

Short-term Effect of Nitrogen Addition on Microbial and Root Respiration in an Alpine Spruce Ecosystem

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Abstract: Soil respiration plays an important role in the carbon (C) flux of the global C cycle and is greatly affected by nitrogen (N) additions in the form of deposition or fertilization. The aim of this study was to investigate the response of total soil respiration (Rs), microbial respiration (Rm), and root respiration (Rr) to short-term N addition and the potential mechanisms of short-term N deposition influencing soil respiration in an alpine spruce ecosystem. Four N treatment levels (0, 50, 100, 150 kg N ha⁻¹ year⁻¹) were applied monthly in a *Picea balfouriana* (commonly known as “alpine spruce”) plantation beginning in November 2013 and Rs, Rm, and Rr were measured from May to November 2014. The results show that simulated N depositions stimulate Rs, Rm, and Rr and the beneficial effects decreased along N gradients from hourly to seasonal scales. The seasonal temperature coefficients (Q_{10}) of Rs, Rm, and Rr ranged from 2.50 to 3.8, 2.99 to 4.63, and 1.86 to 2.96, while the diurnal Q_{10} ranged from 1.71 to 2.04, 1.89 to 2.32, 1.42 to 1.75, and there was a similar trend with soil respiration along N gradients. In addition, Rr showed significant positive correlation with fine root biomass, and Rm was likely driven by soil enzyme related to the microbial C cycle in the growing season. Our results indicate that short-term N addition stimulated fine root biomass and soil enzymatic activity to bring about a potential increase in soil respiration rates under low-N addition, while the opposite occurred under high-N addition.

Keywords: Nitrogen addition, Microbial respiration, Root respiration, Q_{10} , Alpine forest ecosystem.

Introduction

More than 1500 Pg of carbon (C) are stored in soil carbon sinks, approximately twice and three times more than the C storage in the atmosphere and vegetation, respectively [1]. Global estimates of the soil respiration rate range from 50 to 98 Pg C year⁻¹ [23], which is approximately 60-90% of the ecosystem respiration [25]. The CO₂ that is released from the soil has a great impact on the CO₂ concentrations of the atmosphere and contributes positive feedback to global climate change [11]. Studying the C fluxes of soil respiration and their variable patterns under environmental factors is vital to evaluating the global C balance.

Soil temperature was the main factor to drive the daily and seasonal patterns of soil respiration. In alpine forest ecosystems, low soil temperatures repress respiration, and respiration would increase exponentially with increases in soil temperature [31]. Water is another important factor affecting soil respiration, especially in monsoon areas [20]. In these ecosystems, soil moisture often influences soil respiration interacted with soil temperature, making the soil respiration rate unable to reach its maximum at optimal temperature [39]. The nitrogen (N) is also an important factor affecting soil respiration. The reactive deposition of N has gradually increased and will persist in the following decades because of human activities, such as the production and combustion of fossil fuels since the 1850s [10]. The addition of different N nutrients to forest soils has been concluded to have positive [4, 31], negative [20], or no significant [16] effects on soil respiration. It remains a scientific challenge for us to understand how N application would alter soil C cycling and the soil CO₂ flux after increasing atmospheric reactive nitrogen entering the soil.

Soil respiration consists of two components: R_r released from plant roots, and mycorrhizal microorganisms and R_m from the decomposition of plant residues and soil organic matter [31]. N addition influences R_m by altering soil enzymatic activities, microbial communities, and plant decomposition rates [6, 37]. Moreover, Tu et al. [31] believed that short-term N addition would stimulate R_m through increasing microbial biomass, available nutrient concentration, and extracellular enzymatic activities under N additions. The complexity of factors influencing R_m made the responses of R_m to N addition inconsistent. Additionally, N addition could decrease fine root biomass [16, 20], resulting in corresponding changes in R_r. Thus, a further study of the different responses of R_s, R_m, and R_r after N addition may contribute to achieving a comprehensive understanding of the impacts of increasing N enrichment on soil respiration and C cycling.

Compared to the N-polluted regions of the Eastern United States and Western Europe (typically 47 kg N m⁻² year⁻¹), the alpine forest on the Qinghai-Tibetan Plateau receives only 4.6 kg N ha⁻¹ year⁻¹ atmospheric wet N deposition due to being far from industrial areas and remaining relatively undisturbed by humans [14]. Therefore, it was an ideal region to demonstrate the actual impacts of increasing N deposition on CO₂ fluxes. N addition in some N-saturated ecosystems or high N addition in N-unsaturated ecosystems would become a limiting factor because of the C-limited state when soil demand for N is gradually satisfied [26]. Alpine areas differ from warm temperate zones not only in climate conditions, but also by being more N-limited than C-limited [30]. Studies to date have shown that short-term N addition would stimulate fine root growth [4, 31] and soil enzymes related to microbial activity [27] to promote soil respiration. Therefore, we conducted an N addition experiment investigating the effects of N deposition on microbial and root respiration in an alpine spruce forest ecosystem.

