



## Effects of nitrogen addition on soil inorganic N content and soil N mineralization of a cold-temperate coniferous forest in Great Xing'an Mountains



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### ABSTRACT

Nitrogen (N) plays an important role in carbon and nutrient cycling in forest ecosystems. Previous studies have shown that increased atmospheric N deposition has led to changes in forest soil N transformations and soil N availability. However, knowledge gap exists about the impacts of N deposition on soil N dynamics of forest, especially for the boreal forests in China. In order to explore such impacts, a low-dose (Control, 0 kgN hm<sup>-2</sup> years<sup>-1</sup>, Low N—10 kgN hm<sup>-2</sup> years<sup>-1</sup> and High N, 40 kgN hm<sup>-2</sup> years<sup>-1</sup>) and multiple forms (NH<sub>4</sub>Cl, KNO<sub>3</sub> and NH<sub>4</sub>NO<sub>3</sub>) of simulated atmospheric N deposition experiment was conducted from 2010–2012 in a cold-temperate coniferous forest in the Great Xing'an Mountains. This paper reports inorganic N (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) content, net ammonification rate, net nitrification rate and net N mineralization rate in the 0–10 cm mineral soil during the growing season from May to September in 2012, the third year after N addition. Results that during the growing season, soil inorganic N was dominated by NH<sub>4</sub><sup>+</sup>-N, showing a significant seasonal variation. Soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N responded differently to exogenous N addition. Soil NH<sub>4</sub><sup>+</sup>-N content was more affected by the forms of N addition, while soil NO<sub>3</sub><sup>-</sup>-N content was more affected by the doses of N addition. Relative to control, NH<sub>4</sub><sup>+</sup>-N content of 0–10 cm mineral soil increased greatly with NH<sub>4</sub><sup>+</sup>-N addition, which was completely different from the results observed in the first growing season of N addition in that no obvious effects of N addition on NH<sub>4</sub><sup>+</sup>-N content of 0–10 cm mineral soil was observed. In agreement with results observed in the growing season, NO<sub>3</sub><sup>-</sup>-N content of 0–10 cm mineral soil still showed a trend of enrichment with low dose N addition. For the N-limited forest ecosystem, soil net ammonification with a significant seasonal variation contributed most to soil net N mineralization, and soil net ammonification increased under NO<sub>3</sub><sup>-</sup>-N addition, especially in the case of low dose N addition. Similar to soil net ammonification, soil net nitrification rates increased more under low-dose N addition compared to high-dose N addition. The possible explanation for our results is that a shift of 0–10 cm mineral soil NH<sub>4</sub><sup>+</sup>-N in response to N addition over time could be attributed to continue NH<sub>4</sub><sup>+</sup>-N addition-induced increase of litterfall decomposition and decrease of microbial N immobilization and autotrophic nitrification due to a reduction in soil pH. Continual NO<sub>3</sub><sup>-</sup>-N addition might decrease the number and activity of autotrophic nitrifying bacteria, showing a positive effect on net ammonification in the 0–10 cm mineral soil. Compared with low doses of N addition, the effect of high doses of NH<sub>4</sub><sup>+</sup>-N addition on soil acidification could be higher and high doses of NO<sub>3</sub><sup>-</sup>-N addition might suppress the activity of nitrifying bacteria, increase the risk of leaching and denitrification, and stimulate microbial N immobilization in the N-poor soil. Our results, to some extent, suggest that increasing atmospheric N deposition will promote the accumulation of 0–10 cm mineral soil NH<sub>4</sub><sup>+</sup>-N and promote soil net N mineralization of the cold-temperate coniferous forest in Great Xing'an Mountains.

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### 1. Introduction

In general, N is the key nutrient limiting factor affecting productivity and ecological processes of forest ecosystems [1–3]. As reported by many studies, soil N availability affects forest productivity [4],

biogeochemical cycling [5] and greenhouse gas emissions [6] both directly and indirectly. Traditionally, it was assumed that only the inorganic forms of N made available via the mineralization of soil organic matter are available for plants [7]. Recent studies, however, have shown that some plants can directly take up organic N compounds [8,9]. Bioavailable N is estimated at less than 1% of total N in forest soils, and sharp competition in this part of N exists between plants and soil microbes [10]. Thus, much attention have been paid to N dynamics in forest soils.

