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 ¹⁵N 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 NH₄Cl–N ha^{−1} yr^{−1}, respectively. To get a better resolution of mechanistic changes in soil N cycling and retention processes under NH₄⁺ additions, we used a conceptual ¹⁵N tracing model to quantify process-specific and pool-specific N transformation rates in soils. Gross N mineralization rates decreased at high NH₄⁺ additions, which were paralleled by a reduction in fungal biomass and mineralization of recalcitrant organic N. Gross NH₄⁺ immobilization rates did not show a change with increasing NH₄⁺ additions. Interestingly, soil NO₃[−] production (heterotrophic, autotrophic, and gross nitrification) and retention (NO₃[−] immobilization and dissimilatory nitrate reduction to ammonium) showed insensitivity to increasing additions of NH₄⁺. 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 NH₄⁺ additions. Because of the low autotrophic nitrification potential and the lack of NH₄⁺ limitation to autotrophic nitrifiers, autotrophic nitrification was unresponsive to NH₄⁺ additions. NO₃[−] immobilization rates appeared to be controlled by the NO₃[−] produced from heterotrophic nitrification, as indicated by the positive relationship between NO₃[−] immobilization and heterotrophic nitrification ($R^2 = 0.59$, $p = 0.015$), thus showing a lack of a change under increased NH₄⁺ additions. DNRA seemed to be inherently less responsive to environmental changes such as NH₄⁺ deposition. Our work demonstrates that enhanced NH₄⁺ deposition has a low potential to stimulate soil NO₃[−] production and weaken soil retention of NO₃[−] in this, and perhaps other subtropical/tropical acidic forest ecosystems.

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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 (NH₄⁺) supplied by N mineralization strongly influences NH₄⁺ immobilization (Booth et al., 2005). With high rates of N mineralization and ample NH₄⁺ 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 NH₄⁺ 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 (NO₃[−]) 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 $\text{NH}_4\text{Cl-N ha}^{-1} \text{ yr}^{-1}$, 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 $12.6 \pm 1.5 \text{ kg N ha}^{-1} \text{ a}^{-1}$, with a $\text{NH}_4^+ \text{-N/NO}_3^- \text{-N}$ 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 $\text{N ha}^{-1} \text{ yr}^{-1}$ and 120 kg $\text{N ha}^{-1} \text{ yr}^{-1}$ 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 ($>22.2\text{--}35 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) (Liu et al., 2013; Jia et al., 2014), and the level of low N addition to subtropical/tropical forest ecosystems ($50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) (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 ($100\text{--}150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) (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

Table 1Soil properties at 0–10 cm depth after 2.5 years of NH_4^+ addition.

NH_4^+ addition	Soil pH 1:2.5 (H_2O)	Soil NH_4^+ mg N kg^{-1} soil	Soil NO_3^- mg N kg^{-1} soil	Soil C g C kg^{-1} soil	Soil N g N kg^{-1} soil	Soil C: N ratio
Control	5.0 $\pm$ 0.2	11.7 $\pm$ 2.1	8.3 $\pm$ 1.0	14.5 $\pm$ 0.5	0.9 $\pm$ 0.1	16.3 $\pm$ 1.9
Low N	4.6 $\pm$ 0.0	10.3 $\pm$ 1.4	9.7 $\pm$ 1.4	14.4 $\pm$ 2.7	0.9 $\pm$ 0.2	16.5 $\pm$ 0.5
High N	4.7 $\pm$ 0.3	10.9 $\pm$ 1.7	10.5 $\pm$ 1.6	12.2 $\pm$ 2.7	0.8 $\pm$ 0.2	15.5 $\pm$ 1.0

Values are means with standard errors (SE; $n = 3$).

KCl at a soil/extractant ratio of 1: 5 after shaking for 60 min at 250 rpm and 25 °C, and the concentrations of NH_4^+ and NO_3^- were assayed with a continuous-flow analyzer (Skalar, Breda, the Netherlands).