Materials and methods

Site description

The study was conducted in Honglashan, Tibet, China (29°16'N, 98°41'E, 3792 m.a.s.l.). The study area is a large-scale, complicated ecotone that is located southeast of Tibet Province, ranging 80 km east to west and 30 km north to south, with an area of approximately 1853 km². The region has a mid-subtropical, humid, mountainous climate. The mean annual temperature was 6.5 °C, and the mean annual precipitation was 509 mm in 2014. The vegetation was dominated by alpine spruces. At the time of our study, the plant density was 1056 trees ha⁻¹, and the mean diameter at breast height was 13.1 cm. The vegetation and soil carbon stock were 49.13±12.28 and 120.32±33.00 trees ha⁻¹, respectively, in September

2013 ($n = 3$). The soil properties at the study site were measured using the samples that were collected in the control plots (0-10 cm depth) in September 2013 and showed that the soil pH, organic C, total N, total P, C/N ratio, and bulk density were 6.10 ± 0.07 , $36.94 \pm 15.96 \text{ g kg}^{-1}$, $2.81 \pm 1.00 \text{ g kg}^{-1}$, $0.58 \pm 0.09 \text{ g kg}^{-1}$, 12.15 ± 1.54 , and $1.10 \pm 0.22 \text{ g cm}^{-3}$, respectively ($n = 3$).

Experimental design

Twelve plots were established within the study site in September 2013, each measuring $3 \times 3 \text{ m}$ at approximately 5 m intervals. The individual plots were separated from each other by impermeable plastic covers at 0-1 m in soil depth. Plots were divided into four treatments (3 plots per treatment) as follows: (1) Control (no N added), (2) Low-N ($50 \text{ kg N ha}^{-1} \text{ year}^{-1}$), (3) Medium-N ($100 \text{ kg N ha}^{-1} \text{ year}^{-1}$), and (4) High-N ($150 \text{ kg N ha}^{-1} \text{ year}^{-1}$). Ammonium nitrate (NH_4NO_3) was applied monthly in equal applications from November 2013 to October 2014. In each application, the fertilizer was weighed, dissolved in 1 L of water, and applied to each plot using a portable sprayer. The control plots received 1 L of water without fertilizer. In November 2013, each $3 \times 3 \text{ m}$ plot was randomly divided into two sections for measuring R_r and R_m , respectively. The detailed method of determining R_r and R_m referred to the paper published by Tu et al. [31].

Measurement protocols

Soil fluxes were determined between 8:00 and 10:00 approximately every 15 days depending on the weather conditions on each measuring date using Li-8100. Diurnal measurements at the same site from June to October 2014 showed that the average soil respiration of the diurnal cycle occurred between 8:00 and 18:00. The soil respiration rates were expressed as $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. The probe of temperature and moisture was inserted to monitor soil temperature and moisture near the chamber each time. Most research has defined the diameter of roots that are thinner than 2 mm as fine roots [13, 16]. Fine roots were mainly collected in the 0-10 cm soil horizon and separated by washing and sieving through a 0.42 mm sieve, dried at 65°C for 48 h, and weighed. The fine root biomass was expressed as the weight of the root biomass in the unit area (g m^{-2}). Soil samples for enzymatic assay were collected from the 0-10 cm horizon in November. Three soil enzymes that are closely related to the soil C cycle were chosen for analysis in this study. The polyphenol oxidase activity, glucosidase activity and cellulase activity were measured by the methods mentioned by Guan et al. [12]. Kang and Freeman [15] found that the mean activity of four soil enzymes could be used as an indicator for litter decomposition rates. We suppose that it is likely more helpful to explain variations of R_m through an average of soil enzymes than a single soil enzyme along N gradients. Therefore, the soil enzymatic activity that are mentioned in this paper were the average of three soil enzymatic activities.

Statistical analyses

Statistical analyses were carried out using SPSS v19.0 (SPSS Inc., Chicago, USA). A repeated-measure ANOVA and Fisher's LSD tests were performed to examine soil temperature, soil moisture, R_s , R_r , R_m , fine root biomass and mean soil enzymatic activity for different treatments. Significance was determined at $\alpha = 0.05$. The exponential equation between soil respiration and temperature was $R = \alpha e^{(\beta T)}$ [17], where R is the rate of the total or components of soil respiration ($\mu\text{mol m}^{-2} \text{ s}^{-1}$), T is the soil temperature at a 10 cm depth ($^\circ \text{C}$), and α and β are fitted parameters. The Q_{10} value was calculated as $Q_{10} = e^{10\beta}$.

Results

Microclimate and biotic factors

Precipitation was mainly concentrated in July, August, and September when air temperature was high, showing obvious seasonal variations in the experimental site (Fig. 1). Precipitation was 408.2 mm, and the mean air temperature was 11.7 °C during the study period (half a year). The soil temperature at a 10 cm soil depth ranged from 2.0 °C in November to 14.1 °C in July in the control plots (Fig. 2A). The seasonal variation in soil moisture was similar to that of precipitation, ranging from 2.9 to 40.5 cm³ H₂O 100 cm³ soil (Fig. 2B). Soil temperature and moisture were not significantly different among the plots during the study period ($p = 0.1484$ and 0.2764 , respectively, see Table 1).

