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

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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
nitrarte 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 would not stimulate soil NO$_3^-$ immobilization potential of 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.

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KCl at a soil/extractant ratio of 1: 5 after shaking for 60 min at 250 rpm and 25 °C, and the concentrations of NH₄⁺ and NO₃⁻ were assayed with a continuous-flow analyzer (Skalar, Breda, the Netherlands).

2.3. ¹⁵N tracing experiment

There were two ¹⁵N treatments (each with three replications), of which either ammonium (¹⁵NH₄NO₃) or nitrate (¹⁵NO₃⁻) were labeled with ¹⁵N 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 ¹⁵NH₄NO₃ labeling, and the remaining three for ¹⁵NO₃⁻ 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 ¹⁵NH₄NO₃ or ¹⁵NO₃⁻ solution was added to each conical flask at a rate of 2.86 μmol N g⁻¹ dry soil (20 μg NH₄⁻N g⁻¹ dry soil and 20 μg NO₃⁻ 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 NH₄⁺ and NO₃⁻. A detailed description of laboratory ¹⁵N 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 NH₄NO₃. N was added to the plantation floor in the form of NH₄⁺ only. However, we used either ¹⁵NH₄NO₃ or ¹⁵NO₃⁻ as N tracer, and thus there may be an additive effect of NH₄⁺ and NO₃⁻ on microbial cycling of N in the soil. Actually, we addressed this concern by taking the 1: 1 ratio of NH₄⁺ to NO₃⁻ 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 NH₄⁺ and NO₃⁻ were determined using methods described above. The isotopic compositions of NH₄⁺ and NO₃⁻ were determined using an automated C–N analyzer coupled to an isotope ratio mass spectrometer (Europa Scientific Integra, UK). For isotopic analysis, NH₄⁺ and NO₃⁻ were separated by distillation with MgO and Devarda's alloy (Zhang et al., 2009, 2012b, 2013).

2.4. ¹⁵N tracing model

The ten simultaneously-occurring gross N transformations in the soil were quantified with a process-based ¹⁵N tracing model (Fig. 1) (Müller et al., 2007): M_Nlab = mineralization of recalcitrant organic N to NH₄⁺; M_Nrec, mineralization of labile organic N to NH₄⁺; M_NH₄, immobilization of NH₄⁺ to recalcitrant organic-N; M_NO3, immobilization of NH₄⁺ to labile organic-N; R_NH₄/NO3, release of adsorbed NH₄⁺; A_NH₄, adsorption of NH₄⁺ on cation exchange sites; O_NH₄, oxidation of NH₄⁺ to NO₃⁻ (heterotrophic nitrification); O_NO3, oxidation of recalcitrant organic N to NO₃⁻ (heterotrophic nitrification); D_NO3, dissimilatory NO₃⁻ reduction to NH₄⁺. The transformation rates were calculated either by zero (M_Nrec and O_NO3) or first-order (M_Nlab, M_NH₄/NO3, M_NO3, Nlab, Nrec, and D_NO3) 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_Nlab = M_Nlab + M_Nrec), gross nitrification/ gross NO₃⁻ production (O_NH₄ + O_NO3), gross NH₄⁺ immobilization (I_NH₄ = I_NH₄/NO3 + I_NH₄/Nlab), gross NO₃⁻ immobilization (I_NO3 = I_NO3/NO3 + I_NO3/Nrec), and gross NO₃⁻ retention (I_NO3 + D_NO3) were calculated. The respective turnover rates of soil, labile and recalcitrant organic N was estimated: turnover rate of organic N = total N (M_Nlab + O_NO3), turnover rate of labile organic N = labile organic N / M_Nlab, and turnover rate of recalcitrant organic N = recalcitrant organic N / (M_Nrec + O_NO3). In this study, labile and recalcitrant organic N were considered to equal 1% and 99% of soil organic N (= 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 (μmol N g⁻¹ dry soil) and ¹⁵N excess (at. % excess ¹⁵N) of NH₄⁺ and NO₃⁻ (mean ± 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 NH₄⁺ and NO₃⁻ and their respective ¹⁵N enrichments. To obtain the most suitable model that was able to simulate

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**Table 1**  Soil properties at 0–10 cm depth after 2.5 years of NH₄ addition.

| NH₄ addition | Soil pH 1:2.5 (H₂O) | Soil NH₄ mg N kg⁻¹ soil | Soil NO₃ 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 ± 0.2           | 11.7 ± 2.1              | 8.3 ± 1.0              | 14.5 ± 0.5           | 0.9 ± 0.1           | 163 ± 1.9        |
| Low N        | 4.6 ± 0.0           | 10.3 ± 1.4              | 9.7 ± 1.4              | 14.4 ± 2.7           | 0.9 ± 0.2           | 165 ± 0.5        |
| High N       | 4.7 ± 0.3           | 10.9 ± 1.7              | 10.5 ± 1.6             | 12.2 ± 2.7           | 0.8 ± 0.2           | 155 ± 1.0        |

