralization in different alpine ecosystems, we sampled soils in each alpine ecosystem (**Table 1**) and incubated them in laboratory at different temperature and moisture gradient. The chosen four alpine ecosystems were an alpine forest dominated by *Abies georgei var.smithii*, an alpine shrub dominated by *Lonicera*, an alpine meadow dominated by *Kobresia pygmaea*, and an alpine steppe dominated by *Stipa pures*. [13].The altitudes of forest and shrub sites are lower and that of meadow and steppe sites are higher than 4000 meters. The forest site lies in the southeast of Tibetan Plateau, and the annual precipitation and temperature of that is 705 mm and 0.6°C respectively; Shrub site lies in the south of Tibetan Plateau, the annual precipitation is lower 268 mm and the annual temperature is higher 4.4 °C than that of forest. The annual precipitation and temperature of meadow are 450 mm and -2.8°C respectively, while that of steppe is 288 mm and -3.4°C respectively.

### 2.2 Sample collection and laboratory incubation

Based on ecosystems type, we sampled upper 20 cm soils (no including the litters) by PVC pipes (inner diameter is 5 cm, length is 20cm) along Qinghai-Tibet road from July to August of 2004. In each ecosystem we sampled 39 soil cores (each treatment have 3 replicates), totally sampled 156 soil cores. Before sampling we got rid of the above plants and litters and put the PVC pipes into upper 20 cm soils, and pulled out the PVC pipes with undisturbed soil cores, and sealed the PVC pipes with fresh-keeping film (waterproof and breathable), and quickly went back to laboratory to keep the soil cores under 4 °C circumstances.

The soil cores were incubated in the laboratory in 4 constant incubators under 4 temperatures (5°C, 15°C, 25°C and 35°C) and 3 moisture (**a**: low moisture; **b**: middle moisture; **c**: high moisture) gradients. The moisture gradients are regulated as follow: (a) low moisture: the natural water content of undisturbed soil cores by no adding any water; (b) middle moisture: half saturated water content of soil cores by adding water; (c) high moisture: saturated water content of soil cores by adding water. When finished regulating soil moisture, we put the 144 PVC pipes into 4 constant incubators incubating for 30 days. The initial NH<sub>4</sub>-N and NO<sub>3</sub>-N contents were analyzed using left 12 soil cores; when finishing incubation the final NH<sub>4</sub>-N and NO<sub>3</sub>-N contents would also be analyzed.

### 2.3 Chemical analyses

Soil organic carbon (SOC) was measured by K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-extra heating method [14]; soil total N (TN) was measured by kjeldahl N method [14]; soil mechanical composition was measured by Laser Intensity Meter; soil pH was measured by acidometer; soil bulk density was measured by cutting ring method [14]; soil water content was measured by oven drying method [14]; NH<sub>4</sub>-N and NO<sub>3</sub>-N were measured by distillation method [15].

### 2.4 Calculation and statistical analyses

Soil net mineralization rate and soil net nitrification rate are calculated as following<sup>[8]</sup>:

$$N_{min}=(N_2-N_1)/d \quad (1)$$

$$N_{nit}=(N_2'-N_1')/d \quad (2)$$

Where  $N_{min}$  is soil net N mineralization rate ( $mg \cdot kg^{-1} \cdot d^{-1}$ ),  $N_1$  is the initial inorganic N content (NH<sub>4</sub>-N + NO<sub>3</sub>-N) before incubation,  $N_2$  is the final inorganic N content (NH<sub>4</sub>-N + NO<sub>3</sub>-N) after incubation;  $N_{nit}$  is soil net N nitrification rate ( $mg \cdot kg^{-1} \cdot d^{-1}$ ),  $N_1'$  is the initial NO<sub>3</sub>-N content before incubation,  $N_2'$  is the final NO<sub>3</sub>-N content after incubation;  $d$  is the incubation days ( $d$ ) [8].

Statistical analyses of data were conducted by software of Microsoft Excel 2003 and SPSS 13. Figures were processed by software of SigmaPlot 10.