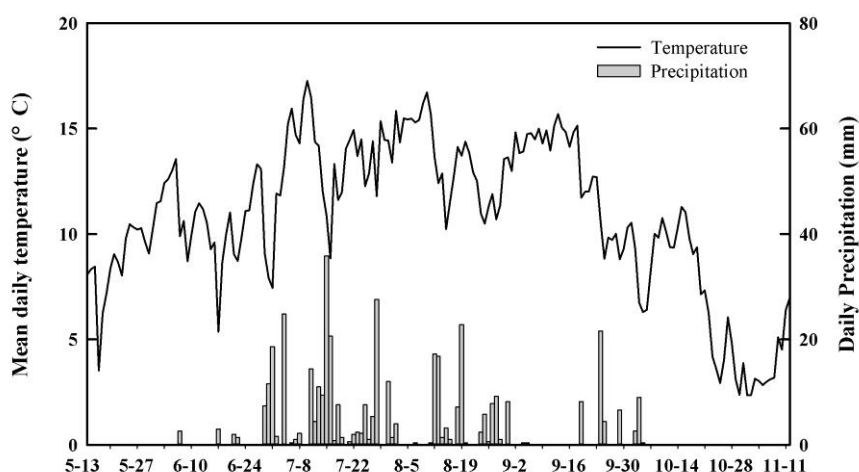


Fig. 1 Seasonal variation in air temperature and precipitation in an alpine spruce plantation in the south-eastern Tibetan Plateau from May to November 2014.

Bars indicate \pm SE, $n = 3$.

Table 1. Results of repeated measures ANOVA of soil temperature (°C), soil moisture (cm³ H₂O cm³ soil), and total and component soil respiration (μ mol CO₂ m⁻² s⁻¹) in an alpine spruce plantation in the south-eastern Tibetan Plateau.

Treatment/effect	ST	SM	Rs	Rm	Rr
Control	8.86 \pm 0.69 ^a	15.04 \pm 2.38 ^a	0.74 \pm 0.07 ^b	0.49 \pm 0.05 ^b	0.25 \pm 0.03 ^b
Low-N	8.80 \pm 0.67 ^a	16.99 \pm 2.29 ^a	1.22 \pm 0.13 ^a	0.84 \pm 0.09 ^a	0.38 \pm 0.05 ^a
Medium-N	8.58 \pm 0.67 ^a	18.02 \pm 2.19 ^a	1.30 \pm 0.11 ^a	0.84 \pm 0.09 ^a	0.46 \pm 0.04 ^a
High-N	8.56 \pm 0.69 ^a	18.44 \pm 1.86 ^a	0.96 \pm 0.09 ^b	0.59 \pm 0.06 ^b	0.37 \pm 0.05 ^a
Time effect	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Time\timesN effect	$p = 0.2516$	$p = 0.0081$	$p = 0.0002$	$p < 0.0001$	$p = 0.0907$
N effect	$p = 0.1484$	$p = 0.2764$	$p = 0.0026$	$p = 0.0051$	$p = 0.0192$

ST: soil temperature at 10 cm below the surface; SM: soil moisture in 0-10 cm horizon (%)

The biomass was 47.01 g·m⁻² in the control plots. Mean activity of soil β -glucosidase, soil polyphenol oxidase and soil cellulase were 0.28 in control plots. Although low-N and medium-N additions increased fine root biomass and mean enzymatic activity, there was an obvious decrease in fine root biomass and mean enzymatic activity after high-N addition compared to medium-N addition (Fig. 3A and 3B).

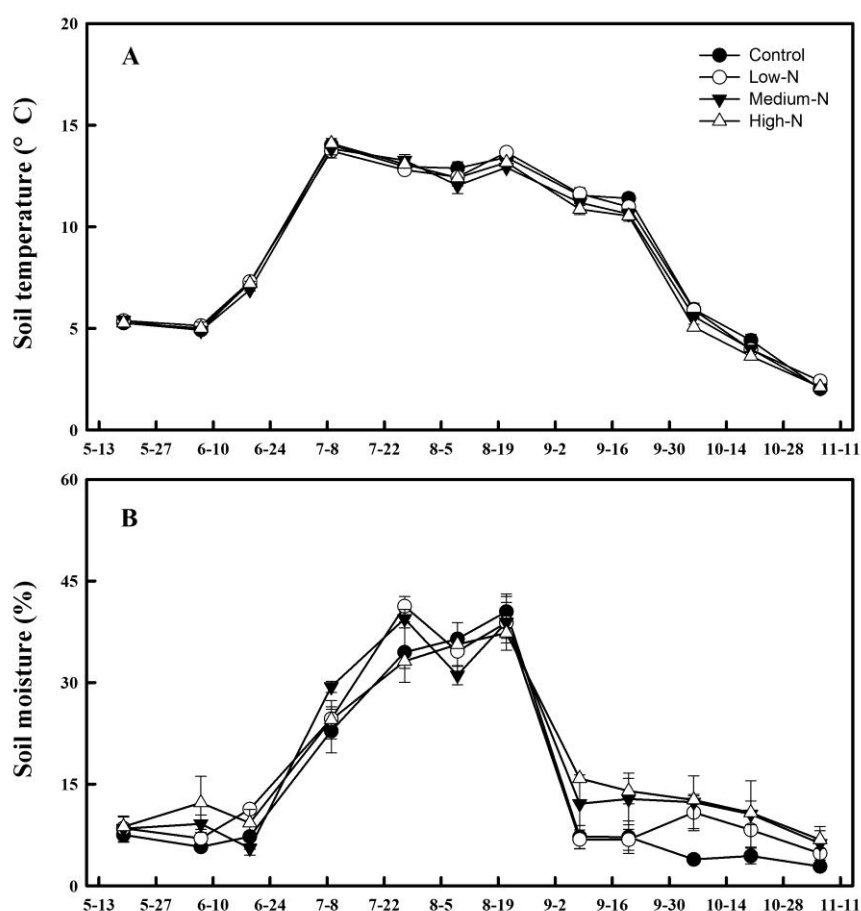


Fig. 2 Seasonal variations in soil temperature and soil moisture in an alpine spruce plantation in the south-eastern Tibetan Plateau from May to November 2014. Error bars indicate \pm SE, $n = 3$.