2.3. ^{15}N tracing experiment

There were two ^{15}N treatments (each with three replications), of which either ammonium ($^{15}\text{NH}_4\text{NO}_3$) or nitrate ($\text{NH}_4^{15}\text{NO}_3$) were labeled with ^{15}N at 10 at.% excess. For each plot, the sieved soil was placed in four sets of conical flasks (six conical flasks per set, three of the six conical flasks for $^{15}\text{NH}_4\text{NO}_3$ labeling, and the remaining three for $\text{NH}_4^{15}\text{NO}_3$ labeling; each conical flask containing fresh soil with the equivalent of 20 g of dry soil) (see supporting information, Fig. S1). After sealing with parafilm with five pin holes for gas exchange, the conical flasks were preincubated in the dark for 24 h at 25 °C. After pre-incubation, three ml of $^{15}\text{NH}_4\text{NO}_3$ or $\text{NH}_4^{15}\text{NO}_3$ solution was added to each conical flask at a rate of 2.86 $\mu\text{mol N g}^{-1}$ dry soil (20 $\mu\text{g NH}_4^+ \text{N g}^{-1}$ dry soil and 20 $\mu\text{g NO}_3^- \text{N g}^{-1}$ dry soil). The conical flask was incubated in the dark for 144 h at 25 °C after adjusting the soil to 60% water holding capacity (WHC) and sealing with parafilm (with five pin holes for air exchange). Soil extractions were carried out at 0.5, 48, 96, and 144 h after label addition to determine the concentrations and isotopic compositions of NH_4^+ and NO_3^- . A detailed description of laboratory ^{15}N tracing study on each soil sample can be found in Fig. S1 (see supporting information).

It should be noted that field treatment effects may be somewhat masked by laboratory additions of NH_4NO_3 . N was added to the plantation floor in the form of NH_4^+ only. However, we used either $^{15}\text{NH}_4\text{NO}_3$ or $\text{NH}_4^{15}\text{NO}_3$ as N tracer, and thus there may be an additive effect of NH_4^+ and NO_3^- on microbial cycling of N in the soil. Actually, we addressed this concern by taking the 1: 1 ratio of NH_4^+ to NO_3^- in field samples into consideration (Table 1). Previous studies have suggested that cold storage, soil sieving and laboratory incubation all would produce effects on microbial cycling of N (Johnson et al., 2005; Huygens et al., 2007; Arnold et al., 2008). However, laboratory measurements of gross N transformations allow identifying the direction of the change in *in-situ* N transformations in treatments (Paterson, 2003).

Concentrations of NH_4^+ and NO_3^- were determined using methods described above. The isotopic compositions of NH_4^+ and NO_3^- were determined using an automated C–N analyzer coupled to an isotope ratio mass spectrometer (Europa Scientific Integra, UK). For isotopic analysis, NH_4^+ and NO_3^- were separated by distillation with MgO and Devarda's alloy (Zhang et al., 2009, 2012b, 2013).

2.4. ^{15}N tracing model

The ten simultaneously-occurring gross N transformations in the soil were quantified with a process-based ^{15}N tracing model (Fig. 1) (Müller et al., 2007): M_{Nrec} , mineralization of recalcitrant organic N to NH_4^+ ; M_{Nlab} , mineralization of labile organic N to NH_4^+ ; $I_{\text{NH}_4\text{Nrec}}$, immobilization of NH_4^+ to recalcitrant organic-N; $I_{\text{NH}_4\text{Nlab}}$, immobilization of NH_4^+ to labile organic-N; $R_{\text{NH}_4\text{ads}}$, release of adsorbed NH_4^+ ; A_{NH_4} , adsorption of NH_4^+ on cation exchange sites; O_{NH_4} , oxidation of NH_4^+ to NO_3^- (autotrophic nitrification); O_{Nrec} , oxidation of recalcitrant organic-N to NO_3^- (heterotrophic nitrification); $I_{\text{NO}_3\text{Nrec}}$, immobilization of NO_3^- to recalcitrant organic N; and D_{NO_3} , dissimilatory NO_3^- reduction to NH_4^+ .

