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ORIGINAL PAPER

Nitrogen additions inhibit nitrification in acidic soils in a subtropical pine plantation: effects of soil pH and compositional shifts in microbial groups

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Abstract Plantation forests play a pivotal role in carbon sequestration in terrestrial ecosystems, but enhanced nitrogen (N) deposition in these forests may affect plantation productivity by altering soil N cycling. Hence, understanding how simulated N deposition affects the rate and direction of soil N transformation is critically important in predicting responses of plantation productivity in the context of N loading. This study reports the effects of N addition rate (0, 40, and 120 kg N ha^{-1} a^{-1}) and form (NH_4Cl vs. $NaNO_3$) on net N mineralization and nitrification estimated by in situ soil core incubation and on-soil microbial biomass determined by the phospholipid fatty acid (PLFA) method in a subtropical pine plantation. N additions had no influences on net N mineralization throughout the year. Net nitrification rate was significantly reduced by additions of both NH_4Cl (71.5) and $NaNO_3$ (47.1%) during the active growing season, with the stronger inhibitory effect at high N rates. Soil pH was markedly decreased by 0.16 units by NH_4Cl additions. N inputs significantly decreased the ratio of fungal-to-bacterial PLFAs on average by 0.28 (49.1%) in November. Under NH_4Cl additions, nitrification was positively related with fungal biomass and soil pH. Under $NaNO_3$ additions, nitrification was positively related with all microbial groups except for bacterial biomass. We conclude that simulated N deposition inhibited net nitrification in the acidic soils of a subtropical plantation forest in China, primarily due to accelerated soil acidification and compositional shifts in microbial functional groups. These findings may facilitate a better mechanistic understanding of soil N cycling in the context of N loading.

Keywords Acidification · Atmospheric nitrogen deposition · Microbial functional group · Nitrification · Soil nitrogen transformation

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Introduction

Plantation forests represent a large proportion of the total forest area worldwide (FAO 2006) and play a pivotal role in carbon sequestration in terrestrial ecosystems (Winjum and Schroederb 1997). Atmospheric nitrogen (N) is increasingly deposited on subtropical highly weathered soils where plantation forests predominate (Hansen et al. 2013). N is quantitatively and functionally the most important nutrient for plant growth and N deficiency frequently limits forest productivity (Reich et al. 1997; Burton

et al. 2007). However, persistently elevated N deposition may affect forest productivity via altering soil N cycling (Vitousek et al. 1997; Matson et al. 1999, 2002). Hence, understanding how simulated N deposition affects soil N transformations is critically important in predicting the potential responses of productivity of subtropical plantation forests in the context of N loading.

Soil N transformations (e.g., mineralization and nitrification) are highly sensitive to N deposition and mediated by both biotic (microorganisms) and abiotic (acidification) factors (Zhu et al. 2013). Hence, N transformation rates are tightly correlated to microbial biomass, enzymatic activities, and group composition (e.g., fungi to bacteria ratios) (Wallenstein et al. 2006). Additionally, microorganisms generally present varying degrees of sensitivities to acidity (e.g., acid-tolerant vs. acid-adaptive). Evidence has shown that bacterial groups were more affected by soil pH than fungal groups (Fierer et al. 2009). Thus, N deposition induced changes in soil pH will inevitably induce shifts in the relative abundance of different microbial groups and therefore in corresponding N transformations, especially nitrification (Yao et al. 2011).

Different forms of inorganic N addition may have contrasting effects on soil N transformations. Addition of N as ammonium (NH_4^+) can produce more protons than nitrate (NO_3^-), regardless of being taken up by plants or being nitrified (Matson et al. 1999). Therefore, microbial groups may respond differentially to inputs of NH_4^+ and NO_3^-, which have varying potentials to incur soil acidification. Furthermore, it has been observed that microorganisms preferentially assimilate NH_4^+ over NO_3^-, due to the lower energy expenditure of NH_4^+ assimilation (Recous et al. 1992). However, studies on acidic soils have shown that microorganisms utilize equal or greater amounts of NO_3^- compared to NH_4^+ (Zhang et al. 2011a, b; Zhu et al. 2013), which may result from selective NO_3^- uptake by fungi (Marzluf 1997). To clarify this paradox, further research is needed to unravel the N form effects on microbial functional groups and associated soil N transformations.