Soil respiration and its components under N addition

R_s , R_r , and R_m showed strong seasonal patterns, with the highest rates in the growing season and the lowest rates in the winter (Fig. 4), generating a great significant level ($p < 0.0001$) across the repeated-measure ANOVA analysis (Table 1). In the control plots, low-N and high-N plots, R_s reached the highest rate of 1.37 ± 0.23 , 2.40 ± 0.36 , and $1.76 \pm 0.12 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in August and the lowest rate of 0.30, 0.37, and $0.34 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in October. However, the highest rate of R_s in the medium-N plot occurred in July, which was $2.72 \pm 0.25 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. The highest rates of R_m and R_r occurred in July and August, respectively.

The repeated-measure ANOVA indicated that N addition had significant effects on soil respiration ($p < 0.05$). R_s , R_m , and R_r increased significantly with low-N addition, increased slightly but not significantly with medium-N addition ($p > 0.05$), and decreased significantly with high-N addition, except for R_r (see Table 1).

The diurnal variation of soil CO_2 fluxes of the alpine spruce plantation during the growing season of 2014 is shown in Fig. 5. In the five measurement days, the soil CO_2 fluxes increased from 8:00, peaked mainly between 12:00 and 16:00, and subsequently gradually decreased in each treatment plot. Compared to the control plots, low-N and medium-N promoted soil CO_2 release, but high-N decreased soil respiration rates.

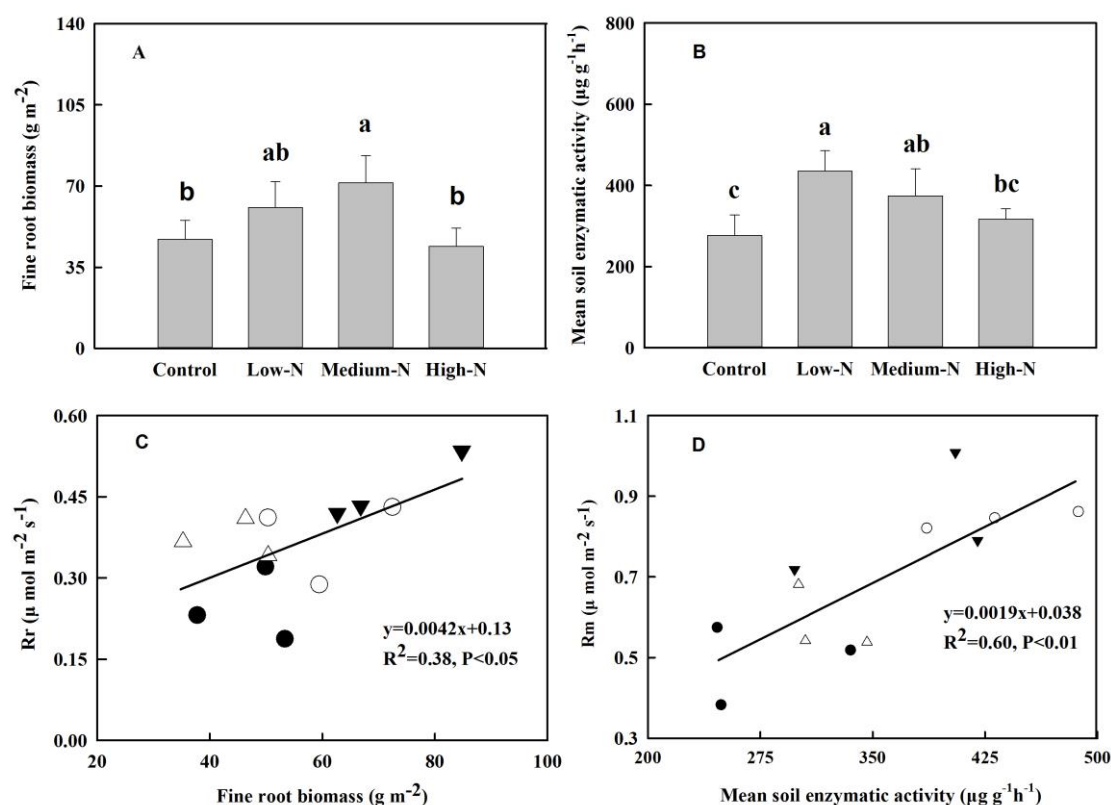


Fig. 3 Comparisons of fine root biomass and mean soil enzymatic activity and their relationships to soil respiration among treatments. Bars indicate \pm SE. Different letters denote significant differences ($p < 0.05$) between treatments. Dark circles (●) represent Control, open circles (○) represent Low-N, dark triangles (▼) represent Medium-N, and open triangles (▽) represent High-N. Rm and Rr represent microbial respiration and root respiration.

