

Combined effects of nitrogen addition and organic matter manipulation on soil respiration in a Chinese pine forest

Jinsong Wang^{1,2} · L. Wu³ · Chunyu Zhang¹ · Xiuhai Zhao¹ · Wensheng Bu⁴ · Klaus V. Gadow^{5,6}

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Abstract The response of soil respiration (R_s) to nitrogen (N) addition is one of the uncertainties in modelling ecosystem carbon (C). We reported on a long-term nitrogen (N) addition experiment using urea ($\text{CO}(\text{NH}_2)_2$) fertilizer in which R_s was continuously measured after N addition during the growing season in a Chinese pine forest. Four levels of N addition, i.e. no added N (N0: $0 \text{ g N m}^{-2} \text{ year}^{-1}$), low-N (N1: $5 \text{ g N m}^{-2} \text{ year}^{-1}$), medium-N (N2: $10 \text{ g N m}^{-2} \text{ year}^{-1}$), and high-N (N3: $15 \text{ g N m}^{-2} \text{ year}^{-1}$), and three organic matter treatments, i.e. both aboveground litter and belowground root removal (LRE), only aboveground litter removal (LE), and intact soil (CK), were examined. The R_s was measured continuously

for 3 days following each N addition application and was measured approximately 3–5 times during the rest of each month from July to October 2012. N addition inhibited microbial heterotrophic respiration by suppressing soil microbial biomass, but stimulated root respiration and CO_2 release from litter decomposition by increasing either root biomass or microbial biomass. When litter and/or root were removed, the “priming” effect of N addition on the R_s disappeared more quickly than intact soil. This is likely to provide a point of view for why R_s varies so much in response to exogenous N and also has implications for future determination of sampling interval of R_s measurement.

Keywords Soil respiration · Organic matter treatment · Nitrogen supply · Interactive effect · Chinese pine forest

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✉ Xiuhai Zhao
zhaoxh@bjfu.edu.cn

- ¹ Key Laboratory for Forest Resources and Ecosystem Processes, Beijing Forestry University, Beijing 100083, China
- ² Synthesis Research Center of Chinese Ecosystem Research Network, Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China
- ³ Sustainable Soils and Grassland Systems, Rothamsted Research, North Wyke, Okehampton, Devon EX20 2SB, UK
- ⁴ College of Forestry, Jiangxi Agricultural University, Nanchang 330045, China
- ⁵ Department of Forestry and Wood Technology, University of Stellenbosch, Stellenbosch, South Africa
- ⁶ Faculty of Forestry and Forest Ecology, Georg-August-University Göttingen, Büsgenweg 5, D-37077 Göttingen, Germany

Introduction

Increased anthropogenic nitrogen (N) deposition, primarily due to artificial fertilizer application and fossil fuel combustion (Pinder et al. 2012), has been considered as a major scientific challenge and has affected carbon (C) balance of terrestrial ecosystems by influencing plant growth and C emission (Schlesinger and Andrews 2000; Luo et al. 2006; Reay et al. 2008). Understanding how N deposition affects terrestrial C balance is critically important to improve our modelling ability of terrestrial C cycle (Chapin et al. 2009).

Forest soils, which retain a larger and more persistent pool of C than that contained in vegetation, represent a potentially important reservoir for increased terrestrial ecosystem C storage in response to N deposition (Janssens et al. 2010; Lovett et al. 2013). Soil respiration (R_s), the primary pathway by which plant-fixed carbon dioxide (CO_2) returns to the atmosphere (Gaumont-Guay et al. 2009), with approximately 80–98 Pg C emitted to the atmosphere annually (Bond-Lamberty

and Thomson 2010), is presently increasing globally by 0.1 Pg C y^{-1} . Therefore, changes in soil N availability could profoundly alter Rs processes and, in particular, strongly affect the direction and extent of the C balance response (Johnson and Curtis 2001).

A growing body of evidence has confirmed that Rs is often reduced by N addition (Liu and Greaver 2010; Lu et al. 2011). However, the mechanisms that cause the decline in the Rs are not well understood. It is widely agreed that inhibition of soil microbial activity (Demoling et al. 2008; Zhao et al. 2014; Li et al. 2015) and suppression of fine root biomass and mycorrhizal respiration (Sun et al. 2014) result in a reduction of Rs and could be responsible for the decrease in the Rs when soil N availability exceeds the plant requirements (Bowden et al. 2004; Ramirez et al. 2010). As the widespread uncertainties surrounding Rs response to anthropogenic N deposition, the effects of N deposition on the Rs and soil C cycling should require more detailed studies in different forest ecosystems and regions (Zhang et al. 2014).

