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Soil microbial biomass, phosphatase and their relationships with phosphorus turnover under mixed inorganic and organic nitrogen addition in a *Larix gmelinii* plantation

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ABSTRACT

Atmospheric nitrogen (N) deposition can affect soil microbial biomass, phosphatase activity, and the concentrations of organic phosphorus (P), inorganic P and available P, but the changes in soil microbial biomass under mixed inorganic and organic N addition and their relationships with soil P turnover in forest ecosystems are not fully understood. In this study, a simulated N deposition experiment was conducted in a *Larix gmelinii* plantation in Northeastern China to investigate the variation of soil microbial biomass, phosphatase activity, P forms and available P concentrations as well as the relationships between these features. The experiment was arranged in a randomized block design with three replications for each treatment, including the control (no N addition, CK), total inorganic N (NH_4NO_3, TIN), mixed inorganic and organic N (inorganic N and organic N in the ratio of 7:3, ION), and total organic N (urea and glycine 1:1, TON). The results indicated that there was a significant difference in the response of soil biochemical characteristics to different types of N addition. Soil total microbial biomass, the biomass of bacteria and actinomycetes, alkaline phosphomonoesterase (AlP) activity, as well as oxalate-extracted aluminum (Al), cMonoesters, cDiesters and orthophosphate concentrations were significantly increased by the addition of mixed inorganic and organic N, whereas they were not affected by single inorganic or organic N addition. Compared to the CK treatment, the increase in soil microbial biomass and Al oxides concentrations under ION treatment significantly increased soil cMonoesters and cDiesters concentrations, and the increased bacterial biomass contributed to the increase of soil AlP activity. Moreover, the ION treatment also significantly increased soil total P concentration and enhanced microbial immobilization of P compared to the CK treatment. However, there was no significant difference in available P concentration between the ION treatment and other treatments, which could be attributed to increased orthophosphate concentration under the ION treatment. Overall, the findings suggest that bacteria and AlP can play important roles in maintaining soil P availability by accelerating P turnover under conditions of increased atmospheric N deposition in a *Larix gmelinii* plantation.

1. Introduction

Atmospheric nitrogen (N) deposition has been increasing in China due to the increase in the use of N fertilizers and fossil fuel combustion (Liu et al., 2013). Previous studies have reported that the increased atmospheric N deposition can have dramatic impacts on soil microbial groups and biomass as well as on relevant biological process in terrestrial ecosystems. However, most studies focused on the response of soil microbes and C cycling and N turnover in the ecosystem (Corre et al., 2003; Bowden et al., 2004; Waldrop et al., 2004; Du et al., 2014; Gao et al., 2016), while the effect of N deposition on soil microbes and phosphorus (P) turnover in the ecosystem remains unclear.

Soil P is one of the major nutrients that plants need for growth, and the P taken up by plants is mainly derived from soils (Schachtman et al., 1998; Achat et al., 2010). In terrestrial ecosystems, the changes in soil microbial groups and biomass under N deposition may play an important role in soil P turnover, since microbes can affect microbial immobilization of P, microbial phosphatase activity and microbial decomposition of plant litter, etc. (Kuperman, 1999; Mo et al., 2008; Tripathi et al., 2008; Marklein and Houlton, 2012). Previous studies have found that the addition of exogenous N in N-limited ecosystems can alleviate microbial N-limitation and increase soil microbial biomass

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in the short term before N saturation (Johnson et al., 1998; Lv et al., 2017). The increase of microbial biomass under exogenous N addition may aggravate the microbial immobilization of P, and result in the reduction of soil available P concentration (Nziguheba et al., 2000; Lupwayi et al., 2007). Meanwhile, other studies have found that exogenous N addition can stimulate microbes to allocate excess N to phosphatase enzymes, thus increasing soil phosphatase activity and accelerating the soil organic P mineralization process and soil inorganic P return (Olander and Vitousek, 2000; Marklein and Houlton, 2012). Soil organic P and inorganic P forms can be identified with the solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy, of which organic P determined by ^{31}P NMR primarily consists of monoesters, diesters and phosphonates, while inorganic P consists of orthophosphate, pyrophosphate and polyphosphate (Turner et al., 2003a,b; Cade-Menun, 2015). The organic P forms can be hydrolyzed by phosphatase to produce inorganic P, which may increase soil available P concentration (Nannipieri et al., 2011). However, although the effect of N deposition on soil microbial biomass, phosphatase activity, P forms and available P concentration were separately studied in terrestrial ecosystems, the N used was mainly single inorganic N or organic N (Turner et al., 2003a; DeForest et al., 2004; Farrer et al., 2013; Liu et al., 2014; Yang et al., 2015; Tian et al., 2017). However, actual atmospheric N deposition consisted of inorganic N and organic N, of which organic N accounts for 36.1% of total N deposition on average, with urea and amino acids as the main organic N constituents (Cornell et al., 2003). Based on this, single inorganic N or organic N addition may not accurately reflect the effect of atmospheric N deposition on soil biological and chemical properties. Currently, studies on the effect of mixed inorganic and organic N addition on soil properties in forest ecosystems mainly have focused on its effect on soil microbial biomass and relevant enzymatic activity as well as C cycle processes (Guo et al., 2011a,b; Du et al., 2014). Little is known about the changes in the microbial biomass in N-limited *Larix gmelinii* plantations after five years of mixed inorganic and organic N addition or how these changes affect soil P cycling as well as available P concentration.