Temperature sensitivity of total respiration and its components

Rs, Rr, and Rm exponentially showed significant correlations with soil temperature in the growing season ($p < 0.05$) (Table 2). The temperature coefficients (Q_{10}) of Rs, Rm, and Rr were 2.50, 2.99, and 1.86, respectively, in the control plots. The Q_{10} value of Rm was higher than that of Rr. Low-N addition increased the Q_{10} values of Rs and two components of Rs. With N addition increasing in the medium-N and high-N plots, the Q_{10} values decreased greatly, whereas the Q_{10} value of Rr in high-N was higher than that in medium-N. Linear regression analyses indicated that Rs and its components increased significantly with soil moisture in all of the treatments except for Rm in the high-N plots ($p < 0.05$) (Table 3).

The diurnal patterns that were observed in the study significantly ($p < 0.05$) correlated with the diurnal variation of soil temperature at a 10 cm depth (Table 2). The determination coefficients (Q_{10}) of Rs, Rm, and Rr were 1.71, 1.89, and 1.43 in the control plots, respectively. Compared to the control plots, low-N addition increased the Q_{10} values of Rs and two components of Rs (Fig. 5). With increasing N addition, the Q_{10} values decreased greatly in the medium-N and high-N plots. Linear regression analyses showed that Rs and its components increased significantly with soil moisture in all of the treatments in partial plots ($p < 0.05$) (Table 3).

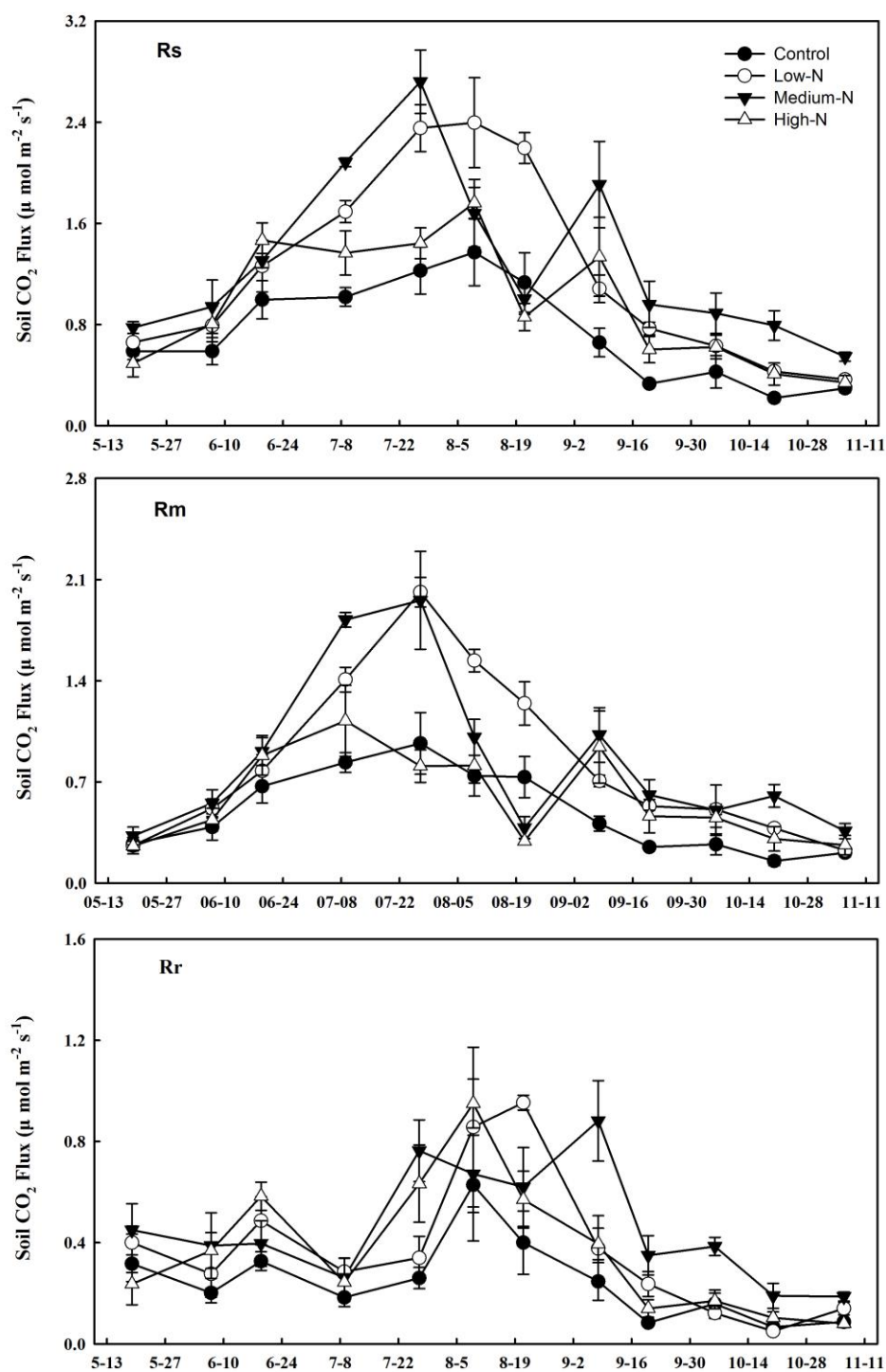


Fig. 4 Seasonal variations in total soil respiration and different components of soil respiration in an alpine spruce plantation in the south-eastern Tibetan Plateau from May to November 2014. Error bars indicate $\pm\text{SE}$, $n = 3$.