adsorbed NH_4^+ ; A_{NH_4} , adsorption of NH_4^+ on cation exchange sites; O_{NH_4} , oxidation of NH_4^+ to NO_3^- (autotrophic nitrification); O_{Nrec} , oxidation of recalcitrant organic N to NO_3^- (heterotrophic nitrification); $I_{\text{NO}_3\text{Nrec}}$, immobilization of NO_3^- to recalcitrant organic N; and D_{NO_3} , dissimilatory NO_3^- reduction to NH_4^+ . The transformation rates were calculated either by zero (M_{Nrec} and O_{Nrec}) or first-order (M_{Nlab} , $I_{\text{NH}_4\text{Nrec}}$, $I_{\text{NH}_4\text{Nlab}}$, O_{NH_4} , $I_{\text{NO}_3\text{Nrec}}$, and D_{NO_3}) kinetics. It should be noted that gaseous N losses and N leaching can not be simulated by this model. Based on available N cycling parameters, gross N mineralization ($M_{\text{tot}} = M_{\text{Nlab}} + M_{\text{Nrec}}$), gross nitrification/gross NO_3^- production ($O_{\text{NH}_4} + O_{\text{Nrec}}$), gross NH_4^+ immobilization ($I_{\text{NH}_4} = I_{\text{NH}_4\text{Nrec}} + I_{\text{NH}_4\text{Nlab}}$), gross NO_3^- immobilization ($I_{\text{NO}_3} = I_{\text{NO}_3\text{Nrec}}$), and gross NO_3^- retention ($I_{\text{NO}_3} + D_{\text{NO}_3}$) were calculated. The respective turnover rates of soil, labile and recalcitrant organic N was estimated: turnover rate of organic N = total N / ($M_{\text{tot}} + O_{\text{Nrec}}$), turnover rate of labile organic N = labile organic N / M_{Nlab} , and turnover rate of recalcitrant organic N = recalcitrant organic N / ($M_{\text{Nrec}} + O_{\text{Nrec}}$). In this study, labile and recalcitrant organic N were considered to equal 1% and 99% of soil organic N ($\approx$ soil total N) (Jinbo Zhang, personal communication) due to the difficulty in separating soil organic N into different fractions to estimate the two conceptual organic N-pool sizes (Huygens et al., 2007). The data supplied to the model were the concentrations ($\mu\text{mol N g}^{-1}$ dry soil) and ^{15}N excess (at. % excess ^{15}N) of NH_4^+ and NO_3^- (mean $\pm$ standard deviations).

The model calculated gross N transformation rates by simultaneously optimizing the kinetic parameters for each individual process via minimizing the misfit between modeled and observed concentrations of NH_4^+ and NO_3^- and their respective ^{15}N enrichments. To obtain the most suitable model that was able to simulate

Fig. 1. Conceptual ^{15}N tracing model (Müller et al., 2007). N_{rec} , recalcitrant organic N; N_{lab} , labile organic N; NH_4^+ , ammonium; NO_3^- , nitrate, M_{Nrec} , mineralization of recalcitrant organic N to NH_4^+ ; M_{Nlab} , mineralization of labile organic N to NH_4^+ ; $I_{\text{NH}_4\text{Nrec}}$, immobilization of NH_4^+ to recalcitrant organic-N; $I_{\text{NH}_4\text{Nlab}}$, immobilization of NH_4^+ to labile organic N; $R_{\text{NH}_4\text{ads}}$, release of adsorbed NH_4^+ ; A_{NH_4} , adsorption of NH_4^+ on cation exchange sites; O_{NH_4} , oxidation of NH_4^+ to NO_3^- (autotrophic nitrification); O_{Nrec} , oxidation of recalcitrant organic-N to NO_3^- (heterotrophic nitrification); $I_{\text{NO}_3\text{Nrec}}$, immobilization of NO_3^- to recalcitrant organic N; and D_{NO_3} , dissimilatory NO_3^- reduction to NH_4^+ .