Alteration in the amount of soil organic matter (SOM) input can have profound effect on the Rs. Forest litter layer is an important source of SOM as aboveground litter provides fresh labile organic C to the growth of soil microorganisms. It is generally accepted that microbes are C-limited (Kamble et al. 2013), and therefore litter layer removal may alter Rs directly, by controlling C substrate availability. Aboveground litter layer also regulates soil microclimate (Sayer et al. 2012), and litter removal can affect Rs indirectly, via interactions with soil physical and chemical properties, such as soil temperature, moisture, and pH (Xu et al. 2013). Root and root exudates are another source of SOM. Generally, the exclusion of roots eliminates the supply of root exudates that are respired by soil microorganisms (Zhang et al. 2014). The absence of roots may also curb fungal growth, resulting in a decrease in fungal biomass and change of bacterial community structures and thus affects Rs (Brant et al. 2006). Many studies have addressed the separate effect of litter and/or root removal and N addition on the Rs (Wan and Luo 2003; Bowden et al. 2004; Sun et al. 2014; Fan et al. 2015); however, the combined effects of SOM manipulation and N addition on the Rs are still not well understood. Therefore, further investigations of the differential responses of Rs to N addition and SOM changes may be the key to achieve a more comprehensive understanding of the effects of increased N deposition on the Rs and forest C cycling.

We used a long-term N addition experiment to assess Rs continuously after N enrichment in a Chinese pine (*Pinus tabulaeformis* Carr.) forest that received organic matter manipulation: both aboveground litter and root removal, only aboveground litter removal and intact soils. Our hypotheses were as follows: (1) N addition would stimulate Rs in intact soils because the study area

is N-limited; (2) when litter and roots were both removed, Rs would be inhibited by N addition due to the suppression of microbial activities; (3) when only litter was removed, although microbial activity was suppressed, however, N addition still could be a good facilitator for the Rs in this N-limited forest; and (4) the duration of N addition effects on the Rs would vary in different organic matter treatments. When litter and/or root were removed, we expected Rs would respond quickly to N addition and the “priming” effect of N addition on the Rs would disappear more quickly than intact soil. Our main objectives were (a) to understand the variations in the Rs responses to N addition in the various organic matter treatments, (b) to investigate to what extent N addition affects the Rs on different sample dates after N addition application and (c) to explore the mechanisms underlying the combined effects of N addition and organic matter manipulation on the Rs.

Materials and methods

Study site

The field work was conducted at the Taiyue Mountain Ecosystem Research Station located in Shanxi Province, in north China ($36^{\circ} 18' \text{ N}$ and $111^{\circ} 45' \text{ E}$; 1560 m a.s.l.). The region has a warm-temperate semi-arid continental monsoon-affected climate, with a mean annual temperature of 9.9° C . The highest monthly average temperature of 22.4° C occurred in July, whereas the lowest monthly average temperature of -4.6° C occurred in January. The mean annual precipitation was 548 mm, and the mean relative humidity was 65 %. Over a year, the distribution of precipitation was relatively uneven. The wet season was from July to September, which accounted for more than 60 % of annual precipitation. The soil was brown forest soil.

The vegetation cover represented a warm-temperate, deciduous broad-leaved forest zone. The forest canopy was primarily *Pinus tabulaeformis*, *Quercus mongolica*, and *Populus davidiana*. The mean height of the overstory was 18.0 m with a mean breast-height diameter of 23 cm. The understory trees were primarily *Ostryopsis davidiana*, *Lespedeza bicolor*, *Hippophae rhamnoides*, *Corylus mandshurica*, *Swida bretchneideri*, and *Rosa xanthina*. The mean height of the understory was 2.2 m with a mean breast-height diameter of 2.0 cm. *Carex tristachya* and various other species of the family *Cyperaceae* were the common herbs.

Experimental design and treatments

The experiment used a split-plot design with N addition as a whole-plot variable and organic matter treatment as

the split-plot variable. Three blocks of plots were established for N addition and organic matter treatments. The size of each block was 100 m (length) × 10 m (width) and the block was laid out in the same horizontal plane in order to minimize the effect of stand slope on the Rs. Each block was then divided into 12 2 m × 2 m subplots for the Rs measurements, the subplots were distributed randomly in each block (Fig. 1). Each subplot had a 2-m buffer between subplots.