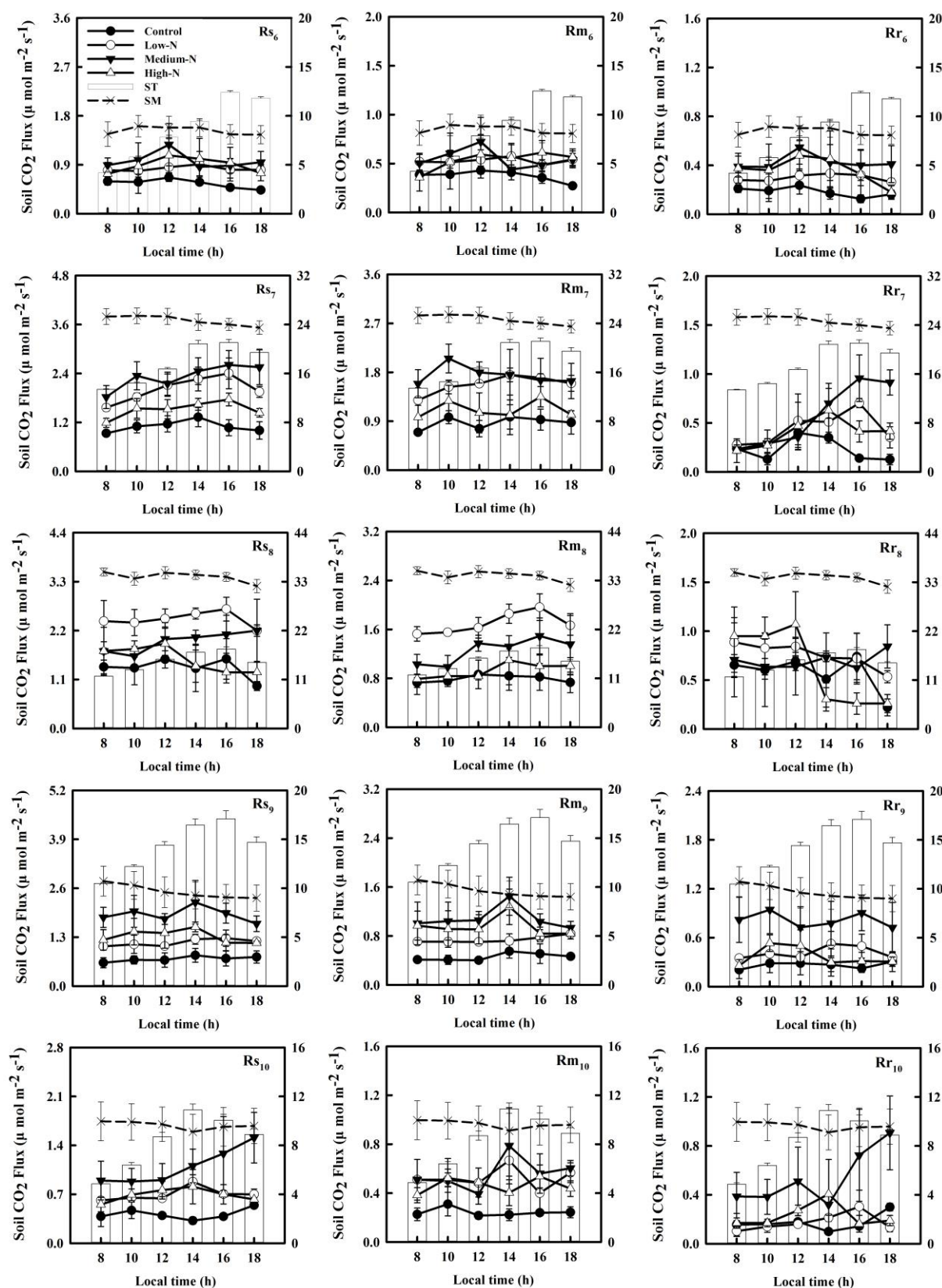


Fig. 5 Diurnal variations in total soil respiration and different components of soil respiration and soil temperature at a 10 cm depth (column) in an alpine spruce plantation in the south-eastern Tibetan Plateau from June to October 2014. ST represents soil temperature at 10 cm depth; SM represents soil moisture at 0-10 cm depth (%). Error bars indicate \pm SE, $n = 3$.

Table 2. The analytic results of temperature sensitivities of the total soil respiration and its components for seasonal and diurnal variation

R component	Treatment	Seasonal variation			Diurnal variation		
		<i>p</i>	<i>R</i> ²	<i>Q</i> ₁₀	<i>p</i>	<i>R</i> ²	<i>Q</i> ₁₀
Rs	Control	< 0.0001	0.39	2.50	< 0.0001	0.23	1.71
	Low-N	< 0.0001	0.66	3.98	< 0.0001	0.46	2.04
	Medium-N	< 0.0001	0.52	2.72	< 0.0001	0.40	1.95
	High-N	< 0.0001	0.43	2.35	< 0.0001	0.37	1.64
Rm	Control	< 0.0001	0.46	2.99	< 0.0001	0.32	1.89
	Low-N	< 0.0001	0.67	4.63	< 0.0001	0.50	2.17
	Medium-N	< 0.0001	0.45	3.63	< 0.0001	0.43	2.32
	High-N	0.0006	0.29	2.28	< 0.0001	0.35	1.76
Rr	Control	0.0471	0.11	1.86	0.0404	0.05	1.43
	Low-N	0.0021	0.25	2.96	0.0001	0.15	1.75
	Medium-N	0.0036	0.22	1.90	0.0209	0.059	1.42
	High-N	0.0015	0.26	2.45	0.0351	0.05	1.41

