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Are nitrate production and retention processes in subtropical acidic forest soils responsive to ammonium deposition?

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ABSTRACT

Changes in soil N-cycling and retention processes in subtropical/tropical acidic forest ecosystems under anthropogenic N inputs are not well understood. We conducted a laboratory ^{15}N tracing study on an acid soil (pH values: 4.6 to 5.0) from a subtropical forest fertilized for more than 2.5 years at a rate of 0, 40, and 120 kg NH_4Cl–N ha^{-1} yr^{-1}, respectively. To get a better resolution of mechanistic changes in soil N cycling and retention processes under NH_4^+ additions, we used a conceptual ^{15}N tracing model to quantify process-specific and pool-specific N transformation rates in soils. Gross N mineralization rates decreased at high NH_4^+ additions, which were paralleled by a reduction in fungal biomass and mineralization of recalcitrant organic N. Gross NH_4^+ immobilization rates did not show a change with increasing NH_4^+ additions. Interestingly, soil NO_3^- production (heterotrophic, autotrophic, and gross nitrification) and retention (NO_3^- immobilization and dissimilatory nitrate reduction to ammonium) showed insensitivity to increasing additions of NH_4^+. The mechanisms behind the lack of response of heterotrophic nitrification were unclear, but possibly related to the absence of significant changes in soil C: N ratio and soil acidity under increased NH_4^+ additions. Because of the low autotrophic nitrification potential and the lack of NH_4^+ limitation to autotrophic nitrifiers, autotrophic nitrification was unresponsive to NH_4^+ additions. NO_3^- immobilization rates appeared to be controlled by the NO_3^- produced from heterotrophic nitrification, as indicated by the positive relationship between NO_3^- immobilization and heterotrophic nitrification ($R^2 = 0.59$, $p = 0.015$), thus showing a lack of a change under increased NH_4^+ additions. DNRA seemed to be inherently less responsive to environmental changes such as NH_4^+ deposition. Our work demonstrates that enhanced NH_4^+ deposition has a low potential to stimulate soil NO_3^- production and weaken soil retention of NO_3^- in this, and perhaps other subtropical/tropical acidic forest ecosystems.

1. Introduction

Nitrogen (N) mineralization is at least a two-step process: the depolymerization of N-containing soil polymers into organic N-containing monomers (amino acids, amino sugars, nucleic acids, etc.) and subsequent ammonification (Schimel and Bennett, 2004). The depolymerization step, critical in N cycling, is controlled by extracellular enzymes which are often produced by fungi (Jones et al., 2004; Schimel and Bennett, 2004). Ammonium (NH_4^+) supplied by N mineralization strongly influences NH_4^+ immobilization (Booth et al., 2005). With high rates of N mineralization and ample NH_4^+ availability, autotrophic nitrification rates in many subtropical/tropical acidic forest soils (soil pH < 5.0) remain low (Huygens et al., 2008; Zhang et al., 2013), indicating that autotrophic nitrification rates in these acidic soils might not be controlled by NH_4^+ availability (Zhao et al., 2007). In subtropical/tropical acid forest soils (soil pH: <5.0) with high soil C: N ratios (>15.0) and fungal biomass, nitrification tends to be heterotrophic, and microbial immobilization of nitrate (NO_3^-) dominates over dissimilatory

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nitrate reduction to ammonium (DNAR) in N retention (Huygens et al., 2008; Zhang et al., 2013; Zhu et al., 2013). Forest soils exhibiting high rates of heterotrophic nitrification generally have higher NO_3^- immobilization rates, but this is not the case for forest soils with high rates of autotrophic nitrification (Huygens et al., 2007, 2008; Zhang et al., 2013; Zhu et al., 2013), suggesting that NO_3^- immobilization and heterotrophic nitrification might be functionally linked in forest soils. Many studies document the occurrence of DNAR in tropical/subtropical forest soils, a potential N conservation mechanism that redirects NO_3^- flow towards NH_4^+ (Silver et al., 2001, 2005; Rütting et al., 2008). Huygens et al. (2008) pointed out that DNRA also depends directly on heterotrophic nitrification for substrate generation.

Subtropical/tropical forest ecosystems are projected to receive enhanced N deposition (Galloway et al., 2008; Liu et al., 2013), but changes in the processes, rates and controls of soil N-cycling in these ecosystems under anthropogenic N inputs are less well understood (Silver et al., 2005; Corre et al., 2010; Cusack et al., 2011; Baldos et al., 2015; Gao et al., 2015). For example, Corre et al. (2010) reported that during 9 years of N addition to an old-growth lowland forest with net primary production not limited by N, microbial biomass decreased with increased soil acidity, but gross N mineralization rates increased. Although this increase of gross N mineralization was attributed to increased substrate quality, no direct evidences were provided (e.g. which organic N pool was affected in the soil). Despite widespread evidence for N deposition-induced nonlinear increase in net production and loss of NO_3^- from subtropical/tropical soils (Silver et al., 2005; Corre et al., 2010; Baldos et al., 2015), it remains uncertain to what extent this increase results from stimulation of either autotrophic or heterotrophic nitrifiers, or from the saturation of N uptake by plants, heterotrophic microbes, mycorrhizae, and abiotic reactions (Perakis et al., 2005). The low autotrophic nitrification potential of subtropical/tropical acidic forest soils suggests that autotrophic nitrification in these soils is unlikely to be stimulated by N deposition. Moreover, soil C: N ratio (Templer et al., 2012) and fungal biomass (Frey et al., 2004; Demoling et al., 2008) are often observed to decrease with N deposition. This indicates that N deposition will not stimulate heterotrophic nitrification to affect NO_3^- production and retention in subtropical/tropical acidic forest soils.