In September 2009, organic matter manipulation (each with four replicates) was applied to the subplots in each block 2.5 years prior to N addition: (1) both aboveground litter and belowground root removal (LRE), (2) only aboveground litter removal (LE), and (3) intact soil (CK). For the LE treatment, the organic layers above the mineral soil were removed, and a 2 m × 2 m litter trap was placed 1 m above the ground to intercept fall foliage. To initiate root removal, the perimeters of the subplots were trenched to 1 m. To prevent roots from outside of the subplots from penetrating into the subplots, a 0.5-mm thick polyethylene sheet was placed along the bottom and sides of the trench before backfilling. The root-free plots were kept free of vegetation by cutting plant regrowth manually throughout the study periodically, with minimal disturbance to the soil. In each subplot, a collar, 20 cm inner diameter × 10 cm height, was inserted 5–6 cm into the soil. The Rs measurements were initiated 24 h after the installment of the collars. The location of the collar did not change during Rs measurements.

Four levels of N addition (each with three replicates) were investigated in each block: no added N (N0: 0 g N m⁻² year⁻¹), low-N (N1: 5 g N m⁻² year⁻¹), medium-N (N2: 10 g N m⁻² year⁻¹), and high-N (N3: 15 g N m⁻² year⁻¹). In each Rs subplot, the fertilizer of urea was weighed, mixed with 1 l of water and applied to each subplot using a portable sprayer. With the sprayer, two passes were made over each subplot to ensure an even distribution of the fertilizer. The N0 subplot received 1 l of water without the addition of any N.

In October 2009, three soil samples were collected in each subplot (0–20 cm) 2.5 years prior to N addition. All samples in each subplot were combined into one sample, were sieved (2 mm) to remove coarse fragments, and were air-dried to analyse for pH and nutrient content. The soil pH was measured with a glass electrode in a 1:2 soil-to-water ratio. The total N was measured using Kjeldahl’ digestion with a salicylic acid modification (Pruden et al. 1985), and available

phosphorus and potassium were measured using the NaOH fusion method. Soil organic carbon was measured following the method described by Kalembasa and Jenkinson (1973). The soil characteristics of each N level collected prior to N addition are presented in Table 1.

Soil respiration measurements

N fertilizer treatments were being initiated 2.5 years following organic matter manipulation. The first N addition was applied on 30th June 2012 and implemented at the end of each month from July to October 2012. The first Rs measurement was conducted on 1 July 2012 and was measured continuously for 3 days following each N addition application and was monitored approximately 3–5 other times each month during the observation period. The first Rs measurement was conducted 33 months after root trenching, and it was assumed that all roots in the LRE subplots were died and there was no root respiration.

The Rs was measured with an automated soil CO₂ flux system (LI-8100, LI-COR, Nebraska, USA) from 08:00 to 18:00 h, and the rates were averaged for data analysis. The Rs measurements were accompanied by recordings of soil temperature at 10 cm depth and volumetric soil water content in the top 5 cm of the soil profile (Echo EC-5, Decagon Devices Inc, Pullman, WA, USA). The cumulative soil CO₂ efflux from the various organic matter treatment subplots was calculated as the mean of the cumulative CO₂ effluxes of the three replicates for each N level.

Fine root biomass measurements

Fine root (<2 mm) biomass was measured in November 2012 using an auger with a 9.3-cm inner diameter. In each LE and CK subplot, three soil cores to a depth of 10 cm were collected. The roots were collected by washing over a 0.5-mm sieve. To determine dry weight, the samples were oven-dried at 85 °C until constant weight was reached.

Soil microbial biomass measurements

Three soil cores of 2.5 cm diameter were randomly collected from the top soil (0–10 cm) in each subplot in November 2012 and were mixed to form a composite sample. After removing roots and plant residues, these composite samples were immediately sieved through a 2-mm mesh sieve in the field and refrigerated. Soil

Fig. 1 The schematic design of nitrogen addition subplots for Rs measurement

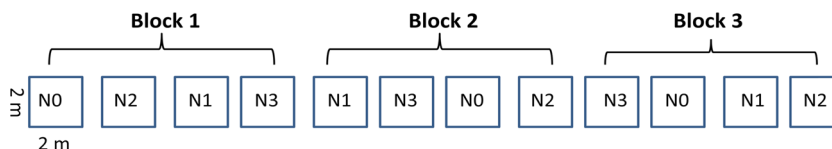


Table 1 Soil conditions (0–20 cm) conducted 2.5 years prior to N addition in the Chinese pine forest

Nitrogen level	Soil bulk density (g cm ⁻³)	Soil pH	Total nitrogen (g kg ⁻¹)	Soil organic carbon (g kg ⁻¹)	Available phosphorus (mg kg ⁻¹)	Available potassium (mg kg ⁻¹)
N0	0.94 ± 0.03a	7.1 ± 0.3a	1.9 ± 0.3a	45.3 ± 5.9a	5.7 ± 0.12a	214.2 ± 9.8a
N1	0.97 ± 0.04a	7.1 ± 0.2a	2.0 ± 0.3a	44.0 ± 6.1a	5.8 ± 0.13a	215.7 ± 9.7a
N2	1.07 ± 0.05a	7.2 ± 0.2a	1.9 ± 0.3a	44.1 ± 5.8a	5.7 ± 0.09a	218.4 ± 9.9a
N3	1.06 ± 0.05a	7.3 ± 0.2a	2.0 ± 0.3a	46.0 ± 5.7a	5.7 ± 0.08a	215.5 ± 11.0a

Values are means ± SE ($n = 3$). Different letters in the same column represent significant differences at the 0.05 level

microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were measured using the chloroform direct-fumigation extraction method (Vance et al. 1987).

