Effects of long-term nitrogen addition on soil respiration and its components in a boreal forest

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Research

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Abstract

Background Atmospheric nitrogen (N) deposition in boreal forest ecosystems increased gradually with the development of industry and agriculture, but the effects of N input on soil CO₂ fluxes in these ecosystems were rarely reported in previous studies. To evaluate the effect of N addition on soil respiration is of great significance for understanding the distribution of soil carbon (C) on the N gradient in forest ecosystems.

Results In this study, four treatment levels of N addition (0, 25, 50, 75 kg N ha⁻¹ yr⁻¹) were applied to natural *Larix gmelinii* forest in Greater Khingan Mountains of northeast China. We focused mainly on the dynamics of soil respiration (Rs), heterotrophic respiration (Rh), autotrophic respiration (Ra), microbial biomass C and N (MBC and MBN) and fine root biomass (FRB) in a growing season. We found that low N addition significantly increased Rs, Rh and Ra, but with the increase of N addition, the promotion effect was gradually weakened. Medium N increased the temperature sensitivity (Q₁₀) of Rs and Rh components, while medium N and high N significantly reduced the Q₁₀ of Ra. Ra was positively correlated with FRB; Rh was positively correlated with soil MBC and MBN; and R_s was probably driven by Ra from May to July, while by Rh in August and September.

Conclusions Long-term N addition alleviated microbial N limitation, promoted soil respiration and accelerated soil C and N cycle in boreal forest ecosystems.

Background

Over the past century, anthropogenic nitrogen (N) deposition has been increased dramatically and is expected to intensify further in the coming decades (Liu et al. 2013; Basto et al. 2015). The surge in N deposition has greatly affected the terrestrial carbon (C) cycle, especially soil respiration (Rs) (Greaver et al. 2016). Carbon stored in soil is about twice as much as that in atmosphere, and soil respiration is the main way for C to be released from soil system to atmosphere (Gaumont-Guay et al. 2016; Zhong et al. 2016). Therefore, even a small change in Rs can have an important impact on the C cycle. Since Rs is composed of two different components: autotrophic respiration (Ra, root and associated rhizosphere respiration) and heterotrophic respiration (Rh, microbial decomposition of plant debris and soil organic matter) (Bahn et al. 2010), predicting the response of Rs to N enrichment is inherently challenging, which limits our understanding of whether soil is a C sink or source in the context of increased N deposition. Understanding the relative sensitivity of Ra and Rh to N enrichment is the key to evaluate soil C storage and stability under the condition of enhanced N deposition.

In order to study the relationship between increased N deposition and soil respiration, extensive studies have been carried out. However, these studies have yielded contradictory results. It has been reported that soil respiration increased (Gao et al. 2014; Zhou and Zhang 2014; Zhai et al. 2017), decreased (Mo et al. 2007; Sun et al. 2014) and stabilized (Zhu et al. 2015; Liu et al. 2017a) with N addition. This is mainly due to the differences in vegetation types, soil microbial communities and other soil parameters (Gao et al. 2018). Additionally, the effects of N addition on Rs in different terrestrial ecosystems are also inconsistent. For example, Tian et al. (2018) conducted a meta-analysis on the response of soil respiration to N addition in China's forest ecosystems, and the results showed that the soil respiration of temperate forests had a nonlinear response to N addition gradient, which increased at low N levels and decreased at high levels. However, Rs in subtropical and tropical forests decreased with the increase of N addition. Zhou et al. (2014) also showed that N addition reduced soil respiration by 11.2% – 17.8% in subtropical and temperate forests, and increased by 7.9% in cold temperate forests. The internal factor causing this difference may be the difference in response of Ra and Rh to N addition (Graham et al. 2014). The response of Rs to environmental change largely depends on the comprehensive response of Ra and Rh. Although many studies have evaluated the different reactions of Rh and Ra to N addition (Tian et al. 2016), the understanding of the mechanism of the reaction of Rs to N addition is still limited. The separation of Rh and Ra from Rs also helps us to have a deeper understanding of soil C stability. The availability of soil nutrients can regulate the direction and size of soil respiration. Especially in the N-limited ecosystem, moderate input of exogenous N can increase the content of soil N, thus reducing the soil C:N, which is conducive to improving soil microbial biomass and activity, and promoting soil respiration (Ramirez et al. 2012; Zhou et al. 2019). However, the sustained high level of N input will exceed the biological demand, resulting in the N saturation of the ecosystem (Penuelas