## 3. Results and discussions

### 3.1 Basic soil properties of experiment sites

**Table 2.** The basic soil chemical properties of experiment sites in alpine ecosystems

Ecosystem	Soil type	SOC (%)	TN (%)	C/N	Initial NO <sub>3</sub> -N ( $mg \cdot kg^{-1}$ )	Initial NH <sub>4</sub> -N ( $mg \cdot kg^{-1}$ )
forest	Brown earths	10.01	0.65	15.47	18.64	17.86
shrub	Cold brown calcic soils	2.98	0.12	25	22.95	5.74
meadow	Felty soils	3.44	0.23	14.83	16.79	9.21
steppe	Dark frigid calcic soils	0.11	0.02	7.33	15.3	5.74

The basic soil chemical properties of experiment sites in the four alpine ecosystems on the Tibetan Plateau were investigated (**Table 2.**). The soil types of forest and shrub are Brown earths and Cold brown calcic soils, and that of meadow and steppe are Felty soils and dark frigid calcic soils. The nutrient element contents of these soils varied greatly. There was a sharp difference in the SOC and TN contents between alpine ecosystems. The highest SOC (10.01%) and TN (0.65%) contents were found in forest ecosystem, and the lowest SOC (0.11%) and TN (0.02%) contents were found in steppe ecosystem, and the middle contents were found in shrub and meadow ecosystems. The studies showed the soil C/N ratio greater than 25 would exhibit net N immobilization; otherwise the C/N ratio less than 25 would exhibit net N mineralization. That is to say, to a certain extent the C/N ratio can indicate whether would exhibit soil net N mineralization or immobilization. The soil C/N ratios of experiment sites

in this study were all less than 25 (**Table 2.**), which meant that was theoretically more beneficial to N mineralization. If the inorganic N in the soils could not meet microbes' demands, the net N immobilization process would be dominant in the soil N cycling [4]. After satisfying their demands for inorganic N, the soil microbes would release inorganic N to soils by N mineralization process.

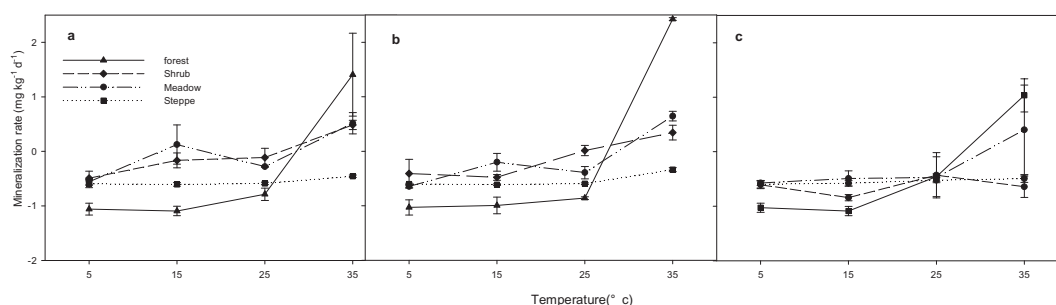
The soil bulk density varied insignificantly in four alpine systems on the Tibetan Plateau (**Table 3.**). In forest and meadow ecosystems, the bulk densities were less than 1 g.cm<sup>-3</sup>, which maybe arose from the fact many roots lying in these soil layers; and the soil bulk densities in both of shrub and steppe ecosystems were greater than 1 g.cm<sup>-3</sup>. The forest soil was found mild acid soil based on soil pH, but the shrub, meadow and steppe soils are alkaline soils (**Table 3.**). The soil pH differences in the alpine ecosystems led to the variations in the soil microbes' type and quantity. The sand and silt contents in four alpine ecosystems were all above 99% and the clay contents were less than 1 % (**Table 3.**), these soil textures were more in favour of N mineralization. The soil development on the Tibetan Plateau was so short that alpine soils were mainly composed of coarse silt and sand components, which was also good for the N mineralization on the Tibetan Plateau.

**Table 3.** The basic soil physical properties of experiment sites in alpine ecosystems

Ecosystem	Bulk density (g.cm <sup>-3</sup> )	pH	Mechanical composition (%)		
			Clay	Silt	sand
forest	0.68	5.12	0.31	79.29	20.40
shrub	1.26	7.81	0.21	42.96	56.84
meadow	0.95	7.03	0.41	41.12	58.47
steppe	1.43	8.08	0.02	6.61	93.37

### 3.2 The response of soil net N mineralization to temperature and moisture

The responses of soil net N mineralization rate  $N_{min}$  to temperature and moisture differed greatly in alpine forest, shrub, and meadow and steppe ecosystems (**Figure 1**), and the soil net N mineralization positively correlated with temperature in the three moisture gradients ( $R=0.79$ ,  $P<0.01$ ).