Statistical analyses

A three-way analysis of variance was used to investigate the effects of month (M), N level (N), and organic matter treatment (O) on soil temperature, soil moisture, and Rs. One-way analysis of variance was applied to test the effect of the addition of N on fine root biomass and soil microbial biomass. The significance level was set at 0.05, and all statistical tests were performed using SPSS (ver. 16.0) and R2.15.2 (<http://www.R-project.org/>).

Results

Microclimate and soil properties

Dynamics of soil temperature at the N addition levels in the various organic matter treatment subplots after each fertilizer application are shown in Fig. S1. A three-way ANOVA showed that soil temperature had significant monthly variation (Table 2), and the highest temperature was in July and the lowest temperature was in October. Neither organic matter treatment nor N addition had any significant effect on soil temperature ($P > 0.05$).

Table 2 Results (F -values) of three-way ANOVA for soil temperature at 10 cm depth (T), soil moisture at 5 cm depth (W) and soil respiration rate (R_s) from July to October in 2012

Source of variation	Soil temperature at 10 cm (T)	Soil moisture at 5 cm (W)	Soil respiration rate (R_s)
Month (M)	2590***	144.8***	192.0***
Nitrogen level (N)	0.3	10.7	41.3***
Organic matter treatment (O)	2.0	147.7***	193.4***
$N \times O$	0.2	1.3	18.9***
$N \times M$	0.2	0.7	0.9
$O \times M$	1.8	12.4***	10.9***
$N \times O \times M$	0.1	0.8	1.1

*** $P < 0.001$

Soil moisture also had significant monthly variation (Table 2, Fig. S2). The highest moisture level was observed in September (40.8 %), and the lowest in July (33.8 %). The organic matter treatment had a significant effect on soil moisture in different months (Table 1). In general, the average soil moisture in the LRE subplots was significantly higher than that in the LE or CK subplots ($P < 0.05$). N addition had no significant effect on soil moisture throughout the study period ($P > 0.05$).

N addition significantly decreased soil pH and available potassium (Table S1). N addition had a negative effect on soil organic carbon (SOC) but SOC was only significantly declined in the N3 level ($P < 0.05$). Soil total nitrogen was significantly increased by N addition ($P < 0.05$).

Combined effects of organic matter manipulation and nitrogen addition on Rs

The Rs showed significant monthly dynamics, with the highest value observed in August and the lowest observed in October (Table 2 and Fig. 2). The organic matter treatment had a significant effect on Rs ($P < 0.05$). During the entire observation period, the average Rs rates in the LRE, LE and CK subplots were 1.77, 2.21 and 3.11 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. In general, the average Rs rates in the CK subplots were significantly higher than those in the LRE and LE subplots ($P < 0.05$).

N addition also significantly affected the Rs values ($P < 0.05$), with decreased rates of Rs in the LRE subplots.

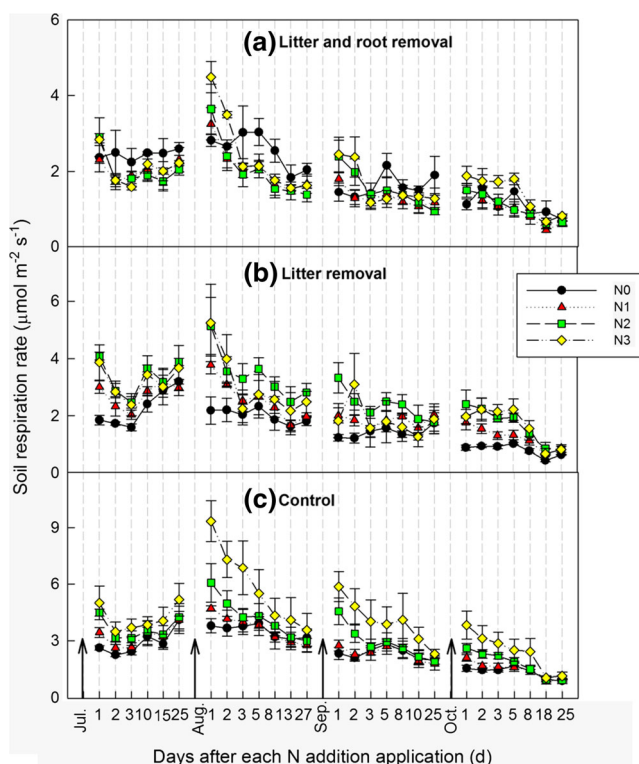


Fig. 2 Variation in the rates of Rs at four levels of N addition in the various organic matter treatment subplots