et al. 2012), which may lead to changes in Rs. Rs is controlled by many factors, including root biomass, soil organic C (SOC), soil nutrient content, soil temperature and moisture, microbial biomass and activity. Any changes in these biological and abiotic factors may affect the global C cycle and climate change (Deng et al. 2009; Zhou and Zhang 2014). Rh is related to microbial decomposition of soil organic matter (SOM), while Ra is related to the respiration of plant roots and rhizosphere microorganisms (Zhang et al. 2019a). Atmospheric N deposition can directly or indirectly affect soil microbial communities and activities, thus affecting soil microbial biomass (Zhang et al. 2010). Therefore, microbial biomass C (MBC) and N (MBN) can be used as indicators to measure soil microbial activity. With the change of soil nutrient availability, plants will also change their investment in underground fine root biomass to adapt to the environment based on the consideration of energy cost (Wang et al. 2014). All these factors indicate the different sensitivity of root and microbial activity to N enrichment.

Although Rs has been widely studied in many forest ecosystems, little attention has been paid to the seasonal variation of soil CO₂ flux in forest ecosystems under different N gradients, especially in boreal forest ecosystems. *Larix gmelinii* forest is the typical zonal vegetation in boreal zone of China, which not only plays an important role in the ecological balance of the boreal biomes, but also has a pivotal impact on China and global climate change. For this reason, four levels of N addition have been applied in the natural *Larix gmelinii* forest in Greater Khingan Mountains of northeast China since 2011, including control (CK, 0 kg N ha⁻¹ yr⁻¹), low nitrogen (LN, 25 kg N ha⁻¹ yr⁻¹), medium nitrogen (MN, 50 kg N ha⁻¹ yr⁻¹) and high nitrogen (HN, 75 kg N ha⁻¹ yr⁻¹). The response of Rs, Rh, Ra, fine root biomass (FRB), MBC, MBN and Q₁₀ to N addition in a growing season was studied to explore the effects of N input on soil CO₂ flux and C cycle. We hypothesized that (1) LN increased Rs, Rh and Ra; (2) Rh was positively correlated with microbial biomass, and Ra was positively correlated with FRB; (3) MN increased the Q₁₀ of Rs.

Methods

This study was carried out in a *Larix gmelinii* forest of the Nanwenghe National Natural Reserve in the Greater Khingan Mountains, Northeast China (51°05'-51°39' N, 125°07'-125°50' E) (Fig. 1). The climate in this area is a typical continental climate in the cold temperate zone. The annual average temperature is - 2.4°C, the highest average temperature in July is about 18.6°C, and the lowest average temperature in January is - 26.3°C. The annual precipitation is about 500 mm, the frost free period is 90-100 days, the plant growth period is about 110 days, and the annual sunshine time is 2500 hours. The soil is dark brown sandy gravel soil with an average depth of 20 cm. The dominant tree species was *Larix gmelinii*, whose growth density is 2852 (±99) trees/ha, with an average DBH of 8.98 (±0.32) cm.

Experimental design

Our plots have been built since May 2011. Nitrogen was uniformly distributed with sprayer once a month during growing season (May to September), which has been continuously applied for 7 years. We randomly set up twelve 20 m × 20 m sample plots, in which the buffer zone between any two plots is larger than 10 m, so as to avoid disturbing the nearby plots (Fig. 2). Based on the current N deposition rate (25 kg N ha⁻¹ yr⁻¹) in northern China (Liu et al. 2013), three N addition treatments were randomly conducted in four blocks of each plot: control (CK, 0 kg N ha⁻¹ yr⁻¹), low N (LN, 25 kg N ha⁻¹ yr⁻¹), medium N (MN, 50 kg N ha⁻¹ yr⁻¹) and high N (HN, 75 kg N ha⁻¹ yr⁻¹). Each treatment group was repeated three times to simulate the future trend of atmospheric N deposition 1, 2 and 3 times. In this study, NH₄NO₃ was used as N source. Before each application of N, NH₄NO₃ was weighed according to the amount of N applied, mixed with 32L water, and evenly sprayed on the forest floor of each plot. In order to eliminate the difference of water content, same amount of pure water was sprayed on the control field.