the observed data, the number of possible N transformations, the possible kinetic settings of individual processes (zero/first-order kinetics/Michaelis-Menten kinetics) and N pools were varied and evaluated. The final model was identified based on Akaike's information criterion (AIC) (the smallest AIC) (Cox et al., 2006). Initially, all parameters (N pools and N transformations) from the conceptual model (Fig. 1) were included in the optimization run and the kinetic settings adjusted to reach an AIC as low as possible. Those parameters approaching zero were considered to not significantly improve the model fit and were excluded in the following step. In general, N transformations (e.g. M_{Nrec} and O_{Nrec}) originating from large pool sizes are appropriately described by zero-order kinetics, while N transformations (M_{Nlab} , I_{NH4_Nrec} , I_{NH4_Nlab} , I_{NO3_Nrec} , and D_{NO3}) originating from small pool sizes are likely to follow first-order kinetics (Myrold and Tiedje, 1986). Michaelis-Menten kinetics, rather than zero- or first-order kinetics, could be more appropriate to describe NH_4^+ oxidation if the activity of nitrifying microbes undergoes a rapid change from non- NH_4^+ limiting conditions (zero-order kinetics) to NH_4^+ limiting conditions (first-order kinetics) (Müller et al., 2007). A detailed description of stepwise modification in parameters and their kinetic settings to find the lowest AIC can be found in previous studies (Müller et al., 2007; Rütting and Müller, 2007; Inselsbacher et al., 2013). The model parameters were optimized with the Markov Chain Monte Carlo Metropolis algorithm (MCMC-MA), which has been described in detail by Müller et al. (2007). To get a better resolution of soil processes, and in line with previous studies, soil organic N pool was conceptually divided into two fractions, a labile and a recalcitrant pool (Müller et al., 2007; Inselsbacher et al., 2013; Zhang et al., 2013). The initial (i.e. $t = 0$) pool sizes of mineral N (^{14}N and ^{15}N) were estimated based on Müller et al. (2004). In brief, the initial concentrations of NH_4^+ and NO_3^- were obtained by extrapolating the data at $t = 0.5$ h and 24 h (48 h for the incubation experiment) back to $t = 0$ h. The initial values of the NH_{4ads} were measured as the difference between applied NH_4^+ and the initial concentrations of NH_4^+ . The optimization procedure samples the probability density function (PDF) for each process, from which the average and standard deviation of each process is calculated. For N transformations following first-order kinetics, average gross rates were calculated by integrating the gross rates over the entire experimental period, divided by the incubation time (Müller et al., 2007; Inselsbacher et al., 2013). The gross N transformation rates were expressed in units of $mg\ N\ kg^{-1}\ dry\ soil\ d^{-1}$. The MCMC-MA routine is programmed in the software Matlab (Version 7.2, The Math Works Inc.), which calls models that are separately set up in Simulink (Version 6.4, The Math Works Inc.).

2.5. Data analysis

All the data were tested for normality (Shapiro–Wilk test) and homogeneity of variance before analyses (Levene-test). If preconditions of ANOVA were not met, we employed the Kruskal–Wallis H test with paired comparisons for testing differences among treatments. Data showing normal distribution and homogeneity were tested by the one-way analysis of variance (ANOVA) with Least Significant Difference (LSD) test to compare the differences between treatments. N dose was set as main effects. To explore the potential relationships between gross N transformation rates and soil characteristics, and among N transformation rates, linear regression and Spearman's rank correlation were performed for parameters that showed normal and non-normal distributions, respectively, with experimental plots (9 plots) as the experimental unit. We tested for significant differences at $\alpha = 0.10$. All analyses were conducted using SPSS version 20.0 (IBM Co., Armonk, NY, USA).