Table 3. The regression analysis results of seasonal and diurnal variation between total soil respiration, its components and soil moisture

R component	Treatment	Seasonal variation		Diurnal variation	
		Equation	<i>R</i> ²	Equation	<i>R</i> ²
Rs	Control	$Y = 2.50SM + 0.36$	0.68**	$Y = 7.78SM - 0.35$	0.69**
	Low-N	$Y = 3.27SM + 0.66$	0.40**	-	NS
	Medium-N	$Y = 3.08SM + 0.75$	0.34**	-	NS
	High-N	$Y = 2.57SM + 0.49$	0.31**	$Y = 5.94SM + 0.03$	0.32*
Rm	Control	$Y = 1.71SM + 0.24$	0.64**	$Y = 5.57SM - 0.30$	0.77**
	Low-N	$Y = 2.55SM + 0.33$	0.32**	-	NS
	Medium-N	$Y = 2.35SM + 0.42$	0.29**	$Y = 4.11SM + 0.29$	0.43**
	High-N	-	NS	-	NS
Rr	Control	$Y = 0.80SM + 0.13$	0.35**	$Y = 2.21SM - 0.05$	0.23*
	Low-N	$Y = 0.72SM + 0.33$	0.17*	-	NS
	Medium-N	$Y = 0.73SM + 0.33$	0.45**	-	NS
	High-N	$Y = 1.71SM + 0.06$	0.29**	$Y = 4.70SM - 0.52$	0.40**

Note: $Y = aSM + b$, where Y is the soil respiration and its components; SM is soil moisture at 0-10 cm depth; * significant impact at $p < 0.05$; ** significant impact at $p < 0.01$; NS – no significant impact.

Discussion

Effects of N addition on soil respiration and its components

We hypothesized that individual applications of N addition would lead to short-term increases in soil respiration, depending on the degree of amendment-induced C limitation. Compared to warm temperate forests, alpine forest soils are N- rather than C-limited [30]. Therefore, it is expected that short-term soil respiration would increase in response to fertilizer application in the new plots more strongly than in chronically N-treated plots.

The results in the spruce forest showed that N addition did promote Rs and its components. In the study region, N addition in the spruce forest stimulated Rs by 64.9%, 75.7%, and

29.7% for low-N, medium-N, and high-N treatment, respectively (Fig. 2). However, the positive effects weakened along N gradients in the study area, which was inconsistent with the results of the other short-term N additions in the experiments of previous studies [31, 36].

The following reason may explain the inconsistent weakened effects. The Tibetan Plateau is more sensitive to global change than areas in low-altitude regions [35], resulting in sensitive responses of the soil C cycle to global changes such as increasing N depositions. Therefore, we speculated that it gradually transforms N limitations to C limitations, weakening the positive response in R_s and its components (R_m and R_r) to simulated N. Although there were no inhibitions to soil respiration observed for the forest with N additions in the first year, decreases are assumed to occur in the future. Similar effects were observed in the results reported by Zhou and Zhang [39]. Since fine root respiration and associated microbial activity are two major sources of soil respiration [16], it is not surprising that the variations of soil respiration along an increasing N gradient in the spruce forest stands can ultimately be attributed to the sensitivity of fine roots and microbial activity to N addition.

Similar to R_s , our results indicated that simulated N deposition stimulated R_m in the spruce forest; however, the increasing effects declined along N gradients (71.4%, 71.4%, 20.4% for low-N, medium-N, high-N treatment, respectively). Soil enzymatic activity, mainly released by microorganisms [38], as a sensitive factor reflecting microbial activity and degradation of organic matter [34], has been found to be promoted by N addition [9]. The responses of weakened mean soil enzymatic activity (Fig. 3B) were speculated to be attributed to the reduced positive effects of R_m on N addition in the study area. Linear regression analysis also indicates a positive correlation between mean soil enzymatic activity and R_m ($p < 0.05$, Fig. 3D). These inhibitory results have been proved in many short-term N deposition experiments [4, 6, 39]. Further studies are needed to explore the reasons behind reductions in soil enzymatic activities under high-N additions. Moreover, the flux of CO_2 derived from root and rhizosphere microorganisms is an important part of soil respiration in the spruce plantations. In this paper, N addition stimulated R_r and the beneficial effects declined along N gradients in the spruce forest stands, which agreed with the results reported by Zhou and Zhang [39]. Although Sun et al. [28] suspected that the increased R_r caused by N addition may not be attributed to fine root biomass but rhizospheric microbial activity, the variations in fine root biomass were believed to be the reason for the patterns of R_r after N addition in most studies [16, 31, 36]. In this paper, similar to R_a , fine root biomass showed weakened increased effects along N gradients and a positive relationship with R_r in the two alpine forests ($p < 0.05$) (Fig. 3A and 3C), resulting in reduced beneficial effects of R_r after N addition.