Soil respiration measurement

To separate the Rh and Ra from the Rs, we randomly trenched one 1m×1m area to 30 cm depth (approximately the bottom of the root zone in study area) in each experimental subplot in May 2015 (12 subplots × 3 replicates=36 trenches total), and lined the trench with landscaping cloth to enable lateral water movement while limiting the growth of the roots into the trenched subplots. In order to reduce disturbance, the trenches were backfilled according to its original soil profile. Then, through periodic manual removal, all the trenched subplots were free of seedlings and herbs throughout the experiment. Polyvinylchloride collars (PVC, 20 cm in diameter and 6 cm in height) were installed inside and outside the trench to measure Rh and Rs respectively.

PVC collar was inserted into soil 2-3 cm deep. In order to reduce the effect of dead roots caused by trenching, Rs was measured 2 years after trenching. During the growing season, Rs and Rh were measured twice a month using li-8100 (Li-cor Inc., USA) from May to September 2018. Measurements were taken between 09:00 and 12:00, as soil flux over these hours has been shown to represent the mean of the whole day (Sheng et al. 2010). The interval between the two measurements is two weeks, and the average of this two times represents the monthly Rs and Rh. We repeated each measurement for each collar three times to obtain the average Rs rate. During each Rs and Rh measurement, the soil temperature and volumetric water content at the 5 cm depth were monitored simultaneously with the measurement of Rs by using a Li-Cor thermocouple (Omega Engineering Inc. USA) and soil moisture probes (Deltat Devices Ltd., Cambridge, England) connected to Li-8100. Soil CO₂ efflux rates measured in the trenched subplots were represented as the Rh, and the difference between the soil CO₂ efflux rates in the out and trenched plots was represented as the Ra (Rs-Rh). The precipitation and atmospheric temperature were retrieved from the positioning station of Nanwenghe in Greater Khingan Mountains from May to September of 2018.

Soil and fine root sampling and analysis

During the growing season from May to September, five soil cores were randomly collected from each sample plot with a soil auger (diameter of 6 cm) every month, mixed to form a composite sample by putting it in a sterile plastic bag, sealed and transported to the laboratory for storage at 4°C. After that, the soil samples were divided into three parts through 2 mm soil sieve. Soil inorganic N was extracted with 2 mol/L KCl solution, and soil inorganic N (NH₄⁺, NO₃⁻) and total C (TC), total N (TN) and total phosphorus (TP) were determined by continuous flow automatic analyzer (SKALAR SAN⁺, Netherlands). The others was used to analyze soil microbial biomass (MBC, MBN), which was extracted and measured by chloroform fumigation (Vance, Brookes and Jenkinson 1987). The pH value of soil was determined after the last part was dried. Soil pH was measured by pH meter (SX7150, China) in 1:2.5 (soil:water) suspension.

To measure fine root (< 2 mm) biomass, a sharp-edge steel soil corer (6 cm diameter) was used to collect soil samples to a depth of 20 cm. Soil cores were sampled at three random locations from each plot, which were immediately transferred to the laboratory, and the roots were removed from the cores by careful hand-sorting. Fine roots were dried at 70°C until they reached a constant weight.

Statistical analyses

Repeated measurement ANOVA was conducted to examine the effects of N addition on Rs, Rh, Ra, soil temperature and moisture. The effects of different treatments on FRB, soil physicochemical properties, soil microbial biomass, Rs, Rh, Ra and Q₁₀ were studied by one-way ANOVA and Tukey HSD test. The relationship between Rs, Rh, Ra and soil physicochemical properties, microbial biomass and FRB was further discussed by using linear regression model.

We fitted the measured soil temperature and soil moisture with R as an exponential equation ($R = ae^{bTW^c}$) and obtained the Q₁₀ value from the b coefficient ($Q_{10} = e^{10b}$).