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Mineralization, nitrification, denitrification and dissimilatory nitrate reduction to ammonium (DNRA) are the main N transformation processes in forest soils. Net dynamics of soil inorganic N as  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N is a result of N transformations, plant uptake, abiotic and biotic immobilization, gaseous losses and leaching [6]. Accordingly, carbon/nitrogen ratios (C/N ratios) of substrate being decomposed and decomposer, biomass of microorganisms and fine root, soil temperature and moisture, soil pH, soil inorganic N content, microbial respiration and adenosine triphosphate levels are factors affecting soil N transformations and N budgets in forest ecosystems [11,12]. In the context of increased global atmospheric N deposition [13,14], N deposition as a driving factor can lead to changes in many properties of forest ecosystems, including structure, function and C and N cycles [15–17]. N saturation may happen when exogenous N input exceeds biological needs and abiotic immobilization of forest ecosystems, along with which are a series of nonlinear responses such as a nonlinear changes in  $\text{NO}_3^-$ -N leaching and net nitrification, N mineralization, gaseous N losses and soil acidification [3]. In terms of N mineralization, Aber et al. [3] firstly considered that long-term N deposition may gradually increase soil N mineralization in N-limited temperate forests. However, more studies have shown that soil N mineralization in response to N deposition increased initially and then decreased [6]. Already in 1998, Gundersen et al. [18] have pointed out that in N-rich forest soils, in-situ net N mineralization rates may decline with increasing N input; in N-poor forest soils, in-situ net N mineralization rates may increase with increasing N input. In agreement with Gundersen et al. [19], Corre et al. found that in German spruce forest soils, gross N mineralization and gross nitrification rates increased up to intermediate N enrichment level and somewhat decreased at highly N enriched condition. The possible explanations for the above responses could be that N deposition or N addition can directly supply soil and litter with mineral N, and further result in changes in the C/N ratios of substrate being decomposed and litter fall, microbial activity, microbial community composition, soil pH and enzyme activity [6]. In short, great uncertainties exist in both responses and mechanisms, and more data from long-term N addition experiments in various types of forest ecosystems should be accumulated to assess the effects of N deposition on N dynamics and so the fate of deposited N as well.

Compared with Europe and North America, there are fewer studies examining the effects of N deposition on the properties of forest ecosystems, including structure, function and ecological processes in China [6, 20]. In fact, followed by Europe and North America, China has become third largest area of N deposition in the world [21]. It was found that the rates of N deposition had reached up to  $38.4 \text{ kg N hm}^{-2} \text{ a}^{-1}$  in the Guangdong Dinghushan Nature Reserve; N deposition in the Great Xing'an Mountains was estimated at a range from 9.87 to  $14.25 \text{ kg N hm}^{-2} \text{ a}^{-1}$  [22], which was slightly higher than that in regions such as Scandinavia, Canada and Alaska's boreal forests. It is expected that increased N availability would promote tree growth and productivity of N-poor boreal forest ecosystems. However, as mentioned above, excessive N input may have inverse effects, including soil acidification and promotion of  $\text{N}_2\text{O}$  emission, on forest ecosystems. For the boreal coniferous forest in the Great Xing'an Mountains, little is known about the degree to which increased N deposition may affect its biomass, productivity and nutrient cycling. Therefore, it is urgent to conduct long-term field studies to evaluate the potential influences of enhanced N deposition on the functions, services and ecological processes of the boreal coniferous forest.

In May, 2010, we started a simulated atmospheric N deposition experiment in the boreal coniferous forest in the Great Xing'an Mountains. In this study, we analyzed the seasonal dynamics of soil net ammonification, net nitrification, net mineralization rates and soil inorganic N content and their responses to N addition in the third growing season (May to September) after N addition. The main purposes were to reveal the effects of atmospheric N deposition on N availability and net transformations in the boreal coniferous forest. Our results will help to better assess and predict the effects of increased atmospheric N deposition

caused by human activities on nutrient dynamics and associated ecological processes of boreal forest ecosystems in China.

