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Ammonium fertilization causes a decoupling of ammonium cycling in a boreal forest

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ABSTRACT

The forest-floor organic layer of the boreal coniferous forest is generally characterized by large mineral-N pools (especially ammonium), high rates of gross N mineralization, and low rates of autotrophic nitrification and nitrate immobilization. As atmospheric N deposition increases in boreal regions, it is expected to increase N losses from the forest-floor organic layer, which could affect the N status and microbial N cycling of the underlying mineral soil. To test this possibility, we conducted a long-term experiment, starting in 2010, consisting of three N addition levels (0, 20, and 40 kg NH_4Cl–N ha^{-1} yr^{-1}) in a boreal *Larix gmelinii* forest in the Great Xing'an Mountain, China. We measured mineral N concentrations (2012–2014), the *in-situ* net N-cycling rates (2012 and 2013), the gross N transformation rates (2014), and microbial abundance (2014) in mineral soil (0–10 cm) in the peak growing season. The gross rates of N transformations were quantified via a laboratory, ^{15}N tracing experiment with a process-based ^{15}N tracing model. NO_3^- concentration, *in-situ* net nitrification, heterotrophic nitrification, gross nitrification, NO_3^- immobilization, and dissimilatory NO_3^- reduction to NH_4^+ (DNRA) neither increased nor decreased, suggesting that NO_3^- loss, production and retention were not affected by continual NH_4^+ additions. However, the NH_4^+ concentration and *in-situ* net ammonification rates increased under continued high NH_4^+ additions, reflecting a change in soil NH_4^+ status. As a result, microbial NH_4^+ cycling was in uncoupled state in the high N addition plots (NH_4^+ immobilization rates were incomparable to gross N mineralization rates), but this was not the case for the control and low N addition plots. Interestingly, the NH_4^+ oxidation rates decreased rather than increased with decreased NH_4^+ immobilization rates in the high N addition plots. However, the decreased NH_4^+ oxidation rates were paralleled by a reduction in ammonia-oxidizing archaea (AOA) abundance. Our results indicate that for this boreal coniferous forest, enhanced NH_4^+ deposition could alter mineral soil NH_4^+ status and NH_4^+ consumption. We show that NH_4^+ fertilization could inhibit NH_4^+ oxidation in forest soils.

1. Introduction

Atmospheric deposition of reactive nitrogen (N), has substantially increased over the past century, and is expected to increase further in the coming decades (Galloway et al., 2003, 2008). Nitrogen deposition could shift an ecosystem from previously N-limited into N saturated, driving N loss, soil acidification and productivity reduction (Aber et al., 1998; Fenn et al., 1998). The adverse effects of N deposition might be a particular concern in boreal regions, as ecosystems with short growing seasons and shallow soils have lower capacities to sequester N (Williams et al., 1996; Curtis et al., 2005; Bowman et al., 2008).

Key factors influencing N losses and the functional stability of boreal ecosystems under anthropogenic N inputs could be the changes in soil N cycling and retention (Aber et al., 1998; Fenn et al., 1998; Gundersen et al., 1998). However, information on how boreal

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soil N cycling and retention processes respond to N deposition is currently lacking (Aber et al., 1998; Allison et al., 2008). It has become clear to us that increased N availability and changes in microbial N cycling under N deposition could negatively affect the main root system of trees that are generally distributed in mineral soil, resulting in forest decline (Aber et al., 1998; Janssens et al., 2010). Previous studies have shown that enhanced deposition of N to forest ecosystems can alter soil N cycling and retention not only in the organic layer (if present), but also in the mineral layer, especially when the organic layer is rich in N availability, and is not thick (Corre et al., 2003, 2007, 2010; Zak et al., 2006; Baldos et al., 2015).

Boreal forests are generally dominated by coniferous trees, which produce litter with high contents of lignin and phenolic compounds that are resistant to microbial decay (Aerts, 1995). In coniferous forests, decomposition of litter and humus could be the rate-limiting step for microbial N cycling in the organic layer (Aber et al., 1998; Prescott et al., 1999), while microbial N cycling in the underlying mineral soil might be greatly influenced by mobilized N (Bengtsson and Bergwall, 2000). As needle inputs take a considerable time to decompose (Prescott et al., 1999, 2000), microbial N cycling in the coniferous organic layer is typically unresponsive to N fertilization (Bengtsson and Bergwall, 2000; Allison et al., 2008). However, the coniferous forest organic layer generally has a large mineral-N pool, a high rate of gross N mineralization, and low rates of autotrophic nitrification and NO_3^- immobilization (Gundersen et al., 1998; Bengtsson and Bergwall, 2000; Gao et al., 2013), which creates a potential for high N-leaching under N deposition conditions (Gundersen et al., 1998; Meiwes et al., 1998; Corre et al., 2003). By contrast, the mineral soil of the coniferous forest is often characterized by small mineral-N pools and low rates of net N mineralization (Gundersen et al., 1998; Gao et al., 2013, 2015a,b). Previous studies have suggested that N-cycling microbes in low-N soils are highly sensitive to changes in soil N availability (Boyle et al., 2008; Allison et al., 2009). Thus, it was expected that N deposited to the boreal coniferous forest-floor would induce a change in microbial N cycling in the underlying mineral soil.