During our observations, the average rate of Rs at the N0, N1, N2 and N3 level was 1.91, 1.57, 1.65 and 1.88 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, in the LRE subplots. In the LE subplots, N addition significantly increased Rs and the highest value was observed with the N2 level. In the CK subplots, N addition also significantly increased the Rs ($P < 0.05$), and the rate increased with the level of N addition. The average rate of Rs at the N0, N1, N2 and N3 level was 2.51, 2.65, 3.11 and 4.12 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively.

N addition and organic matter manipulation also had a significant interaction effect on the Rs ($P < 0.05$) (Table 2). Organic matter manipulation interacted with month affecting the Rs; however, nitrogen addition did not interact with month to affect the Rs.

Dynamics of Rs after each N addition application

In the LRE subplots, the N2 and N3 levels increased Rs on the first day after the N addition, then Rs varied slightly at all N levels in July (Fig. 2a). A positive effect of the N3 level on the Rs was observed during the first 1–2 days after N addition in August. The Rs was then inhibited by N addition at all N levels from the third day. A positive effects of the N2 and N3 levels on the Rs were observed during the first 2 days in September. After this, N addition caused a decrease in the Rs. In October, N addition stimulated Rs on the first day, and then the Rs increased at the

N3 level, whereas it was inhibited at the N1 and N2 levels until the fifth day.

In the LE subplots, N addition stimulated Rs after each N application during the study period. The Rs decreased until the third day and then increased again at all N levels in July, which was most likely due to rainfall on the third day with a striking effect on the first 1–2 days after N addition (Fig. 2b). A significant positive effect was also observed on the first 2 days at all N levels in August. After this, the Rs varied very little. In September, the significant positive effect was found on the first day after N addition and the highest rate of Rs was also observed at the N2 level. On the second day, the effect of N2 level weakened, whereas the N3 level significantly stimulated Rs. The effect weakened at all N levels after the second day, with the highest rate of Rs occurring largely at the N2 level. N addition increased Rs, particularly at the N2 and N3 levels in October. The effect gradually weakened at all N levels beginning on the eighth day.

In the CK subplots, Rs increased with N levels after N addition in each month (Fig. 2c). The Rs decreased until the second day and then increased again at all N levels in July, which was most likely due to the rainfall on the third day. The Rs significantly increased at the N2 and N3 levels on the first day, but after this, the effects at the N1 and N2 levels weakened. At the N3 level, however, the effect lasted almost all August and September and at least 8 days into October.

Cumulative Rs after each N addition application

For the entire observation period, N addition suppressed cumulative Rs in the LRE subplots (Fig. 3a). At the end of the study, the cumulative Rs decreased by 22.6, 29.4 and 17.0 % at the N1, N2, and N3 levels, respectively, relative to the cumulative Rs at the N0 level.

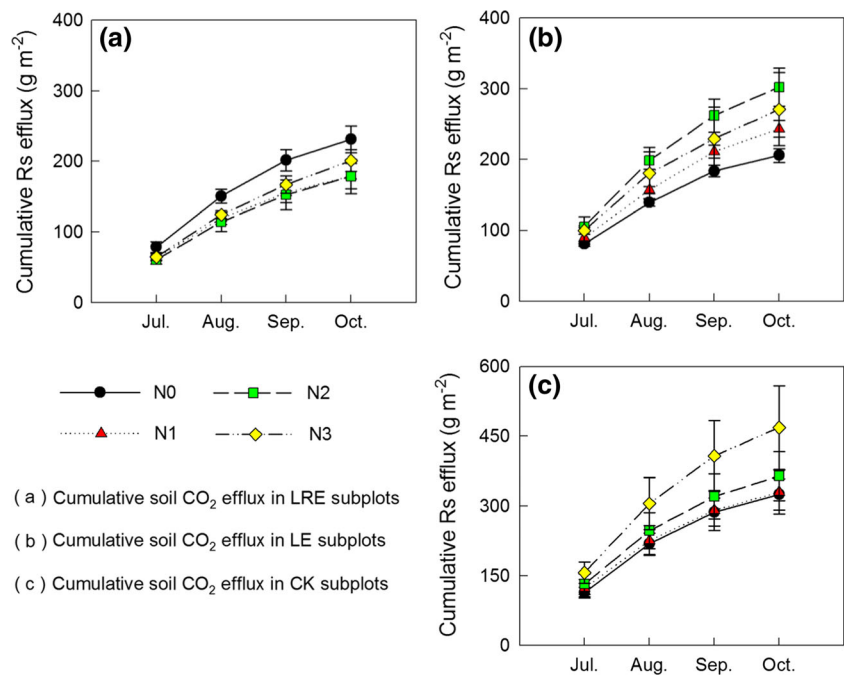
N addition significantly increased the cumulative Rs in the LE subplots (Fig. 3b). By the end of the investigation, the cumulative Rs had increased by 18.2, 46.9 and 31.7 % at the N1, N2, and N3 levels, respectively, relative to the cumulative Rs at the N0 level.