However, inconsistent and contradicting results have been reported regarding N transformations and their responses to N deposition or experimental N addition (Rousk et al. 2010; Yao et al. 2011) and may be ascribed to multiple causes, such as temporal variations in edaphic conditions, differences in rates of the added N, and the N status of forest ecosystems before N addition (Gundersen et al. 1998). Previous studies have demonstrated that net N mineralization is low during the growing season relative to the winter, due to the nutrient immobilization of microorganisms during the summer and nutrient release from dying microorganisms during winter (Hobbie and Chapin 1996). Conversely, net N mineralization is greater during the growing season, which has optimal temperatures and moisture conditions in contrast to the non-growing season (Durán et al. 2013). Net N mineralization also generally increases with an increment of inorganic N that is applied (Heitkamp et al. 2009). Additionally, evidence has shown that N deposition generally stimulates net N mineralization in N-limited forest ecosystems (Vourlitis et al. 2007), but may inhibit it as ecosystems become N-saturated (Gundersen et al. 1998).

Globally, China has the largest area of plantations, with more than half in subtropical regions (Department of Forest Resources Management 2010). Recent ^{15}N-labeling studies have shown that subtropical acidic forest soils in south China are naturally N abundant, due primarily to their microorganism-dominated N-retention mechanism (Zhang et al. 2013). Given the important role of soil microorganisms and enhanced N depositions in subtropical China, different rates and forms of inorganic N fertilizers were applied on acidic soils in 2012 to (1) explore the seasonal pattern of net N mineralization and nitrification, and (2) determine the effects of varying rates and forms of N addition on soil N transformations and the relative effects of associated biotic factors (microbial groups) and abiotic factors (soil pH) in these processes. Specifically, we hypothesized that (1) in the acidic soils of subtropical plantation forests, the active growing season (AGS) would have higher soil N transformation rates than the non-active growing season (NAGS) due to optimal temperature and moisture conditions that occur in the AGS, (2) exogenous N additions would suppress N transformations in acidic soils by accelerating acidification and thus altering microbial functional groups, and (3) ammonium has stronger inhibitory effects on soil N transformations than nitrate, since NH_4^+ is more efficient (higher potential to produce protons) at reducing soil pH.

Materials and methods

Study site and experimental design

The study site is located at the Qianyanzhou (QYZ) Experimental Station of Red Soil and Hilly Land, Chinese Academy of Sciences (CAS), Jiangxi Province, southeastern China (26°44′29.1″N, 115°03′29.2″E, 102 m above sea level). The climate is a subtropical monsoon climate. According to long-term climate records (1989–2008), the annual mean air temperature ranges from 17.4 to 18.9 °C, and the annual precipitation ranges from 945 to 2144 mm, of which approximately 24, 41, 23, and 12% on average falls in spring, summer, autumn, and winter, respectively (Zhang et al. 2011a, b). The lower rainfall and high temperatures in the late summer frequently caused seasonal

droughts at the QYZ site (Wen et al. 2010). Based on the USDA soil taxonomy, the soil that weathers from red sandstone and mud stone is classified as Typic Dystrudepts (Wang et al. 2012). The original vegetation on the gently undulating terrain was evergreen broad-leaved forest, but it was heavily cleared by logging and land conversion to agriculture before the 1980s. The vegetation was restored in approximately 1985 by planting slash pine (*Pinus elliottii*), Masson pine (*Pinus massoniana*) and Chinese fir (*Cunninghamia lanceolata*). The background wet N deposition rate at the site is about 33 kg N ha^{-1} a^{-1} (Zhu et al. 2015; Kou et al. 2017). More information regarding the soil and stand characteristics of the study site can be found in Table 1 and the description of Kou et al. (2015).