Determination of soil gross N transformation rates can provide information on the mechanisms of not only the N cycle of the soil, but also its N status. It has been shown that N-saturated forest soil is generally characterized by an uncoupled microbial N cycle; NH_4^+ and NO_3^- immobilization rates are lower than or do not keep pace with gross N mineralization and nitrification rates, respectively (Corre et al., 2003, 2007, 2010; Venterea et al., 2004).

In this study, we conducted a laboratory ^{15}N tracing study with soil (0–10 cm) from a subtropical forest plantation, which was fertilized for more than 2.5 years at a rate of 0, 40, and 120 kg NH_4Cl–N ha^{-1} yr^{-1}, respectively. To get a better solution of soil N cycling and retention processes, we used a process-based ^{15}N model to quantify process-specific and pool-specific N transformation rates in soils (Müller et al., 2007; Rütting and Müller, 2007). The main objectives were to determine if and how gross rates of soil N transformations (N mineralization, NH_4^+ immobilization, gross nitrification, autotrophic nitrification, heterotrophic nitrification, NO_3^- immobilization and DNRA) would be altered by increasing NH_4^+ additions. We hypothesized that NH_4^+ additions would not stimulate soil NO_3^- production (heterotrophic, autotrophic and gross nitrification) and weaken soil retention of NO_3^- (NO_3^- immobilization and DNRA).

2. Materials and methods

2.1. Site description, experimental design and sampling

The chronic N-fertilization experiment site was established in November 2011 in a 28-year-old subtropical slash pine (*Pinus elliottii*) plantation at the Qianyanzhou Experimental Station of Red Soil and Hilly Land, Chinese Academy of Sciences (CAS), Jiangxi province, southeastern China (115°03′29.2″ E, 26°44′29.1″ N, 102 m a.s.l.). The study site has been described in detailed by Wang et al. (2015). The dominant understory species are *Woodwardia japonica*, *Loropetalum chinense* and *Dicranopteris dichotoma* (Wang et al., 2012). The soil is classified as Typical Dystrudepts Udepts Inceptisols (USDA Soil Taxonomy), derived from weathered red sandstone and mud stone. The study area belongs to subtropical monsoon climate, with mean annual temperature and mean annual precipitation of 17.9 °C and 1475 mm, respectively (Wen et al., 2010). Soil characteristics in the top 0–10 cm were presented in Table 1. The mean (2008–2011) precipitation deposition of dissolved inorganic N (DIN) was 12.6 ± 1.5 kg N ha^{-1} a^{-1}, with a NH_4^+-N/NO_3^--N ratio of 1.9:1 (Zhan et al., 2014).

To explore the nonlinear response of microbial N cycling to N deposition, starting in May 2012 we fertilized 20 m × 20 m plots at the rates of 0, 40 kg N ha^{-1} yr^{-1} and 120 kg N ha^{-1} yr^{-1} in a randomized block design (each with three replicates, totaling 9 plots). N was added as ammonium chloride (NH_4Cl). The three blocks, laid out across a-40 ha, had at least a 10 m buffer zone between any two plots within each block. N solutions were sprayed monthly to the forest floors with a backpack sprayer. Each month, each fertilized plot received 30 L of NH_4Cl solutions, and each control plot received 30 L of water without fertilizer.

The low N treatment was to simulate a future increase in the atmospheric N deposition by 3-fold, corresponding to the annual bulk deposition rates in the subtropical region of China (>22.2–35 kg N ha^{-1} yr^{-1}) (Liu et al., 2013; Jia et al., 2014), and the level of low N addition to subtropical/tropical forest ecosystems (50 kg N ha^{-1} yr^{-1}) (Hall and Matson, 1999; Mo et al., 2008; Baldos et al., 2015). The high N treatment was to stimulate N-enriched conditions, exposing soil microbes to abrupt environmental changes to observe the direction of changes in soil N-cycling (Corre et al., 2010). The high N treatment level at our site, however, was similar to that in other subtropical/tropical forests (100–150 kg N ha^{-1} yr^{-1}) (Hall and Matson, 2003; Mo et al., 2008; Corre et al., 2010).

In late November 2014 (roughly 2.5 years after N addition), 8 samples per plot were collected from the top 0–10 cm soil using a PVC tube (inner diameter of 7 cm; length of 15 cm) after removing the thin litter layer. Soil samples were composited by plot, sieved (2 mm), homogenized, and subsequently divided into two subsamples: one for analysis of soil properties (400 g), and another for laboratory ^{15}N tracing experiment (800 g). The ^{15}N tracing experiment was carried out within two weeks, and soil samples were stored at 4 °C before analyses at the Jiangsu Key Laboratory of Environmental Change and Ecological Construction, School of Geography Sciences, Nanjing Normal University.

2.2. Soil properties

Soil moisture, total C, total N, pH, NH_4^+ and NO_3^- were determined (Table 1). Soil moisture was measured by drying the soil subsamples at 105 °C for 48 h. Total C and N contents were analyzed by an elemental analyzer (Europa Scientific Integra, UK) using air-dried, finely ground soil. Soil pH was determined in a soil (air-dried soil): water ratio of 1:2.5 by a DMP-2 mV/pH detector (Quark Ltd., Nanjing, China). Soil NH_4^+ and NO_3^- were extracted with 2 M