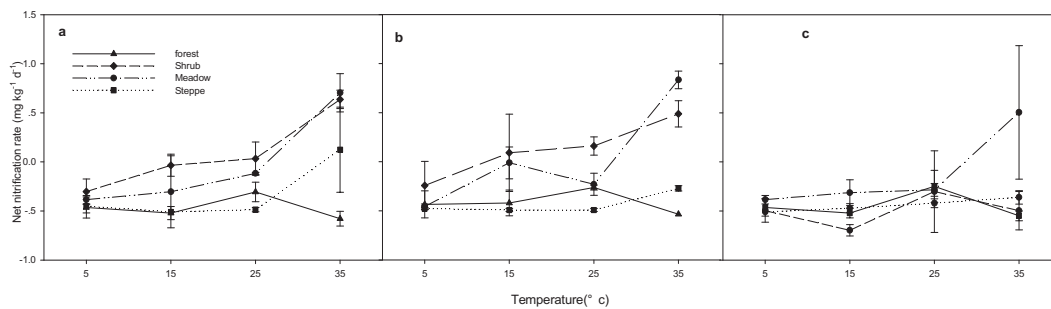
**a:** low moisture; **b:** middle moisture; **c:** high moisture

**Figure 1** Responses of soil net N mineralization rate to temperature and moisture in alpine ecosystems on the Tibetan Plateau

In certain moisture, the  $N_{min}$  responded very actively to the temperature variation, but in different temperature range the response varied greatly. In the three moisture gradients (**Figure 1**), from temperature 5 to 35 °C the  $N_{min}$  rose markedly as the temperature increase in the four alpine ecosystems. Except that in the high moisture from temperature 25 to 35 °C (**Figure 1c**), the  $N_{min}$  decreased as the increase of temperature in shrub ecosystem.

In the forest ecosystem, the  $N_{min}$  rose gently before temperature 25 °C and increased markedly after temperature 25 °C (**Figure 1**), which meant the  $N_{min}$  responded no so actively before temperature 25 °C than after

25 °C. The *Nmin* was negative before temperature 25 °C and positive after temperature 25 °C in the forest ecosystem. Generally the negative *Nmin* meant N immobilization occurred [4, 16, 17], the entire mineralized inorganic N were absorbed by soil microbes. As temperature increased the microbe’s community would grow quickly and the quantity and activity of soil microbes would also be improved, the most direct exhibition to us was the N immobilization dominated in the soil N cycling [18]. The temperature of *Nmin* changed from negative to positive was the critical temperature for N mineralization, less than critical temperature the inorganic N would all be absorbed by the microbes, higher than the critical temperature the inorganic N would be released to soil from microbe’s immobilization. For forest, shrub and meadow ecosystems the critical temperature was 25 °C. Less than Before the temperature 25 °C, the *Nmin* of forest ecosystem was lower than the other three alpine ecosystems in all moisture, while after 25 °C it was higher than the other three ecosystems. In shrub and meadow ecosystems, the *Nmin* rose up and down before 25 °C and increased evidently after 25 °C. In steppe ecosystem, the *Nmin* rose gently as the increase of temperature from 5 to 35 °C, but the *Nmin* was negative, which meant the N immobilization occurred in all conditions and no net N released for the plant growth.



a: low moisture; b: middle moisture; c: high moisture

Figure 2. Responses of soil net N nitrification rate to temperature and moisture in alpine ecosystems on the Tibetan Plateau

The soil net N nitrification of four alpine ecosystems was different from the soil net N mineralization (Figure 2). In the low and middle moisture gradients (Figure 2a,2b), the *N<sub>nit</sub>* increased as the increase of temperature before 25 °C and decreased after 25 °C in the forest ecosystem, while in the other three alpine ecosystems the *N<sub>nit</sub>* increased in all the four temperature gradients. The *N<sub>nit</sub>* increased before 25 °C and decreased after 25 °C of alpine forest, shrub and steppe ecosystems in the high moisture (Figure 2c). The *N<sub>nit</sub>* increased in all the temperature gradients in alpine meadow ecosystem.