The N addition experiment was carried out in a subtropical *P. elliottii* plantation in November 2011. A randomized complete block design with three replicates was employed, and each block was divided into five 20 × 20 m plots. The buffer zone between any two plots was more than 10 m. Topographic position and slope (less than 15°) was considered to ensure uniformity among plots. Within each block, one plot served as the control, receiving ambient N deposition only, and the remaining four plots received ambient N deposition plus randomly assigned chronic atmospheric N deposition (40 vs. 120 kg N ha^{-1} a^{-1} NH_4Cl and 40 vs. 120 kg N ha^{-1} a^{-1} $NaNO_3$, respectively.). Fertilizers were weighed (509.5 g, low NH_4Cl; 1528.5 g, high NH_4Cl; 809.6 g, low $NaNO_3$; 2428.8 g, high $NaNO_3$), fully dissolved in 30 L tap water, and evenly sprayed onto N-addition zone once per month (i.e., 12 equal applications per year), and the control plots were supplied with the equivalent amount of tap water. The understory N addition started on May 1, 2012 and proceeded at 1-month intervals on days without rain. In this study, we divided the year into the active growing season (AGS) from May to October and the non-active growing season (NAGS) from November to April of the following year.

Field sampling and measurements

Net N mineralization and nitrification were measured in situ using an intact soil core incubation technique. Monthly (approximately every 4 weeks) measurements started on April 30, 2012 in the AGS, in March and April in the NAGS, and bimonthly (roughly every 8 weeks) during the rest of the NAGS. Five pairs of 5 cm diameter × 15 cm long polyvinyl chloride tubes were inserted (to 10 cm depth) in the soil at each plot when the incubation began. Five tubes were removed immediately prior to the N additions (before incubation), and the soil in the tube was mixed as one composite sample (approximately 300 g) after hand sorting and removing roots and rocks, and then sent to the laboratory for the extraction of NO_3^- and NH_4^+ to determine the initial inorganic N content. The other five tubes were capped with parafilm, which is permeable to air but not water, and were left in place for incubation (after incubation). Upon completion of in situ incubation, these five tubes at each plot were removed and processed in the same manner as above to determine soil inorganic N content. For each soil composite sample, a subsample (ca. 30 g) was dried in an oven at 105 °C to determine the soil water content, and 50 mL of 2 mol L^{-1} KCl was added to approximately 13–15 g soil to extract mineral soil N (NH_4^+–N and NO_3^-–N).

Monthly net N mineralization rates (mg kg^{-1} d^{-1}) were calculated as (Inorganic-N_A–Inorganic-N_B)/incubation days, and net nitrification rates were calculated as (Nitrate-N_A–Nitrate-N_B)/incubation days, where Inorganic-N_B and inorganic-N_A, represents inorganic N contents before and after incubation, and Nitrate-N_B and Nitrate-N_A represents NO_3^-–N contents before and after incubation. The amounts of ammonium and nitrate were determined with a Flow Auto

Table 1 Soil (0–10 cm) and stand characteristics at the study site at the Qianyanzhou Experimental Station of Red Soil and Hilly Land, Chinese Academy of Sciences, Jiangxi Province, southeastern China

Soil		Stand	
Soil bulk density (g cm^{-3})	1.54 ± 0.12	Forest type	*Pinus elliottii* plantation
pH value (KCl)	3.78 ± 0.01	Stand age (year)	28
NH_4^+–N (mg kg^{-1})	15.9 ± 0.8	Stand density (stems ha^{-1})	833
NO_3^-–N (mg kg^{-1})	2.3 ± 0.3	Mean DBH (cm)	20.9 ± 0.2
Total C (g kg^{-1})	23.5 ± 3.0	Mean canopy height (m)	17.5 ± 0.1
Total N (g kg^{-1})	1.4 ± 0.1	Dominant understory plants	*Woodwardia japonica* (L.f.) Sm
Total P (mg kg^{-1})	200 ± 16		*Loropetalum chinense* (R.Br.) Oliv
C:N ratio	17.1 ± 1.3		*Dicranopteris dichotoma* (Thunb.)
N:P ratio	6.9 ± 0.6		

DBH was measured in December 2011

Analyzer (Bran Luebbe, Germany). Soil pH values were determined in 1 mol L^{-1} KCl extracts (soil to KCl ratio of 1:2.5) with a pH meter (Mettler Toledo, Switzerland). The pH value determined for the KCl extract was lower (by approximately 1.0 unit) than it was with the water extract method in this study. Volumetric soil moisture and temperature at 0–10 cm soil depth were automatically monitored by EM50 (Decagon, USA) at a frequency of 30 min.