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

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**Fig. 1.** Conceptual ¹⁵N tracing model (Müller et al., 2007). N_Nlab, recalcitrant organic N; N_Nrec, labile organic N; NH₄, ammonium; NO₃, nitrate, M_Nlab, mineralization of recalcitrant organic N to NH₄⁺; M_Nrec, mineralization of labile organic N to NH₄⁺; M_NH₄, immobilization of NH₄⁺ to recalcitrant organic-N; I_NH₄/NO3, immobilization of NH₄⁺ to labile organic-N; R_NH₄/NO3, release of adsorbed NH₄⁺; A_NH₄, adsorption of NH₄⁺ on cation exchange sites; O_NH₄, oxidation of NH₄⁺ to NO₃⁻ (heterotrophic nitrification); O_NO3, oxidation of recalcitrant organic N to NO₃⁻ (heterotrophic nitrification); D_NO3, dissimilatory NO₃⁻ reduction to NH₄⁺.
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. \( M_{\text{rec}} \) and \( Q_{\text{rec}} \)) originating from large pool sizes are appropriately described by zero-order kinetics, while N transformations (\( M_{\text{Nlab}} \), \( I_{\text{NH4,Nrec}} \), \( INH4,Nlab \), \( NO3-Nrec \), and \( D_{N3} \)) 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 (\( ^{14}\text{N} \) and \( ^{15}\text{N} \)) 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\(_4\)ads 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 \( ^{15}\text{N} \) 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 \( ^{15}\text{N} \) of the NH\(_4\) pool under NH\(_4\)NO\(_3\) labeling suggested that DNRA rates were negligible (Figs. S2–10; c). The slow increase in \( ^{15}\text{N} \) of the NO\(_3\) pool under \( ^{15}\text{NH4} \)NO\(_3\) labeling indicated low autotrophic nitrification rates (Figs. S2–10; d).

3.2. Mineralization-immobilization turnover

Gross N mineralization rates (\( M_{\text{Nrec}} \)) 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 (\( M_{\text{Nrec}}: 43–52% \)) and labile (\( M_{\text{Nlab}}: 48–57% \)) organic N (Fig. 4). Mineralization of labile organic N was not significantly different across treatments. However, mineralization of recalcitrant organic N (\( M_{\text{Nrec}} \)) 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 (\( I_{\text{NH4,Nlab}}: 72.6–73.7% \)), rather than NH\(_4\) oxidation (\( Q_{\text{NH4,Nlab}}: 2.7–6.3% \)) (Fig. 3). Gross NH\(_4\) immobilization rates (\( I_{\text{NH4}} \)) did not differ significantly across treatments. A higher proportion of the NH\(_4\) produced was immobilized into labile (\( I_{\text{NH4,Nlab}}/M_{\text{lab}}: 40.3–51.8% \)) than recalcitrant (\( I_{\text{NH4,Nrec}}/M_{\text{lab}}: 20.4–33.4% \)) organic N (Fig. 4). \( I_{\text{NH4,Nrec}} \) 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 \( I_{\text{NH4,Nlab}} \) 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 (\( O_{\text{Nrec}} \)) 79.8–91.6% dominated over autotrophic (\( O_{\text{NH4}} \)) 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 (\( I_{\text{NO3}} \)), with 3.7–6.3% being converted into NH\(_4\) via DNRA. \( I_{\text{NO3}} \) DNRA and gross NO\(_3\) retention rates (\( I_{\text{NO3}} + \text{DNRA} \)) 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 ($\text{NH}_4^+$) and nitrate ($\text{NO}_3^-$) in plots receiving low $\text{NH}_4^+$ addition (block 1) after application of 40 mg N kg$^{-1}$ soil as NH$_4$NO$_3$ at 10 atom% $^{15}$N excess.