Where R represents the rate of soil respiration (Rs, Rh, Ra, $\mu\text{mol m}^{-2}\text{s}^{-1}$); T represents the soil temperature at 5 cm depth (°C); W (%) represents soil volumetric moisture at 5 cm depth; and a, b, and c represent model parameters.

All analyses were performed with SPSS 22.0 software package (SPSS, Inc., Chicago, Illinois, USA), and graphs were prepared with SigmaPlot 13.0 software (Systat Software Inc., Chicago, IL, USA).

Results

Effects of N addition on soil physicochemical properties and FRB

MN and HN significantly increased TN, MN significantly increased NH_4^+ , HN significantly increased NO_3^- , while N addition had no significant effect on TC and TP. HN significantly reduced soil pH (Table 1). The highest soil temperature was 13.25°C in August and the lowest was 7.17 °C in May. The soil temperature showed obvious seasonality, and the average temperature in the whole growing season was 10.28 °C (Table 2). The rainfall in growing season was 442 mm, and more than 60% of precipitation occurs in July and September, which leads to more uneven distribution of precipitation in growing season (Fig. 1). Soil moisture content was closely related to precipitation fluctuation in growing season. The high soil moisture month was concentrated in May, July and September (Fig. 1; Table 2), and the highest value in May might be related to snow melting. Soil temperature and moisture among the four treatments did not reach significant levels ($P < 0.05$). LN and MN significantly increased MBC and MBN, and HN significantly decreased MBC (Fig. 4A). LN significantly increased the FRB in all five months of the growing season, MN and HN only significantly increased the FRB in May, but did not significantly affect the FRB in the other four months. Moreover, the interaction of N addition and month had a significant impact on the FRB (Fig. 4B).

Effects of N addition on soil respiration and its components

R_s , R_a and R_h showed seasonal regularity during the growing season, peaking value from June to July, and the lowest value in September (Fig. 3A, B, C). R_h was always higher than that of R_a . In CK, R_a only accounted for 14% of R_s , and even in LN treatment, R_a accounted for only 25.3% (Fig. 3D). LN significantly increased R_s , R_h and R_a by 18.9%, 7.2% and 51%, respectively (Fig. 3A, B, C). LN significantly increased R_s and R_a in all five months of growing season. HN significantly decreased R_s , R_h in June and July, and significantly reduced R_a in June (Fig. 3A, B, C). LN significantly increased R_h in May and June, MN significantly increased R_h in July, but N addition had no significant effect on R_h in August and September (Fig. 3B). Moreover, the interaction of N addition and month had a significant impact on R_s , R_h and R_a (Fig. 3A, B, C).

Effects of N addition on Q_{10} of soil respiration

During the measurement period, the fitted Q_{10} values of R_s ranged from 2.09 to 2.82, the Q_{10} value of R_h ranged from 2.34 to 3.73, and the Q_{10} value of R_a ranged from 0.92 to 2.73. The Q_{10} of R_h was higher than Q_{10} of R_a . MN significantly increased Q_{10} of R_s and R_h , LN significantly increased Q_{10} of R_a , MN and HN significantly decreased Q_{10} of R_a (Table 4).

Correlation between physicochemical properties, FRB and soil respiration

TP was positively correlated with R_s and R_a ($P < 0.05$), but had no correlation with R_h . pH was only positively correlated with R_h ($P < 0.05$), and R_s was positively correlated with MBC and MBN ($P < 0.05$). R_a was positively correlated with MBN ($P < 0.05$), but had no correlation with MBC. FRM was positively correlated with R_a ($P < 0.01$), but had no correlation with R_s and R_h . R_h was positively correlated with MBC ($P < 0.01$). There was no correlation between R_s , R_h , R_a and TC, TN, NH_4^+ , NO_3^- (Table 3).