3. Results

3.1. Dynamics of N pool sizes and ^{15}N enrichment

The stimulated and observed concentrations and isotopic enrichment matched well, and were generally within the range of the observed mean $\pm$ sd (Fig. 2 and Figs. S2–10). NH_4^+ concentrations increased with incubation time in all the soils, with a much lower increase rate in the high N-addition plots (Figs. S2–10; a). Compared to the control plots, NO_3^- concentrations increased at a slightly higher rate in plots receiving N addition (Figs. S2–10; b). The dilution of ^{15}N in the NH_4^+ or NO_3^- pools when the N pool was labeled indicated an inflow of unlabeled NH_4^+ or NO_3^- into the labeled N pool (Figs. S2–10). The very small change in ^{15}N of the NH_4^+ pool under $NH_4^{15}NO_3$ labeling suggested that DNRA rates were negligible (Figs. S2–10; c). The slow increase in ^{15}N of the NO_3^- pool under $^{15}NH_4NO_3$ labeling indicated low autotrophic nitrification rates (Figs. S2–10; d).

3.2. Mineralization-immobilization turnover

Gross N mineralization rates (M_{tot}) in the high N-addition plots were 28.6% lower ($p = 0.055$), while showed no significant changes in the low N-addition plots ($p = 0.938$) compared to the control plots ($3.72 \pm 0.14\ mg\ N\ kg^{-1}\ soil\ d^{-1}$) (Fig. 3). NH_4^+ was produced in roughly equal proportion from mineralization of recalcitrant (M_{Nrec} : 43–52%) and labile (M_{Nlab} : 48–57%) organic N (Fig. 4). Mineralization of labile organic N was not significantly different across treatments. However, mineralization of recalcitrant organic N (M_{Nrec}) was 42.8% lower in the high N-addition plots than in the control plots ($p = 0.095$). The main fate for the NH_4^+ produced was NH_4^+ immobilization (I_{NH4}/M_{tot} ; 72.6–73.7%), rather than NH_4^+ oxidation (O_{NH4}/M_{tot} ; 2.7–6.3%) (Fig. 3). Gross NH_4^+ immobilization rates (I_{NH4}) did not differ significantly across treatments. A higher proportion of the NH_4^+ produced was immobilized into labile (I_{NH4_Nlab}/M_{tot} ; 40.3–51.8%) than recalcitrant (I_{NH4_Nrec}/M_{tot} ; 20.4–33.4%) organic N (Fig. 4). I_{NH4_Nrec} rates showed a trend of rise first and then a fall with increasing NH_4^+ additions (Fig. 4), while there were no significant differences in I_{NH4_Nlab} rates across treatments. The respective turnover rates of soil, labile and recalcitrant organic N did not differ significantly among treatments (Fig. 5).

Gross NH_4^+ immobilization rates in the control plots were not within the standard error limits around the mean of the corresponding gross N mineralization rates, and more so in plots receiving low NH_4^+ addition, and this was not the case in plots receiving high NH_4^+ addition (Fig. 3). However, net gross NH_4^+ production (gross N mineralization rates minus gross NH_4^+ immobilization rates) did not vary significantly among treatments (Fig. 3).

3.3. NO_3^- production and retention

Heterotrophic (O_{Nrec} , 79.8–91.6%) dominated over autotrophic (O_{NH4} , 8.4–20.2%) nitrification in NO_3^- production. Autotrophic, heterotrophic, and gross nitrification rates did not differ significantly across treatments (Fig. 3). 25.6–55.0% of the NO_3^- produced (gross nitrification) was immobilized into recalcitrant organic N (I_{NO3}), with 3.7–6.3% being converted into NH_4^+ via DNRA. I_{NO3} , DNRA and gross NO_3^- retention rates ($I_{NO3} + DNRA$) did not vary significantly across treatments (Fig. 3). Gross NO_3^- immobilization rates in the control and NH_4^+ addition plots were not within the standard error limits around the mean of the corresponding gross nitrification rates (Fig. 3). Net gross NO_3^- production rates (gross nitrification rates minus gross NO_3^- immobilization rates) did not show an increasing trend with increasing NH_4^+ additions (Fig. 3).