Morover, phosphatases in soils mainly consisted of alkaline phosphomonoesterase (AlP) and acid phosphomonoesterase (AcP) (Tabatabai, 1994; Turner and Haygarth, 2005), and there is a difference in their microbial source. It has been reported that bacteria are the main microbial source of AlP (Nannipieri et al., 2011; Fraser et al., 2015), while the main microbial source of AcP is fungi (Turner and Haygarth, 2005; Jiang et al., 2017). Since the adaptation of bacteria and fungi to habitat change is different (Tietema, 1998; Treseder, 2008), changes in bacterial and fungal biomass may occur under deposition of exogenous N, which may result in the different responses of soil AlP and AcP activity to N deposition. However, there are few studies on the relationships between the changes in bacterial and fungal biomass and the variation in AlP and AcP activity under exogenous N deposition.

In addition, exogenous N deposition can also affect soil pH, thus resulting in the changes in soil aluminum (Al) and iron (Fe) concentrations, since the solubility of Al and Fe can be affected by soil pH (Finzi et al., 1998; Guo et al., 2011b; Peng et al., 2017). It has been reported that soil P forms can be strongly adsorbed by Al and Fe oxides in acidic soil (Celi et al., 1999, 2001; Ohno et al., 2011), but the effect of Fe and Al oxides on P forms may be different in different research areas. For example, Turner et al. (2003c) in lowland pasture soils in England and Wales found that Fe oxides had stronger capacity for adsorbing P forms, whereas Murphy et al. (2009) in grassland soils in north-east Ireland observed that Al oxides play a more important role in maintaining P forms. However, little is known about the changes in soil pH, Al and Fe oxides in the *Larix gmelinii* plantations in Northeastern China after five years of mixed inorganic and organic N addition as well as the relative importance of Al and Fe oxides in maintaining soil P forms.

Therefore, the objectives of this study were to investigate the changes in soil microbial biomass, phosphatase activity, Al, Fe, P forms and available P concentrations after five years of mixed inorganic and organic N addition in a *Larix gmelinii* plantation as well as the relationships between these features and to increase our understanding of the variation mechanism of soil available P under increased atmospheric N deposition. We hypothesized that (1) the mixed inorganic and organic N addition would increase soil microbial biomass, phosphatase activity, Al and Fe oxides concentrations, whereas there would be a difference in the responses of different microbial groups and phosphatases as well as Al and Fe oxides; and that (2) the increase in the microbial biomass and phosphatase activity as well as Al and Fe oxides concentrations under mixed inorganic and organic N addition can maintain soil P availability by accelerating P turnover in the temperate forest ecosystem of Northeastern China.

2. Materials and methods

2.1. Site description

This study was conducted in Laoshan Station of Maoershan Forest Farms (45°20′N, 127°34′E, average altitude 340 m), where *Larix gmelinii* is the dominant tree species. The station is located in Shangzhi County, Heilongjiang Province in the Northeastern China, which has a continental monsoon climate. The mean annual temperature is 2.7 °C, and mean annual precipitation varies from 600 to 800 mm with most of it occurring in July and August. The soil type in this study is characterized as a Haplic Luvisol (FAO/ISRIC/ISSS, 1998) with a soil pH (water:soil = 1:1) of 6.28 at a 0–10 cm soil depth as the initial basic soil property before the simulated N deposition experiment commenced.

2.2. Experimental design and soil sampling

This simulated N deposition experiment was initiated in April 2010 in a 33-year-old (by the year 2009) *Larix gmelinii* plantation. The experiment was arranged in a randomized block design with three replications. Each of the experimental plots was 200 m^2 (10 m × 20 m) with a 15 m buffer between plots. The treatments included: (1) control (CK, fertilized with deionized water only); (2) total inorganic N (TIN, $10\ g\ N\ m^{-2}\ a^{-1}$); (3) mixed inorganic and organic N (ION, $10\ g\ N\ m^{-2}\ a^{-1}$, inorganic N: organic N = 7:3); and (4) total organic N (TON, $10\ g\ N\ m^{-2}\ a^{-1}$). For the ION treatment, the ratio of inorganic N to organic N was similar to the average ratio of natural atmospheric organic N deposition (Cornell et al., 2003). In the experiment, NH_4NO_3 was chosen as the inorganic N, while urea and glycine were chosen and mixed equally as the organic N. The required N under TIN, ION and TON treatments was dissolved in 50 L deionized water and equably sprayed on the forest soil in six equal applications monthly over the growing season (May – October) each year, control plots received an equivalent volume as deionized water without N.

Soil sampling was carried out in July 2015. In each plot, ten soil cores (2.6 cm in diameter) were sampled randomly at 0–10 cm depth using an auger. After visible root and plant residues were removed, the soil cores were combined to make composite samples from the same plot, and then divided into three subsamples: one was air-dried for chemical analysis and solution ^{31}P NMR spectroscopy determination; one was freeze-dried and kept at −20 °C for PLFA extraction; and one preserved at 4 °C for analyses of soil microbial biomass C (MBC), microbial biomass P (MBP) and phosphatases.

2.3. Analysis of soil properties

Soil total P was determined by the molybdenum blue colorimetric method (Murphy and Riley, 1962) following perchloric acid ($HClO_4$) digestion (Kuo, 1996). Available P was determined by the same method after extraction by 0.5 M $NaHCO_3$ (Olsen et al., 1954). Soil pH was measured using a 1:2.5 (w:v) soil:water suspension ratio. Oxalate-extractable Al and Fe were extracted with ammonium oxalate/oxalic acid