Discussion

Effects of N addition on R_s

In 2018, we studied the effects of N addition on R_s and its components during growing season through seven consecutive years of N addition gradient experiments. The R_s of *Larix gmelinii* forest was $3.31 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Fig. 3), which was much higher than that of $1.05 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in spruce forest, $0.96 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in Abies forest (Wang et al. 2019a), and the mean value of Chinese coniferous forest ($2.32 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (Dai et al. 2015). In all treatments, the R_s of *Larix gmelinii* forest showed positive responses to low N treatment, which was different from other results (Wang et al. 2019b; Zhang et al. 2019b; Yan et al. 2020). Zhong et al. (2016) considered that forest was less affected by human activities, and had a balanced soil N cycle. The forest R_s was less affected by N, and the variation may be caused by differences of the scale. Meanwhile, due to different vegetation types, soil microbial communities and physicochemical properties, the effects of N addition on soil respiration in different terrestrial ecosystems were not consistent. Boreal forests are generally considered to be N-limited (Vitousek et al. 2010). Our results also showed that MN and HN significantly increased soil TN, MN significantly increased soil NH_4^+ , HN significantly increased NO_3^- (Table 1), and long-term N addition increased soil N availability in *Larix gmelinii* forest.

The sensitivity of Ra and Rh to N addition is different. Rh is the largest contributor of Rs and is not affected by N addition. The higher Rh/Rs ratio indicates that the C released by soil microbial C decomposition is larger than that released by root activity, which may lead to the reduction of soil C fixation.

We found that Rs had obvious seasonal pattern, and N addition did not affect the seasonal pattern of Rs. The highest Rs value was observed in July and the lowest in September (Fig. 3). This seasonal pattern has been reported in previous studies and can be attributed to different mean temperatures (Bondlamberty and Thomson 2010). The positive response of Rs to soil temperature change may be related to the variations of soil matrix availability and microbial abundance (Noh et al. 2017; Sun et al. 2019). Previous studies have shown that warming may increase FRB, root exudates or litter decomposition (Gong et al. 2015; Liu et al. 2017b; Wu and Yu 2019). In our study, FRB in July was significantly higher than that in May (Fig. 4B), and Ra was significantly positively correlated with FRB ($P < 0.05$) (Table 3), which is a potential explanation for the increase of Rs with warming. Compared with May and June, MN significantly increased Rh in July (Fig. 3B), which may be related to the increase of soil moisture in July. Wang et al. (2020) found that precipitation can adjust the saturation threshold of net primary production (NPP) and Rh in response to temperature changes, because resource availability increases with the increase of precipitation, thus reducing the adverse impact of high temperature on NPP and Rh. Similarly, the increase of precipitation or soil moisture may affect the N saturation threshold of Rs by increasing water use efficiency. Our results also showed that LN and MN significantly increased MBC and MBN (Fig. 4A). The Greater Khingan Mountains is a N limited area, nitrogen addition increases the available N in soil, relieves the limitation of microbial N, and increases the fixation of N by microorganisms, thus significantly increasing soil microbial N. When fertilization alleviates nutrient limitation, microbial biomass and cellulose decomposition enzyme capacity increase, which may help to accelerate the decomposition of SOM, and then affect microbial activity and composition (Stark et al., 2014). Cusack et al. (2011) Conducted a 5-year N application experiment, and also found that N application significantly increased the available N content in the soil, provided a rich nutrient source for the growth and reproduction of soil microorganisms, leading to the increase of MBC, nitrogen addition would change soil C storage in terrestrial ecosystem by affecting microbial activity and composition.