Fig. 2. Measured (standard deviation; symbols) and modeled (lines) concentrations and ^{15}N abundance of ammonium (NH_4^+) and nitrate (NO_3^-) in plots receiving low NH_4^+ addition (block 1) after application of 40 mg N kg^{-1} soil as NH_4NO_3 at $10 \text{ atom} \% ^{15}\text{N}$ excess.

Fig. 3. Gross rates of N cycling in the 0–10 cm soil in response to increased NH_4^+ additions. Rates are means with standard errors (mg N kg^{-1} dry soil d^{-1} ; $n = 3$ plots) estimated by laboratory incubation. There was no difference detected among treatments for gross NH_4^+ immobilization and autotrophic nitrification, heterotrophic nitrification, gross NO_3^- immobilization, and DNRA. For gross N mineralization, means followed by different letter indicated significance among treatments ($p < 0.10$).

Fig. 4. Mineralization-immobilization turnover in the 0–10 cm soil in response to increased NH_4^+ additions. Rates are means with standard errors (mg N kg⁻¹ dry soil d⁻¹; $n = 3$ plots) estimated by laboratory incubation. There was no difference detected among treatments for mineralization of labile organic N and NH_4^+ immobilization into labile organic N. For mineralization of recalcitrant organic N and NH_4^+ immobilization into recalcitrant organic N, means followed by the different letter (upper case for mineralization of recalcitrant organic N, and lower case for NH_4^+ immobilization into recalcitrant organic N) indicated significance among treatments ($p < 0.10$).

Fig. 5. The respective turnover rates of labile (N_{lab}), recalcitrant (N_{rec}), and soil (N_{org}) organic N under increased NH_4^+ additions. There were no treatments effects on the turnover rates of labile, recalcitrant, and soil organic N ($p < 0.10$).

We did not find a significant correlation of heterotrophic nitrification to soil C: N ratio. However, there were some significant correlations among N-cycling rates. I_{NO_3} rates were significantly and positively correlated to heterotrophic nitrification ($R^2 = 0.59$, $p = 0.015$) and gross nitrification ($R^2 = 0.64$, $p = 0.009$), respectively. DNRA rates were significantly and negatively correlated to gross nitrification rates ($R^2 = 0.59$, $p = 0.016$).

4. Discussion

4.1. The effects of NH_4^+ additions on mineralization-immobilization turnover

Previous studies have indicated that SOM fractions (Swanston et al., 2004; Janssens et al., 2010; Cusack et al., 2011) and their associated mineralization-immobilization turnover (Corre et al., 2010; Zhang et al., 2012a; Koranda et al., 2014) respond differently to N deposition over time. Thus, in this study, we quantified two specific gross N mineralization rates and two specific gross NH_4^+ immobilization rates, related to either a rapid (N_{lab}) or a slower (N_{rec}) turnover of organic N pool (Fig. 1). Mineralization of

recalcitrant and labile organic N played an equal role in NH_4^+ production, and the NH_4^+ produced was preferentially immobilized into labile organic N (Fig. 4). We observed that compared to mineralization-immobilization turnover originating from labile organic N pool, NH_4^+ additions had more of an effect on mineralization-immobilization turnover originating from recalcitrant organic N pool, and that the effect is dose-dependant. However, the respective turnover rates of soil, labile and recalcitrant organic N were not significantly affected by increased NH_4^+ additions (Fig. 5).

Low NH_4^+ additions stimulated NH_4^+ immobilization into recalcitrant organic N ($I_{\text{NH}_4, \text{Nrec}}$) (Fig. 4). Although the increase in $I_{\text{NH}_4, \text{Nrec}}$ rates do not facilitate a rapid recycling of NH_4^+ (Zhang et al., 2012a), mineralization of recalcitrant organic N, gross N mineralization and gross NH_4^+ immobilization did not change at low NH_4^+ additions. The increase of NH_4^+ immobilization into recalcitrant organic N indicates that microbial N demand increases at low NH_4^+ additions (Rütting et al., 2010). At low NH_4^+ additions the increased $I_{\text{NH}_4, \text{Nrec}}$ rates could be also related to reduced (although not statistically significant) immobilization of NH_4^+ into labile organic N ($I_{\text{NH}_4, \text{Nlab}}$) (Fig. 4).