The N2 and N3 levels significantly stimulated the cumulative Rs in the CK subplots (Fig. 3c). By the end of study, the cumulative Rs increased by 18.2, 46.9, and 31.7 % at the N1, N2, and N3 levels, respectively, relative to the cumulative Rs at the N0 level.

Fine root biomass and soil microbial biomass

Fine root biomass in the LE subplots was 88, 98, 98 and 97 g m^{-2} at the N0, N1, N2, and N3 levels, respectively, and the differences between the N levels were not statistically significant ($P > 0.05$, Fig. 4). The biomass of fine roots in the CK subplots was 77, 97, 104 and 152 g m^{-2} at the N0, N1, N2 and N3 levels, respectively. The fine root

Fig. 3 Monthly cumulative Rs efflux at four levels of N addition in the various organic matter treatment subplots during the study period



biomass significantly increased at the N3 level relative to the N0 level ($P < 0.05$).

In the LRE subplots, the MBC at the N1, N2, and N3 levels was reduced by 24.7, 18.1 and 40.9 %, respectively, relative to the MBC at the N0 level (Fig. 5). The MBN at the N1, N2 and N3 levels was reduced by 38.3, 67.8 and 61.9 %, respectively, relative to the N0 level. The N addition increased the MBC/MBN ratio, and the ratio at the N2 level was significantly higher than that at the N0 level ($P < 0.05$).

In the LE subplots, the MBC and MBN values were significantly higher at the N3 level than the values at the N0 level ($P < 0.05$). The N addition decreased the MBC/MBN ratio, but the ratio was not significantly different among the N levels ($P > 0.05$).

The MBC and MBN increased with N addition in the CK subplots. The MBC values at the N3 level were significantly higher than the values at the N0 level ($P < 0.05$). The MBC at the N1, N2 and N3 levels increased by 27.0, 46.6 and 70.3 %, respectively, relative to the N0 level. No significant differences in MBN among the

N levels were found ($P > 0.05$). The N addition increased the MBC/MBN ratio, but no significant differences for the ratio were found between the N levels ($P > 0.05$).

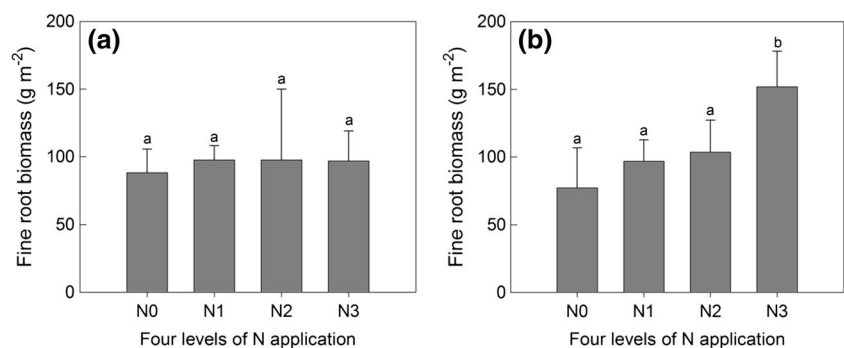
Discussion

Interactive effects of organic matter manipulation and nitrogen addition on the Rs

Despite the substantial number of studies investigated the effects of N addition on the Rs, it is not yet clear if these impacts vary between intact soils and soils where aboveground litter and/or roots have been removed since most of the earlier studies only seem to address the issue with intact soils (Burton et al., 2004; Fang et al. 2012; Du et al. 2014). The study demonstrated that Rs responses to N addition differed in the varying organic matter manipulation.