Effects of N addition on Rh and Ra

The response of Rs to N addition was similar to that of Ra, while Rh had a positive response to LN only in May and June. In July, MN significantly increased Rh, and in May and June HN significantly decreased Rs, Rh and Ra. Ra and Rh showed different responses to N addition (Fig. 3A, B, C). A meta-analysis of Xiao et al. (2020) found that under different N levels, the Rs of LN and MN treatments increased by 14.9% and 5.2%, respectively, while the Rs of HN treatment decreased by 2.9%, who believed that this phenomenon might be related to the dynamic changes of plant roots caused by N addition. Poor soil usually stimulates the growth of plant roots to extract more N to meet their physiological needs (Wright and Westby 2003; Iorio et al. 2011), while the reduction of FRB caused by high N availability can effectively reduce Ra, thus further inhibiting Rs. However, in our study, HN did not reduce the FRB of *Larix gmelinii*, and Ra accounted for 12.52% - 25.3% of the four treatments (Fig. 3D). Rh played a dominant role during the whole growing season. Regression analysis also showed that Rs was significantly positively correlated with MBC and MBN (Table 3). Meanwhile, HN was significantly reduced MBC (Fig. 4A), which confirmed the global evidence that excessive N had a negative impact on soil microbial biomass (Liu and Greaver 2010). Therefore, we infer that the decrease of Rs in June and July under HN treatment may be driven by Rh, and HN may reduce the microbial activity, resulting in the decrease of MBC, then affecting Rh. A recent meta-analysis showed that soil MBC decreased by 11% on average in the world, and the decrease of MBC increased with the augment of N addition rate (Zhang et al. 2018). In addition, there are sufficient evidences that excessive N can change the abundance and composition of microorganisms (Janssens et al. 2010; Geisseler and Scow 2014), through the formation of lignin, alkyl and aromatic C and other refractory compounds, reduce the C utilization rate of soil microorganisms (Guo et al. 2017), and inhibit the enzyme that decomposes complex C (Sinsabaugh et al. 2005), all of which will be detrimental to the growth of soil microorganisms and reduce Rs. In August and September, N addition had no significant effect on Rh, but Rs and Ra had synchronous positive response to low N addition. Therefore, we speculated that Rs was driven by Ra in August and September. This may be due to the drought in August and the low temperature in September, which limited the activity of soil enzymes. Under the influence of low temperature and drought stress, *Larix gmelinii* increased the investment in underground part, resulting in the increase of Ra. At the same time, regression analysis also showed

that Rs and Ra were significantly positively correlated with soil TP (Table 3), and HN significantly reduced soil pH (Table 1). Although N addition did not significantly increase soil TP in our study, long-term N addition led to P limitation has been largely confirmed. Wardle et al. (2004) found that in P limited environment, plant community structure will change to improve P utilization efficiency, and then maintain high biomass. Fujita et al. (2010) found that the content of N in P was high, and its activity was often limited by N supply. Nitrogen addition increased the P activity in soil, and then increased the release rate of P in soil. The decrease of pH value may lead to the increase of Al^{3+} concentration in soil, which has direct and indirect effects on the size, composition and activity of soil microorganisms (Ying et al. 2017).

Effects of N addition on Q_{10}

The temperature sensitivity of Rs (Q_{10}) is an important parameter to infer and predict the potential feedback of climate change. Q_{10} varies with the species and quantity of soil organisms and the quality and quantity of respiratory substrates (Zhou et al. 2009; Stone et al. 2012). Previous studies have reported that N enrichment leads to a decrease in Q_{10} (Zhong et al. 2016; Xiao et al. 2020). However, in our experiment, MN significantly increased the Q_{10} of Rs and Rh, and LN significantly increased the Q_{10} of Ra (Table 4), which was consistent with Wang et al. 2019a. This indicates that global warming will lead to more soil CO_2 emissions under the background of increasing atmospheric N deposition in Greater Khingan Mountains. There are two possible reasons for the increase of Q_{10} . Firstly, the root respiration substrate is derived from the distribution of photosynthetic products to the underground (Meyer et al. 2018), and Ra is closely related to the supply of photosynthetic substrate (Aaltonen et al. 2016). Photosynthesis depends on a variety of enzymes, and higher temperature can accelerate the enzymatic reaction and promote photosynthesis. Therefore, with the increase of temperature, plant photosynthesis leads to the increase of respiration substrate, and Q_{10} may increase. Secondly, the Q_{10} of Rs is affected by temperature. Q_{10} is usually higher with lower temperatures (Davidson et al. 2010). The Q_{10} value of *Larix gmelinii* forest in cold temperate zone was 2.09 ± 0.17 (Table 4), which was lower than that of temperate coniferous forest (3.26 ± 0.32) and subtropical coniferous forest (2.38 ± 0.19) (Song et al. 2014), which was also proved by our experimental results. With the increase of available N content and temperature in soil, the fixation and use of N by microorganism were strengthened, and the activity of enzyme was enhanced. The Rs might be increased by increasing Rh, so the addition of N increased Q_{10} . We also found that MN and HN significantly reduced the Q_{10} of Ra, and the negative reaction of Q_{10} can be attributed to the relative increase of SOM unstable components induced by N addition. The changes of soil microbial community and enzyme activities caused by N addition may also have a significant impact on Q_{10} (Von and kogelknabner 2009). Soil acidification caused by N addition may accelerate the decomposition of refractory organic matter and further reduce the Q_{10} value (Tian and Niu 2012). At present, there are few studies on the mechanism of the influence of N input on Q_{10} value, and the positive response of Q_{10} value to N input needs further experimental study in situ.