Diurnal and seasonal variation of soil respiration

The research results to date indicate that diurnal changes in soil respiration are mainly driven by soil temperature [2]. Significant exponential relationships have been discovered between R_s , R_m , and R_r and soil temperature in this study, which is consistent with many other ecosystems [5, 32]. However, the maximum rates of soil respiration did not occur under maximum soil temperature but at noon and 14:00 in most measuring plots. Furthermore, we discovered a bimodal shape between soil respiration and temperature in some plots (Fig. 5 R_{s7} , R_{s9} , R_{m7} and R_{r9}). Regression analyses showed that R_s , R_m , and R_r were significantly correlated with soil temperature and had partially significant correlations with soil moisture ($p < 0.05$) (Table 2 and 3). The phenomenon indicates that soil respiration might be mainly driven by soil temperature, changing with the variation of soil moisture in a few plots. The results in Fig. 5 showed that soil respiration was mainly driven by soil temperature and

reached its maximum in the highest soil temperature in most plots when water was enough for the soil in July and August, which was consistent with the results of Tu et al. [31]. To the contrary, when soil water content was comparatively low in three other months, soil moisture became a restraining factor affecting soil respiration so that the highest soil respiration appeared slightly before the highest soil temperature or even presented bimodal shapes in some plots, reflecting the complex interactions between soil temperature, soil moisture and soil respiration [19].

Temperature has been considered the most important factor impacting the seasonal variations of soil respiration, which was proved by significant exponential regression between soil temperature and soil respiration (Table 2). However, regression analyses showed that Rs, Rm, and Rr were significantly correlated with soil moisture ($p < 0.05$) (Table 3). A proper increase in soil moisture could stimulate soil respiration. In this study, the largest soil respiration rate did not occur early in July when the soil temperature peaked, but occur late in July when soil moisture reached its maximum (Fig. 2 and Fig. 4). Moreover, soil moisture could be a limiting factor inhibiting soil respiration when exceeding its maximum. When soil moisture exceeded its maximum because of continuous precipitation (Fig. 1) in mid-August, we found that Rs and Rm decreased sharply on August 21, except for in the low-N addition treatments due to the supplement of the stimulating effects on soil respiration. This phenomenon indicates that Rs, Rm, and Rr are probably driven by the interaction of soil temperature and soil moisture under each treatment. This result agrees with other forest studies [7, 29]. The effects of temperature and moisture on soil respiration reflect the South Asian monsoon climate of our study region, with a typical separation of hot and humid, and cold and dry seasons (Fig. 1).

N addition's influence on temperature sensitivity of soil respiration and its components

The diurnal Q_{10} of the soil respiration rate was generally less than 2.0 in most plots (Table 2), which was similar to the results in an alpine steppe ecosystem (2.1) [18], but lower than those figures reported for a spruce forest in the United States (3.3) [24]. The seasonal Q_{10} value of Rs was 2.50 in the control plots (Table 2), which was close to that of a subtropical forest (2.6) [20], slightly higher than the median value reported in a worldwide research summary (2.4) [22], and lower than that in a temperate hardwood forest (3.9) [8]. The Q_{10} of Rs, Rm, and Rr in field experiments has been seldom reported. Boone et al. (1998) suggested that Q_{10} varied among ecosystems and across temperature ranges, partly because the various components of soil respiration had different Q_{10} values [3]. Winkler et al. [33] found the Q_{10} value of Rm (1.7-1.9) over the temperature range of 4 °C to 28 °C was lower than Rm (2.99) in the control plots. Tu et al. [31] found a difference in the Q_{10} values among components of Rs and that the Q_{10} value of Rr (3.19) was slightly higher than Rm (3.09). In this study, the Q_{10} value of Rr (1.86) was lower than Rm (2.99) in the control plots. Mo et al. [20] found that Q_{10} values in high-N situations (150 kg N ha⁻¹ year⁻¹) decreased after three years of N addition in a mature tropical forest. However, low-N addition increased the values of Q_{10} , while medium-N and high-N additions decreased the values of Q_{10} in this study. It has been suggested that increases in soil drying under N addition reduce the Q_{10} of soil respiration due to decreased root and microbial activities [21]. In our experiment, there were no significant effects of N addition on soil temperature or moisture ($p > 0.05$) (Table 1). Therefore, the changes in the temperature sensitivity of soil respiration could not have been caused by soil drying. The changes in the Q_{10} values may be correlated with the change in soil enzymes under N addition.

Conclusions

Studies to date have demonstrated that N addition would stimulate soil respiration due to N limitation under natural conditions in the short term. Consistent with these results, short-term N addition significantly stimulated Rs, Rm, and Rr. However, the increasing effects of soil respiration decreased along N gradients due to a similar responses of soil microbial activity (mean soil enzymatic activity) and belowground biomass (fine root biomass). In this study, Rr and Rm showed significant and positive relations to fine root biomass and mean soil enzymatic activity. The results indicate that low capacity of holding N due to sensitive to global change in the alpine area would make N-limited gradually turn into C-limited during the first year of N addition, leading to a decreasing promoted effect with the increase of N fertilizer application. Additionally, Rs, Rm, and Rr showed a strong seasonal pattern with the highest rates in the growing season and the lowest rates in the winter. Although temperature was the main environmental factor influencing the soil respiration rate, the delay in seasonal variation and bimodal shape in diurnal variation showed that soil moisture was another important driving factor of soil respiration in our study. The interactive effects of temperature and moisture on soil respiration reflect the characteristics of the South Asian monsoon climate in our study region, with high temperatures in the rainy season. We also concluded that the Q_{10} of Rs, Rm, and Rr was strongly affected by N addition, which may be correlated with the change in soil enzymes under N addition. Therefore, the effects of N addition on soil respiration and their temperature sensitivity in this study may support the prediction of C cycling and storage in forest ecosystems under future scenarios of global change.

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