Net N-cycling rates (net N mineralization and net nitrification) provide an index of plant-available N and N leaching (Booth et al., 2005; J.B. Zhang et al., 2012). However, they overshadow the mechanisms and dynamics of the soil internal N cycle (Gao et al., 2015a). Determination of soil gross N transformation rates can provide additional insights into microbially mediated N processes (Booth et al., 2005), as well as the N status of the soil (Corre et al., 2003; Venterea et al., 2004). Thus, an assay of both net and gross N transformations could increase our understanding of the relationship between soil N status and microbial N cycling, and microbial control of plant-available N and N leaching.

The respective gross N-cycling rates of organic and mineral soils are generally estimated via a separation of organic and mineral layers after ^{15}N injection into intact soil cores and incubation (Huygens et al., 2008; Corre et al., 2010; Baldos et al., 2015). The intact-core incubation technique avoids a major disturbance to the soil physical characteristics (Brenner et al., 2005). To minimize the influence of soil heterogeneity, temperature and moisture, gross N transformations are often assayed under controlled laboratory conditions. Although the N transformation rates obtained from laboratory incubations may differ from those derived in the field (Arnold et al., 2008), differences in N-cycling rates among treatments are likely to be identified from laboratory incubation (Paterson, 2003).

Since May in 2010, we have conducted an NH_4^+ addition experiment in a boreal *Larix gmelinii* forest located in the Great Xing'an Mountain region of China. The soil organic layer has a high N content (101.9 ± 10.9 mg NH_4^+-N kg soil^{-1} and 1.9 ± 0.2 mg NO_3^--N kg soil^{-1}) (Gao et al., 2013), and sampling through the growing season showed that experimental NH_4^+ additions have induced a 50% increase in NH_4^+-N concentration in the 0–10 cm mineral soil (Gao et al., 2015b). However, it remained unclear if this change in N status would affect soil internal N cycling.

In this study, we examined the impact of experimental NH_4^+ additions on net N cycling (*in-situ* incubations), gross N transformations (laboratory incubations), and microbial abundance in the 0–10 cm mineral soil in the peak growing season (August). To get a better resolution of soil N cycling and N retention processes, we employed both ^{15}N tracing experiments and full process-based N cycling models to quantify N-pool-specific and process-specific gross N transformation rates. The objective of our study was to determine if and how gross N transformations (N mineralization, NH_4^+ immobilization, gross nitrification, autotrophic nitrification, heterotrophic nitrification, NO_3^- immobilization and DNRA) in the mineral soil respond to experimental NH_4^+ additions.

2. Materials and methods

2.1. Site descriptions and N fertilization experiment

The study site is located on the northwest slope of Great Xing'an Mountains in Inner Mongolia, China (50°49′ – 50°51′E, 121°30′–121°31′N, ~826 m above sea level). The study area belongs to the cold-temperate humid climate. The annual precipitation averages from 450 to 550 mm, with 60% falling from July to August. During the snowfall period (from October to April of the following year), the soil is covered by a 20–40 cm snow layer, corresponding to 12% of the annual precipitation. The annual evaporation is approximately 800–1200 mm. The mean annual sunshine duration is 2594 h, with a frost-free period of approximately 80 days. The annual temperature averages −5.4 °C, with a mean temperature of 11.4–19.5 °C in growing seasons (May to September). The site is occupied by a 150-year-old *Ledum palustre* L. - *Larix gmelinii* (Rupr.) Kuzen. mixed forest. The *L. gmelinii* forest is the dominant boreal forest type and covers 15.6 × 10^6 ha in Northeast China (National Forestry Bureau, 1994). The main canopy layer is dominated by *L. gmelinii* together with the associated tree species mostly *Betula platyphylla* Sukaczev. The understory layer is dominated by *Larix palustre*, *Rhododendron simsii* Planch., and *Vaccinium vitis-idaea L.* The forest-floor (average thickness: 10 cm) comprises a thin O_i horizon dominated by leaf litter, and a thicker O_{ea} horizon interpenetrated by a dense root mat. The underlying mineral soil, with an average thickness of 20 cm, is a podzolic soil derived from granite residual. The terrain is flat, with slopes less than 3°.

Background N deposition rates were estimated at 9.87–14.25 kg N ha^{-1} yr^{-1} in the Great Xing'an Mountain region (Lü and Tian, 2007). To investigate the influence of atmospheric NH_4^+ deposition on microbial N cycling in the boreal coniferous forest soil, we established a NH_4^+ addition experiment in May 2010. We fertilized 20 m × 10 m plots with 0 (control), 20 and 40 kg NH_4Cl–N ha^{-1} yr^{-1} in a randomized block design (three replicates per treatment). There was an interval of 20 m between blocks, and 10 m buffer zones between plots within each block. During the growing season (May to September), NH_4^+ solution (each low-N plot: 33.3 g NH_4Cl–N + 20 L of water; each high-N plot: 66.7 g NH_4Cl–N + 20 L of water) was sprayed onto the forest floor using a backpack sprayer at the beginning of each month. During the non-growing season (from October to April of the following year), fieldwork cannot be carried out because of the extremely harsh weather (low temperature and heavy snow). Thus, the NH_4^+ solution (each low-N plot: 233.3 g NH_4Cl–N + 140 L of water; each high-N plot: 466.7 g NH_4Cl–N + 140 L of water) was sprayed collectively in early October. Control plots received an equal volume