



## The effect of nitrogen addition on soil respiration from a nitrogen-limited forest soil



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### ABSTRACT

We investigated how soil respiration (Rs), heterotrophic respiration (Rh) and rhizosphere respiration (Rr) respond to nitrogen addition in a 21-yr-old larch (*Larix principis-rupprechtii*) plantation in North China. Three levels of nitrogen treatments (control, no nitrogen addition; low-N, 20 kg N ha<sup>-1</sup> yr<sup>-1</sup>; high-N, 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>) were established in May 2010. Rs, Rh and Rr were then measured during the growing seasons from 2011 to 2013. Nitrogen addition significantly reduced Rs by 10.0% under low-N treatment and by 12.5% under high-N treatment. Rh and Rr showed different responses to nitrogen addition. Nitrogen addition had no significant effects on growing season fluxes of Rh during the observation periods, but Rr was decreased by ~37% and ~31% under the low-N and high-N treatments, respectively. Averaged across the three growing seasons, the mean rate of Rs decreased from 2.47 ± 0.39 μmol m<sup>-2</sup> s<sup>-1</sup> in the control plots to 2.22 ± 0.34 μmol m<sup>-2</sup> s<sup>-1</sup> in low-N plot and 2.16 ± 0.30 μmol m<sup>-2</sup> s<sup>-1</sup> in the high-N plot. Rr contributed about 111% and 76% of the observed reduction of Rs in low and high-N plots, respectively. In addition, elevated nitrogen input also reduced the temperature coefficients (Q<sub>10</sub>) of Rh and Rr. Compared to the control, nitrogen additions significantly decreased Q<sub>10</sub> of Rh and Rr in high-N plots by 7% and 13%, respectively. Overall, our results suggest that the reduction in Rs can mainly be attributed to the decrease in rhizosphere respiration in our nitrogen-limited plantation. With global temperature rising, nitrogen deposition may increase carbon sequestration in forest ecosystems, not only by reducing carbon loss by Rs, but also by reducing the temperature coefficients of Rh and Rr.

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### 1. Introduction

Soil respiration (Rs) is the second largest flux in the terrestrial carbon balance, with about 80–98 Pg C being emitted to the atmosphere every year (Bond-Lamberty and Thomson, 2010). Because forests occupy about 30% of the world's land area, respiration from forest soils make a major contribution to the global carbon cycle. The size of this contribution may be altered by N inputs as numerous studies found that Rs is often reduced by anthropogenic nitrogen deposition (Janssens et al., 2010; Liu and Greaver, 2010; Lu et al., 2011; Zhou et al., 2013). Nitrogen deposition, which results from burning fossil fuel, has greatly increased the input of nitrogen

to terrestrial ecosystems (Galloway et al., 2003; Reay et al., 2008) and this may continue to increase in the future (Galloway et al., 2008). However, the underlying mechanisms driving the decline of Rs are not well understood.

Rs is comprised of both rhizosphere respiration (Rr) from roots, mycorrhizae and other microorganisms associated with root systems, and heterotrophic respiration (Rh) from free-living soil bacteria, fungi, and fauna (Hanson et al., 2000; Bond-Lamberty et al., 2004). Nitrogen addition alters Rs through regulating plant and microbial activities that are directly associated with CO<sub>2</sub> production. Nitrogen addition often reduces microbial biomass and microbial activity (Compton et al., 2004; Wallenstein et al., 2006; Demoling et al., 2008; Treseder, 2008), and inhibits enzyme reactions (Waldrop et al., 2004). Thus, many studies have suggested that a reduction in Rh is the primary driver for the decreased Rs observed when nitrogen is enriched (Lee and Jose, 2003; Bowden et al., 2004; Burton et al., 2004; Janssens et al., 2010).

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However, several studies also found that nitrogen addition can greatly reduce Rr (Olsson et al., 2005; Phillips and Fahey, 2007). When nitrogen limitation is alleviated by nitrogen addition, plants tend to invest less photosynthetic products into root systems. This reduction in belowground carbon allocation can lead to a decrease in both root and rhizosphere microbial respiration (Haynes and Gower, 1995; Olsson et al., 2005; Phillips and Fahey, 2007). Although numerous studies have investigated the response of Rs to nitrogen enrichment, few of them measured Rr and Rh in the field, and long-term measurements are especially lacking. This lack of data greatly hinders our ability to predict the future responses of Rs in a changing environment.

At the global scale, nitrogen deposition and temperature are increasing simultaneously. The  $Q_{10}$  value, i.e., the relative change of Rs when temperature increases by 10 K, is affected by substrate availability, enzyme activity and environmental changes (Curiel et al., 2004; Davidson and Janssens, 2006). To predict the changes of Rs in the future, it is important to know whether nitrogen deposition will alter the  $Q_{10}$  value of Rs and its components. However, few studies have addressed the impacts of nitrogen enrichment on the  $Q_{10}$  of Rs, and even less attention has been paid to Rh and Rr. In this paper we aim to produce a better understanding of the mechanisms underlying the response of the carbon cycle to nitrogen deposition by examining how the  $Q_{10}$  values for Rh and Rr respond to different nitrogen addition treatments.