The soil microbial community composition was assessed using phospholipid fatty acids (PLFAs) in June and November 2012. Specifically, lipids were extracted from 8 g soil (sampled from N mineralization composite soils before incubation) using the procedure described by Bååth and Anderson (2003). The abundance of individual fatty acid methyl-esters was expressed as a mole percentage. Nomenclature of fatty acids was made according to Frostegård et al. (1993). PLFAs 16:1ω7c, cy17:0, cy19:0, i15:0, a15:0, i16:0 and i17:0 PLFA are representative of bacteria (Frostegård and Bååth 1996), 18:1ω9 and 18:2ω6 are specific to fungi (Joergensen and Wichern 2008; Frostegård et al. 2011). PLFA 10Me16:0 and 10Me18:0 were chosen to represent actinobacteria (Hossain et al. 2010).

Statistical analyses

Repeated-measures of analysis of variance (RMANOVA) were used to examine effects of N rate, N form, and their interactions on soil N transformations (net N mineralization and nitrification rates). One-way ANOVA was performed to determine the treatment effects on soil pH and microbial functional groups. Where required, data were log (x)-transformed to meet assumptions of normality and homogeneity of variance. Linear regression analyses were conducted on soil microbial groups and soil pH against soil N transformations. Significant differences between means were compared using Tukey's test. All statistical analyses were conducted using SPSS software version 18.0 (SPSS, Chicago, IL, USA).

Results

Net N mineralization and nitrification rates

Monthly N transformation rates presented significant seasonal variations ($P < 0.001$, Table 2), ranging from − 0.16 to 0.52 mg kg^{-1} d^{-1} for net N mineralization rate (Fig. 1a) and − 0.03 to 0.18 mg kg^{-1} d^{-1} for nitrification rate across all sampling months in the control plots (Fig. 1b). Net N mineralization and nitrification rates peaked in June and September and were minimal in December (Fig. 1). Over the sampling period, both net N mineralization and nitrification rates were higher in the AGS than in the NAGS (Fig. 1). Mean net N mineralization rates in the control plots were 0.38 mg kg^{-1} d^{-1} during the AGS and − 0.02 mg kg^{-1} d^{-1} during the NAGS (Fig. 1a). Mean net nitrification rates in the control plots were 0.12 mg kg^{-1} d^{-1} during the AGS and almost negligible during the NAGS (Fig. 1b).

Exogenous N input significantly inhibited nitrification, and the inhibitory effect was more dependent on rate ($P = 0.032$, Table 2; Fig. 1b) than form (marginally significant, $P = 0.054$, Table 2; Fig. 1b) of N. Specifically, net nitrification rate significantly decreased with addition of N as NH_4Cl by 71.5% and $NaNO_3$ by 47.1% during the AGS. The inhibitory effect of N input on nitrification was stronger under the low rate (73.3%) than high rate of N (45.3%), especially under addition of $NaNO_3$. However, exogenous N additions slightly reduced net N mineralization throughout the year (Fig. 1a).

N transformations and microbial community

Microbial PLFA biomass (in nmol g^{-1} dry mass) and the PLFA ratio of fungi to bacteria differed among sampling times (Fig. 2). Although N addition showed a trend of inhibition for all microbial groups, it was not statistically significant (Fig. 2a, b). Specifically, N input slightly decreased the PLFA biomass of all microbial groups in June and fungal PLFA in November. Although N input slightly decreased the fungal PLFA biomass, the ratio of fungi to bacteria decreased significantly by 0.28 (49.1%) on average compared with the control in November ($P = 0.008$, Fig. 2d).