Fig. 3. Gross rates of N cycling in the 0–10 cm soil in response to increased $\text{NH}_4^+$ 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 $\text{NH}_4^+$ immobilization and autotrophic nitrification, heterotrophic nitrification, gross $\text{NO}_3^-$ immobilization, and DNRA. For gross N mineralization, means followed by different letter indicated significance among treatments ($p<0.10$).
plots) estimated by laboratory incubation. There was no difference detected among treatments for mineralization of labile organic N and NH\textsubscript{4}+

For mineralization of recalcitrant organic N and NH\textsubscript{4}+ immobilization into labile organic N. For mineralization of recalcitrant organic N and NH\textsubscript{4}+ immobilization into labile organic N, means followed by the different letter (upper case for mineralization of recalcitrant organic N, and lower case for NH\textsubscript{4}+ immobilization into recalcitrant organic N) indicated significance among treatments (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. \textit{NNO} 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\textsubscript{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\textsubscript{4}+ immobilization rates, related to either a rapid (\textit{Nlab}) or a slower (\textit{Nrec}) turnover of organic N pool (Fig. 1). Mineralization of recalcitrant and labile organic N played an equal role in NH\textsubscript{4}+ production, and the NH\textsubscript{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\textsubscript{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\textsubscript{4}+ additions.

Low NH\textsubscript{4}+ additions stimulated NH\textsubscript{4}+ immobilization into recalcitrant organic N ($\text{INH4}_{\text{Nrec}}$) (Fig. 4). Although the increase in $\text{INH4}_{\text{Nrec}}$ rates do not facilitate a rapid recycling of NH\textsubscript{4}+ (Zhang et al., 2012a), mineralization of recalcitrant organic N, gross N mineralization and gross NH\textsubscript{4}+ immobilization did not change at low NH\textsubscript{4}+ additions. The increase of NH\textsubscript{4}+ immobilization into recalcitrant organic N indicates that microbial N demand increases at low NH\textsubscript{4}+ additions (Rütting et al., 2010). At low NH\textsubscript{4}+ additions the increase in $\text{INH4}_{\text{Nrec}}$ rates could be also related to reduced (although not statistically significantly) immobilization of NH\textsubscript{4}+ into labile organic N ($\text{INH4}_{\text{Nlab}}$) (Fig. 4).

High NH\textsubscript{4}+ additions inhibited mineralization of recalcitrant organic N, resulting in a reduction in gross N mineralization and immobilization of NH\textsubscript{4}+ into recalcitrant organic N (Figs. 3 and 4). At high NH\textsubscript{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\textsubscript{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\textsubscript{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 (Maarouff et al., 2015).

With increasing additions of NH\textsubscript{4}+, microbial NH\textsubscript{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 increasing 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 tropical 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 $^{15}$NH$_4$NO$_3$ additions indicated that autotrophic nitrification rates in soils were low, and were not controlled by NH$_4^+$ availability (Figs. S2–S10; 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.

References

Arnold, J., Corre, M.D., Veldkamp, E. 2008. Cold storage and laboratory incubation of intact soil cores do not reflect in-situ nitrogen cycling rates of tropical forest soils. Soil Biol. Biochem. 40, 2480–2483.

Baldos, A.P., Corre, M.D., Veldkamp, E., 2015. Response of N cycling to nutrient inputs in forest soils across a 1000–3000 m elevation gradient in the Ecuadorian
Abundance. Soil Biol. Biochem. 43, 715–726.
Müller, C., Stevens, R.J., Laughlin, R.J., 2004. A 15N tracing model to analyse N
transformation in old-growth forest soils. Soil Biol. Biochem. 36, 2448–2458.

Moumou, N.L., Nordin, A., Hasselquist, N.J., Bach, L.H., Palmqvist, K., Gundale, M.J.,
2015. Anthropogenic nitrogen deposition enhances carbon sequestration in boreal soils.
Glob. Change Biol. 21, 3169–3180.

Murphy, J., Zhang, W., Zhu, W., Beese, F.O., Corre, M.D., Piñol, J., Yang, Y., Li, D., Wang, H.,
2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China.
Glob. Change Biol. 14, 403–412.

Myrold, D.D., Tiedje, J.M., 1986. Simultaneous estimation of several nitrogen-cycle
processes using 15N - non-techniques. Ecology 67, 1829–1836.

Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S.J., Turnbull, J., Bowman, W.D.,
2002. Variable effects of nitrogen additions on the stability and turnover of soil
carbon. Nature 419, 915–917.

Paterson, E., 2003. Importance of rhizodeposition in the coupling of plant and
microbial productivity. Eur. J. Soil Sci. 54, 741–750.

Piers, S.K., Compton, J.E., Hedin, L.O., 2005. Nitrogen retention across a gradient of
15N additions to an unproductive temperate forest soil in Chile. Ecology 86, 98–105.

Rütting, T., Huygens, D., Müller, C., Cleemput, O., Godoy, R., Boeckx, P., 2008.
Functional role of DNRA and nitrification in a pristine south Chilean
Nahuelbuta forest. Biochemistry 90, 241–258.