## 2. Materials and methods

### 2.1. Site description

The one hectare study site was a 21-year-old larch (*L. principis-rupprechtii*) stand at the Saihanba Ecological Station (42°25'N, 117°15'E, 1505 masl) of Peking University, situated in Saihanba National Forest Park, Hebei Province, China. Saihanba National Forest Park is the largest plantation forest (94,700 ha) in China; the dominant species are *L. principis-rupprechtii* (Prince Rupprecht's larch) and *Pinus sylvestris* var. *mongolica* (Mongolia pine). The topography is flat.

The climate is semi-humid, with a long, cold winter (November–March), and a short spring and summer. From observations at the local weather station over the last 40 years, the mean annual temperature is  $-1.4^{\circ}\text{C}$  ( $-21.8^{\circ}\text{C}$  in January and  $16.2^{\circ}\text{C}$  in July); the mean annual precipitation is 450 mm; and the mean frost-free duration is 81 days (Ma et al., 2014). Snowfall normally begins in November, and snowmelt occurs in early April. In winter, snow depth is typically less than 30 cm (Wang et al., 2010a). Soil at the site is predominantly sandy, with a pH of  $6.42 \pm 0.22$ ; soil bulk density is  $1.38 \pm 0.02 \text{ g cm}^{-3}$ ; soil organic carbon is  $53.23 \pm 5.11 \text{ Mg ha}^{-1}$ ; and available nitrogen content is  $5.52 \pm 0.23 \text{ mg kg}^{-1}$  soil. The ambient nitrogen deposition rate is  $7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (Ma et al., 2014).

### 2.2. Experimental design

The stand was fenced in August 2009 to prevent people entering and to minimize disturbance. The study area is dominated by *L. principis-rupprechtii*, which forms a relatively closed canopy. Ground cover is nearly absent. The stem density is 3160 stems per ha, with mean DBH of  $7.64 \pm 0.14 \text{ cm}$  and height of  $7.78 \pm 0.27 \text{ m}$ .

Nine  $20 \text{ m} \times 20 \text{ m}$  plots were established with three plots for low nitrogen addition (low-N,  $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ), three for high addition (high-N,  $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) and the other three for ambient nitrogen (control, i.e., no nitrogen is added). Over 10 m buffer stripes are set up between the plots. Nitrogen solution has been applied to the soil surface since 2010, in the form of urea using backpack sprayers; six applications were made per year, from early

May to early October. The amount of water added to the soil through each nitrogen application is equivalent to 0.0625 mm rainfall, and the same amount of water was applied to the control plots to prevent the additional water having any relative effect.

### 2.3. Soil carbon flux measurement

To measure Rs, four polyvinyl chloride (PVC) collars (20 cm inside diameter, 11 cm height) were installed at randomly selected positions in each plot. The collars were inserted 8 cm into the soil. The litter layer depth was about 5 cm, indicating that only the roots in the top 3 cm of soil would be injured (cut off) by mechanical insertion of the collars. This was assumed to make no significant impact to Rs.

To measure Rh, we also installed another four deep PVC collars (20 cm inside diameter, 50 cm height) in each plot. Our early survey of root distribution found that almost no roots were present in soils deeper than 35 cm and the soil below 35 cm is predominantly sandy. Those deep collars were therefore inserted 47 cm into the soil, which ensures that almost no roots can grow within the collars. Any living plants inside the collars were eradicated by hand once a week.

Rs and Rh were measured twice a month using a Li-8100 soil  $\text{CO}_2$  Flux system (LI-COR Inc., Lincoln, NE, USA) during the snow-free season from early May to late October. We made measurements five times per day in 2011 and 2012, but three times per day during 2013. Respiration rates were calculated as the means of the three plots. Rr was calculated as the difference between Rs and Rh.

When respiration measurements were made, soil temperature and moisture at a depth of 5 cm, were also recorded automatically by the Li-8100 temperature (8100-201) and moisture (8100-204) probes near each collar. Soil temperature and soil moisture at 5 cm depth were also continuously recorded by an EM-50 (Decagon, USA) every 30 min during the snow-free period.

### 2.4. Fine root biomass and litterfall

Root biomass was determined for each plot in September 2011 and 2013. During each sampling, four soil cores (10 cm diameter, 40 cm deep) were collected from each plot. The samples were divided into three depths (0–10, 10–20 and 20–40 cm). Roots were removed from the cores by hand, with live roots placed into one of two diameter classes:  $<1 \text{ mm}$  and  $>1 \text{ mm}$ . Root mass was determined after oven drying for 48 h at  $65^{\circ}\text{C}$ . The total root mass was estimated as the average of the four samples in each plot during the year.

Two litter traps ( $1 \text{ m} \times 1 \text{ m}$ ) with a mesh size of 0.5 mm were placed randomly in each plot about 0.8 m above the ground surface. The traps were emptied once every two months during the growing season. Litterfall was separated into three components: leaf, small woody material, and miscellaneous (Mo et al., 2008). Litterfall samples were oven dried at  $65^{\circ}\text{C}$  for 48 h and then weighed. The total litterfall mass was estimated as the average of the two samples in each plot during the year.

### 2.5. Soil microbial activity, dissolved inorganic nitrogen and other soil properties

To examine the effects of nitrogen addition on microbial activity in bulk soil, samples were collected in late July and September in 2011, 2012 and 2013. These samples were extracted with a hand auger (internal diameter 4 cm) to a depth of 10 cm from six points in each plot; soil was randomly sampled between the rows of the trees, to minimize the effect of roots. The samples were transferred to a plastic bag inside an icebox and taken to the laboratory immediately following collection. The six soil samples were mixed into a

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