Rs consists of two components. One concerns autotrophic respiration (Ra) derived from plant roots,

Fig. 4 Fine root biomass in only aboveground litter removal subplots (a) and control subplots (b) at four levels of N addition



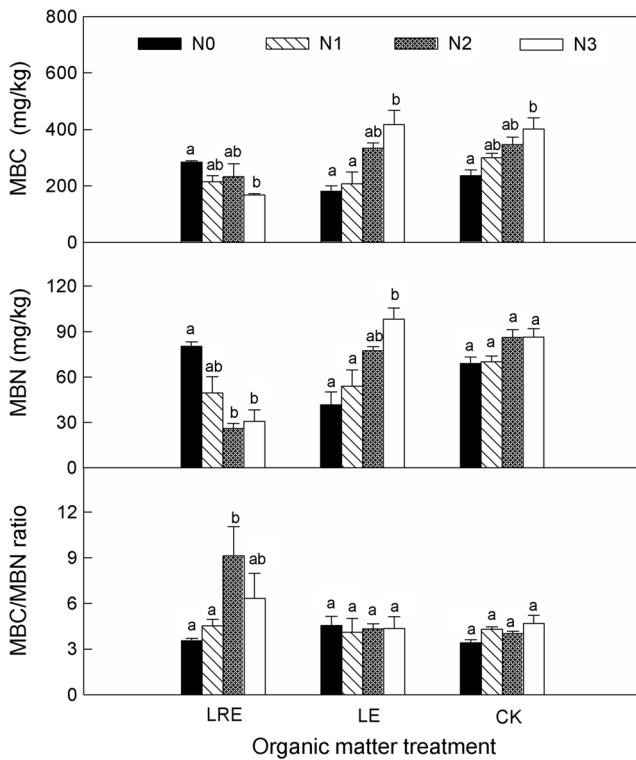


Fig. 5 Dynamics of soil microbial C (MBC), microbial N (MBN) and the MBC/MBN ratio at four levels of N addition in the various organic matter treatment subplots. Different letters on the top of bars represent significant differences between N levels for each organic matter treatment

mycorrhizal fungi, and other associated microorganisms that use C fixed by plant photosynthesis. The other component is heterotrophic respiration (Rh), resulting from decomposition of plant residues and SOM (Ryan and Law 2005). There is a growing recognition that the responses of Ra and Rh to N addition are inconsistent, resulting in uncertainties and variable perspectives to demonstrate the effect of N addition on the Rs (Allison et al. 2008; Samuelson et al. 2009; Tu et al. 2013).

N addition significantly stimulated Rs in the intact soils, which is in agreement with other N-limited forests (Bowden et al. 2004; Cleveland and Townsend 2006; Tu et al. 2013; Sun et al. 2014). The Rs is generally closely correlated with net primary productivity (NPP). Higher NPP leads to more labile C substrate inputs into the soil (Samuelson et al. 2009), thus accelerates the SOM decomposition and CO₂ release (Fontaine et al. 2007). NPP is generally increased under elevated N addition. Aboveground net primary productivity was found increased by 29 % under N supply in a global meta-analysis (LeBauer and Treseder 2008). The observational and experimental studies have also shown that N enrichment stimulates the Rs by increasing fine root biomass and microbial activity in the forests of North America (Aber et al. 1998), Europe (Magnani et al. 2007) and East Asia (Tu et al. 2013).

N addition significantly inhibited Rs when litter and roots were both removed, which implies that N addition has a negative effect on the heterotrophic respiration (Rh) from the decomposition of SOM.

This reduction in heterotrophic activity was described in previous studies that found negative effects of N enrichment on microbial growth after fertilization (Burton et al. 2004; Janssens et al. 2010; Cusack et al. 2011). In a meta-analysis, Liu and Greaver (2010) reported that N addition reduced microbial respiration by an average of 8 %. The addition of N can cause a suite of detrimental effects on microbial activity by increasing osmotic pressures, depleting soil minerals, N toxicity and decreased C availability (Treseder 2008). The N-induced reduction in the Rh in this study was attributed to the decrease in soil microbial activities.

When only aboveground litter was removed, although soil microbial activity was suppressed, however, the N addition still had a good facilitator for the Rs in this N-limited forest. The mechanism to explain this result might be related to the increase in fine root biomass. Rs is generally closely correlated with root biomass, especially the fine root biomass as root respiration from coarse roots is low (Ryan et al. 1996; Pregitzer et al. 1998; Vose and Ryan, 2002). Many studies have reported that the addition of reactive N increased fine root biomass and plant root respiration in N-limited ecosystems (Pregitzer et al. 2000; Cleveland and Townsend 2006). However, in the N saturated ecosystems, the addition of N may decrease or have no effect on fine root biomass (Mo et al. 2007; Jourdan et al. 2008; Wang et al. 2012). How fine root affects Rs in this area subjected to long-term N enrichment, therefore, needs to be further investigated.