## 2. Materials and methods

### 2.1. Site description

The study site was described by Gao et al. [23] in detail. The study site is located at the National Field Station of Daxing'anling forest ecosystems in Inner Mongolia ( $50^\circ 49' - 50^\circ 51' \text{E}$ ,  $121^\circ 30' - 121^\circ 31' \text{N}$ ). The station located on the northwest slope of Xing'an Mountain, and its altitude is 826 m. The terrain is flat with slopes less than  $3^\circ$ . The area belongs to cold temperate humid climate with mean annual temperature of  $5.4^\circ \text{C}$ . Mean annual precipitation ranges from 450 to 550 mm, 60% of them fell in from July to August. During the snowfall period (from the end of September to early May of the following year), the average thickness of snowfall is about 20–40 cm, which accounts for 12% of the total annual precipitation. The annual surface evaporation ranges from 800 to 1200 mm. Its mean annual sunshine is 2594 h and has a frost-free period of 80 day. The soil type belongs to brown coniferous forest soil, and the average thickness of organic and mineral layer is 10 cm and 20 cm, respectively. Soil physical and chemical properties are summarized as follows: humus contents range from 10% to 30%, total N contents range from 2.9 to  $4.7 \text{ g/kg}$ , total phosphorus contents range from 0.5 to  $1.1 \text{ g/kg}$ , pH values range from 4.5 to 6.5, soil bulk density is from 0.15 to  $0.74 \text{ g/cm}^3$ . Vegetation type is a cold-temperate coniferous forest with the forest age of about 200-years, and its dominated species are *Larix gmelini*, *Betula platyphylla*, *Ledum palustre*, *Rhododendron simsii*, and *Vaccinium vitisidaea*.

### 2.2. Experimental design

Simulated atmospheric N deposition experiment started from May, in 2010. Referring to the actual atmospheric N deposition rate ( $8.5 \text{ kg N hm}^{-2} \text{ a}^{-1}$ ) in the Daxing'anling region, two levels of N addition as low N ( $10 \text{ kg N hm}^{-2} \text{ a}^{-1}$ ) and high N ( $40 \text{ kg N hm}^{-2} \text{ a}^{-1}$ ) and three forms of N addition as ammonium chloride ( $\text{NH}_4\text{Cl}$ ), potassium nitrate ( $\text{KNO}_3$ ), ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) were set to simulate the changes of C and N cycles in the boreal forest ecosystems under the scenarios of atmospheric N deposition increased by 1 and 4 times in the future. Each N treatment has three replications. The area of each plot is  $10 \times 20 \text{ m}^2$ , and has an interval of 10 m between two plots. Meanwhile, control plots ( $0 \text{ kg N hm}^{-2} \text{ a}^{-1}$ ) were set to eliminate the effects of micro-topography heterogeneity. In the growing season (from May to September), N fertilizers were dissolved in 20 L water and then were evenly sprayed into each plot using a sprayer at the beginning of each month. Control plots were received the same amounts of water, which is equivalent to an increase in annual precipitation by 24%.

### 2.3. Soil sampling and measurements

We measured in-site net mineralization and nitrification rates at the depth of 0–10 cm of mineral soil using PVC tubes [24]. During the growing season (May–September) in 2012, net rates were measured once a month with an incubation time of rough 30 days. For each plot, six PVC pipes (diameter: 7 cm, length: 15 cm) with parafilms sealed on the top were install into the top 10 cm depth of mineral soil from May to July and three PVC pipes were installed in August and September.

All the samples were sieved (2 mm) to remove stones and roots and then were stored at  $4^\circ \text{C}$  before analysis. Soil samples were extracted with 1 mol/L KCl at a soil:extractant ratio of 1:10. Ammonium ( $\text{NH}_4^+$ -N) and nitrate ( $\text{NO}_3^-$ -N) concentrations in extracts were measured by flow AutoAnalyser (Bran Luebbe, Germany). Soil moisture contents were measured by oven-drying at  $105^\circ \text{C}$  for 48 h to a constant mass. Soil pH values were determined with a soil:water ratio of 1:2.5 using a pH meter (Mettler Toledo, Switzerland).

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