High NH_4^+ additions inhibited mineralization of recalcitrant organic N, resulting in a reduction in gross N mineralization and immobilization of NH_4^+ into recalcitrant organic N (Figs. 3 and 4). At high NH_4^+ additions, the decline in mineralization of recalcitrant organic N could be attributed to either reduced fungal biomass, enhanced stabilization of SOM, or both. Whereas a quick recycling of labile organic N supplies adequate amounts of NH_4^+ , mineralization of recalcitrant requires a depolymerization step which is often carried out by fungal extracellular enzyme (e.g. fungal phenol oxidases and peroxidases of white-rot basidiomycetes) (Carreiro et al., 2000; Frey et al., 2004; Schimel and Bennett, 2004). Our previous field investigations have shown that fungal biomass and fungi to bacteria ratios (Wang et al., 2015) and decomposition of lower-order roots and needles (Kou et al., 2015a, 2015b) exhibited a decreasing trend at high NH_4^+ additions. Moreover, long-term elevated N input has been found to enhance chemical stabilization of organic matter into recalcitrant compounds that are resistant to microbial decay (Neff et al., 2002; Swanston et al., 2004), thereby potentially impairing fungal metabolism (Maaroufi et al., 2015).

With increasing additions of NH_4^+ , microbial NH_4^+ cycling shifted

from a state of decoupling (gross NH_4^+ immobilization rates were incomparable to gross N mineralization rates) to coupling (gross NH_4^+ immobilization rates were comparable to gross N mineralization rates). However, net gross NH_4^+ production rates (gross N mineralization rates minus gross NH_4^+ immobilization rates) were similar across treatments. This shows that net gross NH_4^+ production is not a good indicator of soil N status.

4.2. The effects of NH_4^+ additions on NO_3^- production and retention

Nitrification was almost entirely heterotrophic nitrification, and NO_3^- was almost retained via immobilization into recalcitrant organic N at our site (Fig. 3). The dominance of heterotrophic over autotrophic nitrification in NO_3^- production, and NO_3^- immobilization over DNRA in N retention have been observed in a range of tropical/subtropical forest acidic soils (pH < 5.0) with a high soil C: N ratio (>15) and high fungal biomass (Huygens et al., 2008; Zhang et al., 2013; Zhu et al., 2013). However, we did not observe a significantly positive correlation of heterotrophic nitrification to soil C: N ratio as reported by Zhu et al. (2013). The significantly positive relationship between NO_3^- immobilization and heterotrophic nitrification ($R^2 = 0.59$, $p = 0.015$) might indicate that microbial NO_3^- immobilization depends directly on heterotrophic nitrification for substrate generation. DNRA rates decreased with increasing gross nitrification rates, suggesting that the importance of DNRA in N retention might decrease with increasing NO_3^- availability. This observation does not support the view of Huygens et al. (2008) that DNRA depends directly on heterotrophic nitrification for substrate generation in forest soils.

In line with our hypothesis, NH_4^+ additions did not stimulate soil NO_3^- production (autotrophic, heterotrophic and gross nitrification) and weaken soil retention of NO_3^- (NO_3^- immobilization and DNRA) in the short term (Fig. 3). Gross NO_3^- immobilization rates were incomparable to gross NO_3^- production rates in the control and NH_4^+ addition plots, suggesting that microbial NO_3^- cycling was uncoupled in the soil. Net gross NO_3^- production did not increase with increasing NH_4^+ additions, indicating that increased NH_4^+ additions did not drive microbial NO_3^- cycling to be more open. Our results are thus contrast to the findings of increased gross nitrification and decreased NO_3^- immobilization generally observed in tropical forests after N additions (Hall and Matson, 1999; Silver et al., 2005; Corre et al., 2010; Baldos et al., 2015). Our findings suggest that additional NH_4^+ deposition to this subtropical acidic forest will not stimulate additional leaching losses of NO_3^- .