Varying forms of N addition exerted contrasting influences on the relationships between soil N transformations and microbial groups (Table 3). Specifically, net N mineralization exhibited significant negative relationships with fungal biomass ($P = 0.018$, Table 3), and nitrification presented positive relationships with fungal biomass ($P = 0.038$, Table 3) after ammonium-based N addition. Net N mineralization exhibited significant negative relationships with all microbial groups except for fungal biomass (Table 3), and nitrification had a significant positive relationship with all microbial groups (Table 3) except for bacterial biomass when nitrate-based N was added.

N transformations and soil pH

The response of soil pH to N additions fluctuated little during the entire study. Varying rates and forms of N addition decreased soil pH, and a significant difference was observed after 10 months of N input (Fig. 3). Irrespective of the rate (40 vs. 120 kg N ha^{-1} a^{-1}) applied, ammonium-based N additions caused a significant decline in soil pH by 0.16 units ($P < 0.05$, Fig. 3).

Table 2 Results of repeated measures ANOVA of the effects of month, N rate, N form and their interactions on net N mineralization, nitrification, and content of NH_4^+–N and NO_3^-–N

Source of variation	N mineralization		N nitrification		Soil NH_4^+–N		Soil NO_3^-–N	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
AGS								
Between subjects								
N rate	0.18	0.681	6.67	0.032	1.12	0.320	5.09	0.049
N form	2.79	0.128	4.52	0.054	3.79	0.081	5.26	0.045
N rate × N form	1.43	0.263	4.69	0.059	3.32	0.103	2.50	0.152
Within subjects								
Month	6.00	0.001	27.69	< 0.001	31.35	< 0.001	5.97	< 0.001
Month × N rate	6.00	0.005	5.48	< 0.001	1.06	0.392	0.77	0.583
Month × N form	6.00	0.003	6.72	< 0.001	0.63	0.684	0.16	0.982
Month × N rate × N form	6.00	0.002	3.42	0.013	1.22	0.314	1.46	0.221
NAGS								
Between subjects								
N rate	0.42	0.534	0.23	0.652	4.89	0.053	0.57	0.467
N form	0.70	0.421	0.01	0.906	0.07	0.792	0.10	0.764
N rate × N form	1.29	0.277	1.41	0.261	1.99	0.187	0.56	0.468
Within subjects								
Month	39.13	< 0.001	8.00	0.151	15.60	< 0.001	8.00	0.079
Month × N rate	1.65	0.202	8.00	0.223	5.89	0.003	8.00	0.731
Month × N form	7.71	0.001	8.00	0.184	2.07	0.131	8.00	0.826
Month × N rate × N form	1.10	0.370	8.00	0.457	3.08	0.044	8.00	0.915

AGS active growing season, *NAGS* non-active growing season

The effects of varying forms of N addition on the relationships between soil N transformations and soil pH were divergent (Table 3). Specifically, net N mineralization ($P = 0.041$, Table 3) and nitrification ($P = 0.029$, Table 3) exhibited significant positive relationships with soil pH under ammonium-based N additions. However, no significant relationship between soil N transformations and soil pH were observed with nitrate-based N additions (Table 3).

Discussion

Seasonal variations in net N mineralization and nitrification

Supporting our first hypothesis, net N mineralization and nitrification in both the control and the N addition plots were higher during the AGS than during the NAGS (Fig. 1). This result is consistent with previous studies, suggesting higher mineralization and nitrification rates during the growing season than in the non-growing season (Durán et al. 2013). It has been reported that competition between roots and microorganisms for inorganic N has temporal variations (Xu et al. 2011), which may be associated with soil temperature and moisture. Higher temperatures during the growing season may intensify soil respiration and competition for N between fine roots and microorganisms. Consequently, greater N transformation rates help meet the demand for N by fine roots and/or microorganisms. Conversely, lower N transformation rates were observed in the winter, because both plants and microorganisms are inactive or dormant.