Duration of priming effect of N addition on the Rs

The effect of N addition on the Rs was seasonal, with largest effects during July–September, coincident with peaks in temperature and soil moisture conditions. When litter and/or root were removed, the priming effect of N addition on the Rs disappeared more quickly than intact soil. The negative effect of N addition on the Rh in the LRE subplot was primarily concentrated during the first 1–2 days after each N addition application, and then the effect weakened and any additional N largely inhibited the Rh. Similarly, the positive priming effect of N addition on the Rs in the LE subplot occurred during the first 1–2 days in July–September and during the first 5 days in October, after which the effect weakened and leveled off and any additional N increased Rs, particularly at the N2 level. This might due to N was spayed directly on the mineral soil, which made N addition have a pulse effect on the Rs when litter and/or roots were removed. Aboveground litter layers regulate soil microclimate by forming a buffering

interface between soil surface and atmosphere (Sayer et al. 2007). Therefore, we found that in the intact soils, the duration of positive priming effect on the Rs can last 1 month during July–September and for at least 8 days in October at the N3 level.

The duration of priming effect of N addition on the Rs was different in the varying organic matter treatments, this could have some implications for the future investigation on the sampling interval of Rs measurement. We are aware of a large number of published field and laboratory studies which have examined how N addition affects Rs by periodical measurements of the response in respiratory processes. Sampling Rs measurements after each N addition application varied across studies, the majority showing intervals from 1 week to 1 month (Burton et al., 2004; Mo et al., 2007; Tu et al. 2013; Gao et al., 2014). These sampling intervals probably did not capture the real Rs responses to N addition, especially during the first few days after N supply. Therefore, an increase in the sampling frequency of Rs after N addition would provide a point of view for why Rs varies so much in response to added N.

Factors regulate Rs responses to organic matter manipulation and N addition

Rs can be affected by microclimatic factors, such as soil temperature, soil moisture and pH (Buchmann 2000; Fan et al. 2015). As a biological process, additionally, Rs is closely correlated with plant growth and photosynthetic substrates. Any factors that influence above- and below-ground substrate supply would affect Rs. Organic matter manipulation had no significant effect on soil temperature, which was due to crown closure in the study did not change throughout the observation and forest canopy intercepted solar radiation to maintain a favourable soil environment (Lowman and Schowalter 2012). Soil moisture, however, was significantly increased in the LRE subplot compared to the LE and control subplots due to the inhibition of water transport. Such a change would lead to corresponding changes in the Rs. Although many studies reported that soil microbial biomass was decreased after root trenching (Bowden et al. 2004; Mo et al. 2007; Janssens et al. 2010), root trenching did not significantly reduce the soil microbial biomass in the LRE subplots compared to the control subplots in this study. By contrast, the MBC in the LRE subplots (284 mg/kg) was higher than in the LE subplots (182 mg/kg) or the control subplots (236 mg/kg) without N addition. This might be due to positive priming when root exudates were removed after root trenching (Zhang et al. 2014). Therefore, we assumed that soil moisture might be a factor regulating Rs responses to the organic matter manipulation.

Neither soil temperature nor soil moisture was significantly affected by N addition. N addition significantly decreased soil microbial biomass in the LRE subplots. N addition generally results in a decrease in the microbial biomass. A meta-analysis of 206 published studies has found that microbial biomass decreased by an average of 5.5 % under N addition (Lu et al. 2011). However, N addition significantly increased microbial biomass and Rs in the LE and control subplots, which may be due to N addition satisfies the N demand for the microorganisms and roots in this N-limited forest. N addition also increased fine root biomass in the LE and control subplots although there were no significant differences between the N levels for the fine root biomass. A large number of studies also reported that N addition would inhibit soil microbial activity, fine root biomass and Rs when soil N availability exceeds the amount that plant needs and the forest is N-saturated (Treseder 2008; Thirukkumaran and Parkinson 2000; Ramirez et al. 2010). N deposition is likely to increase rapidly in the region with the expected increase in human activities in the coming decades. Thus, further investigations are required to characterize the ecosystem responses to long-term N addition.

Conclusion

N addition and organic matter manipulation interacted to affect the Rs. N addition significantly stimulated the Rs in the control subplots by increasing fine root biomass and microbial biomass in this N-limited forest. When litter and roots were both removed, N addition significantly inhibited the Rh by decreasing the microbial biomass. Although microbial activity was suppressed when only aboveground litter was removed, N addition still had a good facilitator for the Rs in this N-limited forest. The duration of priming of effect of N addition on the Rs was different in the varying organic matter treatments. When litter and/or root were removed, the priming effect of N addition on the Rs disappeared more quickly than intact soil. This is likely to provide a point of view for why Rs varies so much in response to added N and also has implications for future determination of sampling interval of Rs measurement.

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