Conclusions

In the natural forest of *Larix gmelinii*, Rs and its components showed strong seasonal variation, the highest value in July and the lowest value in September. Soil temperature and moisture are the main driving factors affecting Rs and its composition. In accordance with previous studies, simulated N deposition increased soil N availability, and LN significantly increased Rs and its components, which may be due to the increase of soil enzyme activity and fine root biomass. LN may release more soil CO_2 and accelerate the local C cycle. Nitrogen addition affected the balance between Ra and Rh and its contribution to Rs by changing microbial biomass and root growth. Rs may be driven by Ra from May to July, and then by Rh in August and September. Therefore, further study on the response of soil microbial and fine root traits to N deposition rate can provide a deeper understanding of the dynamics of soil C emission from *Larix gmelinii* forest in boreal zone.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

QW and YX designed the study, were awarded funding, supervised data collection and contributed to and edited manuscripts. QW, GL, YX, TL and XW contributed the whole manuscript preparation and design and wrote the main manuscript text. QW, GL, YX, TL, GY, LW and XW prepared all Figures, GL, YX, GY, LW, XW, TL and QW prepared field experiments, prepared tables and collected literatures. All authors read and approved the final manuscript.

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Tables

Table 1 Basic physicochemical characteristics of the soil from the treatment plots (mean ± SE).

Treatment	TC(g kg ⁻¹)	TN (g kg ⁻¹)	TP (g kg ⁻¹)	NH ₄ ⁺ (mgkg ⁻¹)	NO ₃ ⁻ (mgkg ⁻¹)	pH
CK	46.12±7.85a	2.01±0.32b	0.83±0.14a	6.57±0.81b	3.95±0.47b	5.52±0.12a
LN	44.34±3.15a	2.09±0.17b	0.79±0.19a	8.37±0.87ab	4.15±0.33ab	5.41±0.23ab
MN	45.74±2.05a	2.54±0.22a	0.81±0.17a	10.29±0.82a	4.35±0.34ab	5.29±0.06ab
HN	48.28±4.49a	2.56±0.15a	0.79±0.11a	7.8±1.13b	5.41±0.56a	4.98±0.05b

CK represents the control; LN represents the low N treatment; MN represents the medium N treatment; HN represents the high N treatment; TC represents soil total carbon; TN represents soil total nitrogen; TP represents soil total phosphorous; C:N represents carbon-nitrogen ratio; C:P represents carbon-phosphorous ratio; N:P represents nitrogen-phosphorous ratio. Different superscript letters within each column indicate significant differences (P<0.05) among treatments.

Table 2 soil moisture (%) and soil temperature (°C) under control and N addition treatments in different months (mean ± SE).

Month	soil temperature (°C)				Soil moisture (%)			
	CK	LN	MN	HN	CK	LN	MN	HN
May	7.17±0.45	7.73±0.35	6.78±0.34	6.93±0.14	0.29±0.01	0.27±0.03	0.28±0.01	0.30±0.01
June	10.32±0.26	11.07±0.30	10.89±0.27	10.45±0.24	0.12±0.01	0.11±0.01	0.12±0.01	0.13±0.01
July	12.55±0.40	13.01±0.20	12.27±0.26	12.64±0.45	0.26±0.01	0.27±0.01	0.26±0.02	0.28±0.01
August	13.25±0.44	13.86±0.26	13.10±0.58	14.03±0.12	0.15±0.01	0.17±0.01	0.16±0.01	0.16±0.01
September	8.12±0.16	8.55±0.19	8.34±0.19	8.47±0.14	0.27±0.02	0.26±0.01	0.28±0.02	0.26±0.02

CK represents the control; LN represents the low N treatment; MN represents the medium N treatment; HN represents the high N treatment.