Rütting, T., Müller, C., 2007. 15N tracing models with a Monte Carlo optimization
procedure provide new insights on gross N transformations in soils. Soil Biol.
Biocem. 39, 2351–2361.

Rütting, T., Clough, T.J., Middel, C., Liefeling, M., Newton, P.C.D., 2010. Ten years of elevated
atmospheric carbon dioxide alters soil nitrogen transformations in a shepards-grazed pasture.
Glob. Change Biol. 24, 2530–2542.

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing
planet. Ecology 85, 2359–2365.

Silver, W.L., Herman, D.J., Firestone, M.K., 2001. Dissimilatory nitrate reduction to
ammonium in upland tropical forest soils. Ecology 82, 2410–2416.

Silver, W.L., Thompson, A.W., Reich, A., Ewel, J.J., Firestone, M.K., 2005. Nitrogen
cycling in tropical plantation forests: potential controls on nitrogen retention.
Ecol. Appl. 15, 1604–1616.

Swanston, C., Homann, P., Caldwell, B., Myrold, D., Gannio, L., Sollins, P., 2004. Long-
term effects of elevated nitrogen on forest soil organic matter stability.
Biology and Ecology 7, 229–252.

Templer, P.H., Mack, M.C., Chapin, F.S., Christenssen, L.M., Compton, J.E., Crook, H.D.,
Currie, W.S., Curtis, C.J., Dail, D.B., D’Antonio, C.M., Emmett, B.A., Epstein, H.E.,
Goodale, C.L., Gunderson, P., Hobbie, S.E., Holland, K., Kiparsky, D., Hufnagel, B.A.,
Lamontagne, S., Nadleroff, K.J., Olsenberg, C.W., Perakis, S.S., Schleppi, P., Schimel, J.,
Schmid, K., Sommerkorn, M., Spoelstra, J., Tietema, A., Wessel, W.W., Zak, D.R., 2012. Sinks
for nitrogen inputs in terrestrial ecosystems: a meta-analysis of N-15 tracer field studies.
Ecology 93, 1816–1829.

Vitousek, P.M., Reiners, W.A., 1982. A comparative analysis of potential nitri-
ation and nitrification in forest ecosystems. Ecol. Monogr. 52, 155–177.

Wang, Y., Cheng, S., Fang, H., Yu, G., Xu, X., Xu, M., Wang, Li, X., Si, G., Ceng, J.,
He, S., 2015. Contrasting effects of ammonium and nitrite inputs on soil CO2 emission in a subtropical coniferous plantation of southern China. Biol. Fertil. Soil. 51, 615–622.

Wang, Y., Wang, Z.-L., Wang, H., Guo, C., Bao, W., 2012. Rainfall pulse primarily
drives litterfall respiration and its contribution to soil respiration in a young exotic pine plantation in subtropical China. Can. J. For. Research-Revue Can. De La Forêt. 42, 67–77.

Wen, X.F., Wang, H.M., Wang, J.L., Yu, G.R., Sun, X.M., 2010. Ecosystem carbon changes in a
tropical evergreen coniferous plantation subjected to seasonal drought, 2003–2007. Biogeosciences 7, 357–369.

Zhang, X., He, N., Fang, H., Jia, B., Zhou, M., Wang, C., Zhang, J., Zhao, G.,
Wen, X.F., Wang, H.M., Wang, J.L., Yu, G.R., Sun, X.M., 2010. Ecosystem carbon changes in a
tropical evergreen coniferous plantation subjected to seasonal drought, 2003–2007. Biogeosciences 7, 357–369.

Zhang, X., Yu, G., He, N., Fang, H., Jia, B., Zhou, M., Wang, C., Zhang, J., Zhao, G.,
Wang, S., Liu, Y., Yan, J., 2014. Nitrogen deposition and its spatial pattern in main
forest ecosystems along north-south transect of eastern China. Chin. Geogr. Sci. 24, 854–870.

Zhan, X., Yu, G., He, N., Fang, H., Jia, B., Zhou, M., Wang, C., Zhang, J., Zhao, G.,
Wang, S., Liu, Y., Yan, J., 2014. Nitrogen deposition and its spatial pattern in main
forest ecosystems along north-south transect of eastern China. Chin. Geogr. Sci. 24, 854–870.

Zhang, X., Yu, G., He, N., Fang, H., Jia, B., Zhou, M., Wang, C., Zhang, J., Zhao, G.,
Wang, S., Liu, Y., Yan, J., 2014. Nitrogen deposition and its spatial pattern in main
forest ecosystems along north-south transect of eastern China. Chin. Geogr. Sci. 24, 854–870.