The lack of responses from heterotrophic nitrification and NO_3^- immobilization could be explained by the absence of significant changes in soil C: N ratio and soil pH (Table 1). Despite the previously observed fungal decline in the high N addition plots at the same site (Wang et al., 2015), we did not observe a change in heterotrophic nitrification rates in this study. This indicated that fungal biomass may have less of an effect on heterotrophic nitrification rates compared to other ecological factors such as soil C: N ratio and soil pH (De Boer and Kowalchuk, 2001; Zhu et al., 2013; Zhang et al., 2015). Thus, it remains to be seen whether heterotrophic nitrification and NO_3^- immobilization will change with decreasing soil C: N ratio under long-term NH_4^+ additions.

The small change in ^{15}N of the NO_3^- pool after $^{15}\text{NH}_4\text{NO}_3$ additions indicated that autotrophic nitrification rates in soils were low, and were not controlled by NH_4^+ availability (Figs. S2–10; d). The low autotrophic nitrification rates in this acidic soil could be due to low ammonia (NH_3) availability (Zhang et al., 2012b; Levy-Booth et al., 2014) and high NH_4^+ immobilization (Vitousek and Reiners, 1982; Huygens et al., 2008). The low autotrophic nitrification potential ensures N retention, and reduces the risk of NO_3^- loss under enhanced NH_4^+ deposition. Due to the low autotrophic nitrification

capacity, enhanced NH_4^+ deposition may have a low potential to affect autotrophic nitrification rates via acidifying soil (De Boer and Kowalchuk, 2001).

DNRA retained N, but was not an important N conservation mechanism as suggested in other studies on natural temperate forests (Rütting et al., 2008) or croplands (Chen et al., 2015). DNRA rates were similar across treatments, suggesting that enhanced NH_4^+ deposition would not affect the importance of DNRA in N retention at our site. Silver et al. (2005) even found that deforestation disturbance coupled with fertilization had a minor impact on DNRA in soils. Studies by Zhang et al. (2013) showed that DNRA rates were strikingly similar in mineral soils of 25 forests located in southern (17) and northern (8) China, irrespective of the differences in climate zones, vegetation types (coniferous and broadleaf vegetation), soil properties (e.g. soil pH, N status, C pool, C: NO_3^- ratio) and N transformations. These observations might point to the fact that DNRA is relatively resistant to disturbances or environmental changes, such as N deposition (Bengtsson and Bergwall, 2000; Silver et al., 2005).

5. Conclusions

Our results show that NH_4^+ deposited to this subtropical forest will not affect gross NH_4^+ immobilization rates, even though gross N mineralization rates decline. Gross N mineralization rates declined in the high NH_4^+ addition plots that exhibited reductions in fungal biomass and mineralization of recalcitrant organic N. Our work highlights that at our site, mineralization-immobilization turnover originating from recalcitrant, not labile, organic N pool was less resistant to disturbance by increasing NH_4^+ additions. In line with our hypothesis, elevated NH_4^+ input did not stimulate soil NO_3^- production (heterotrophic, autotrophic and gross nitrification) and weaken soil retention of NO_3^- (NO_3^- immobilization and DNRA). Findings from this and other research indicate a general resistance of NO_3^- production and retention to disturbance by enhanced NH_4^+ deposition in subtropical/tropical acidic forest soils (pH < 5) with a high soil C: N ratio (>15). Our work provides evidence of the dominance of heterotrophic over autotrophic nitrification in NO_3^- production, and NO_3^- immobilization over DNRA in NO_3^- retention in these soils. In these soils, NO_3^- immobilization and heterotrophic nitrification might be functionally linked, and autotrophic nitrification might not be controlled by NH_4^+ availability. Thus, changes in heterotrophic nitrification rates under enhanced NH_4^+ deposition could be the major determinant of the direction and magnitude of the change in NO_3^- production and retention in subtropical/tropical acidic forest soils.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2016.06.002>.

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