### 3.3 The influence of temperature and moisture on soil N mineralization

Table 4. The equations describing the relations of *Nmin* or *Nnit* with temperature and moisture in alpine ecosystems on the Tibetan Plateau

	Ecosystem	Equation	R <sup>2</sup>	P	N
<i>Nmin</i>	forest	$Nmin = 0.008T - 0.104M - 0.585$	0.54*	0.03	36
	shrub	$Nmin = 0.012T - 0.056M + 0.464$	0.72**	0.001	36
	meadow	$Nmin = 0.016T - 0.149M - 0.594$	0.28**	0.001	36
	steppe	$Nmin = 0.005T - 0.025M - 0.531$	0.53*	0.035	36
<i>Nnit</i>	forest	$Nnit = 0.002T - 0.008M - 0.778$	0.42*	0.05	36
	shrub	$Nnit = 0.0111T - 0.06M + 0.901$	0.74**	0.001	36
	meadow	$Nnit = 0.0126T + 0.01M - 0.304$	0.44**	0.001	36
	steppe	$Nnit = 0.005T - 0.0004M - 0.666$	0.53*	0.05	36

Note: *Nmin*: soil net N mineralization rate ( $mg.kg^{-1}.d^{-1}$ ); *Nnit*: soil net N nitrification rate ( $mg.kg^{-1}.d^{-1}$ ); T: temperature (°C); H: moisture (%); \*: significant at  $P < 0.05$ , \*\*: very significant at  $P < 0.01$ , N: Sample size.

The influence degrees of temperature and moisture on the N mineralization differed in different alpine ecosystems (**Figure 1**). The experimental results indicated the N immobilization dominated in the N cycle process, which led to the values of  $N_{\min}$  or  $N_{\text{nit}}$  were negative, so we could not fit the exponential curves as in the other studies. First degree in two variable equations was fitted for the  $N_{\min}$  or  $N_{\text{nit}}$  with temperature and moisture (**Table 4**). The  $R^2$  of fitted equations were all reached significant levels, which explained the fitted curves better in illustrating the relations between the  $N_{\min}$  and  $N_{\text{nit}}$  with temperature and moisture. The variations of independent variable coefficients from the first degree in two variable equations between four alpine ecosystems in this study further indicated the different influencing degrees of temperature and moisture on the soil N mineralization.

### 3.4 The $Q_{10}$ of soil net N mineralization in different alpine ecosystems

The  $Q_{10}$  is the sensitive coefficient of indicating the sensitivity of soil N mineralization to temperature variations. The greater  $Q_{10}$  value means the higher temperature sensitivity, the lower  $Q_{10}$  value means smaller temperature sensitivity.

**Table 5.** The  $Q_{10}$  of soil net N mineralization in different alpine ecosystems on the Tibetan Plateau

Ecosystem	moisture	temperature (°C)		
		5~15 °C	15~25 °C	25~35 °C
Forest	Low	1.03	0.72	1.79
	Middle	0.97	0.86	2.82
	High	1.06	0.42	2.26
Shrub	Low	0.34	0.68	4.30
	Middle	1.17	0.03	2.56
	High	1.39	0.51	1.49
Meadow	Low	0.23	2.23	1.84
	Middle	0.31	1.95	1.64
	High	0.86	0.97	0.85
steppe	Low	1.02	0.97	0.78
	Middle	1.02	0.97	0.57
	High	0.97	0.90	0.94

Note: Low: low moisture; Middle: middle moisture; High: high moisture

The  $Q_{10}$  of 25 to 35 °C in forest and shrub was higher than other temperature ranges, while that of 15 to 25 °C in meadow and 5 to 15 °C in steppe were higher than other temperature ranges (**Table 5**), which meant the responses of microbes to different temperature ranges differed in different alpine ecosystems. The soil physical and chemical properties could affect the species and activities of microbes in alpine ecosystems. The acidic environment was good for fungi growth, and the alkaline soil was good for bacteria<sup>[18, 19]</sup>. The different species of microbes in alpine ecosystem would response to temperature variation differently. In different moisture gradient, the  $Q_{10}$  value of certain ecosystem differed no significantly. The differences of microbes in their activities and growth rates between the alpine ecosystems gave cause for the  $Q_{10}$  variation between the temperature ranges. From this point, the temperature would greatly affect the soil N mineralization than the moisture in the alpine ecosystems.

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