Table 3 The correlation coefficients between soil respiration and soil chemical properties and fine root biomass

	TC	TN	TP	NH ₄ ⁺	NO ₃ ⁻	pH	MBC	MBN	FRM
Rs	0.046	-0.411	0.587*	0.265	-0.301	0.575	0.687*	0.576*	0.637
Ra	0.143	-0.255	0.693*	0.249	-0.463	0.473	0.463	0.643*	0.731**
Rh	-0.006	-0.327	0.462	0.236	-0.292	0.643*	0.746**	0.431	0.422

Rs represents the total soil respiration; Rh represents the heterotrophic respiration; Ra represents the autotrophic respiration; Total C represents the soil total C; Total N represents the soil total N; Total P represents the soil total P; MBC represents the microbial biomass carbon; FRM represents the fine root biomass. ANOVA: *P < 0.05, **P < 0.01, ***P < 0.001

Table 4 Parameter estimates of models for Rs, Rh and Ra as a function of soil temperature (T) and moisture (W) in different treatments (mean ± SE).

	a	b	c	Q ₁₀
Rs=ae ^{bt} w ^c				
CK	0.95±0.03b	0.07±0.01b	-0.27±0.03c	2.09±0.17b
LN	1.36±0.14a	0.09±0.01ab	-0.07±0.09b	2.38±0.15ab
MN	1.61±0.12a	0.10±0.02a	0.23±0.03a	2.82±0.51a
HN	1.48±0.23a	0.08±0.01b	0.11±0.02a	2.12±0.11b
Ra=ae ^{bt} w ^c				
CK	0.02±0.00	0.08±0.00a	-1.67±0.12c	1.73±0.05b
LN	0.21±0.03	0.10±0.00a	-0.35±0.06a	2.73±0.05a
MN	0.26±0.02	-0.049±0.04b	-0.76±0.13b	0.92±0.08c
HN	0.03±0.00	-0.00±0.00b	-1.49±0.04c	0.98±0.10c
Rh=ae ^{bt} w ^c				
CK	1.08±0.01c	0.10±0.01b	0.09±0.01b	2.57±0.23b
LN	1.11±0.01c	0.08±0.01b	-0.06±0.01c	2.34±0.18b
MN	1.21±0.02b	0.13±0.01a	0.35±0.06a	3.73±0.15a
HN	1.57±0.08a	0.09±0.01b	0.36±0.05a	2.57±0.12b

The Q₁₀ value is obtained from b (Q₁₀ = e^{10b}). a, b and c are regression coefficients. CK represents the control; LN represents the low N treatment; MN represents the medium N treatment; HN represents the high N treatment; Different letters among

treatments indicate significant differences ($P < 0.05$). Data were mean $\pm$ SE.

Figures

Figure 1

Precipitation and atmospheric temperature at 2m above ground level during the measurement period in 2018 (data are from Nanwenghe positioning station in Greater Khingan Mountains)

Figure 2

Nitrogen sample plot and diagram of the experimental design in the Greater Khingan Mountains Forest ecosystem, China. CK represents the control; LN represents the low N treatment; MN represents the medium N treatment; HN represents the high N treatment. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 3

Temporal changes in total soil (A), heterotrophic respiration (B), autotrophic respiration (C) and the contribution of autotrophic respiration (Ra) to total soil respiration (Rs) (%), (D) among the different treatments during the measurement periods in the growth season of 2018. The error bars are standard errors. Different lowercase letters represent statistical significances among treatments in same month. CK represents the control; LN represents the low N treatment; MN represents the moderate N treatment; HN represents the high N treatment.

Figure 4

Comparison of microbial biomass C (MBC) and microbial biomass N (MBN) (A) and mean fine root biomass (B) among different treatments. CK represents the control; LN represents the low N treatment; MN represents the medium N treatment; HN represents the high N treatment. Significant differences ($P < 0.05$) among the different treatments are indicated by different letters. Error bars represent SD.