Many studies have shown that N mineralization significantly increases with temperature (Rustad et al. 2001), while warming may stimulate (Verburg et al. 1999) or have no influence on nitrification (Niboyet et al. 2011). We observed that soil temperature rather than moisture had a dominant effect on net N mineralization (Table S1), which confirms that soil temperatures exert greater impacts on soil N transformations than moisture during most of the year. However, in our study, net N mineralization peaked at the beginning of the summer (June), rather than when temperatures were the highest (Fig. 1a), perhaps due to the seasonal drought that occurred later in the summer (Wen et al. 2010), which generally inhibited N mineralization and its sensitivity to warming (Auyeung et al. 2013).

Nitrification rates are positively related with soil moisture, but may progressively decrease as the water content exceeds a specific threshold (Kiese et al. 2008). We noticed that nitrification rates were lowest in June during the AGS, but peaked 3 months later than net N mineralization rates (Fig. 1), probably due to the excessive precipitation for

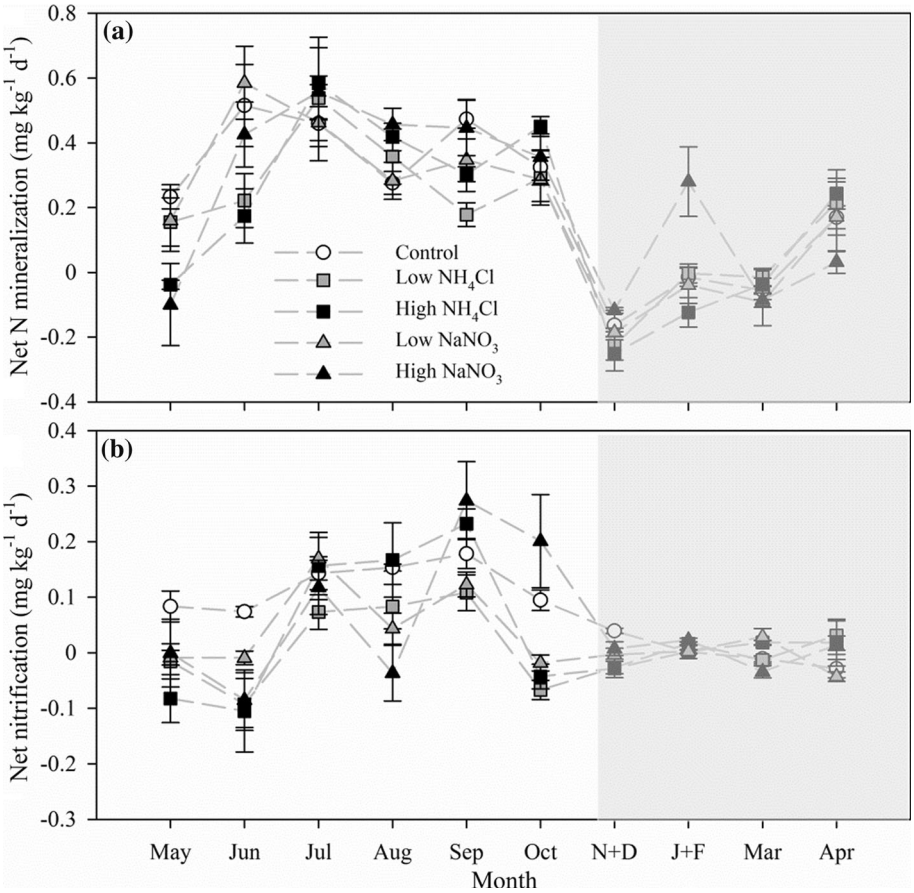

Fig. 1 Temporal variation in net N mineralization (**a**) and nitrification (**b**) rates in control and N-addition (low N: 40 kg N ha^{-1} a^{-1}, high N: 120 kg N ha^{-1} a^{-1}) plots during the study. Months without gray shadows represent AGS (mean monthly soil temperature > 20 °C). Months with gray shadows represent NAGS (mean monthly soil temperature < 20 °C). N + D: November + December, J + F: January + February. Data are mean ± standard error (n = 3)

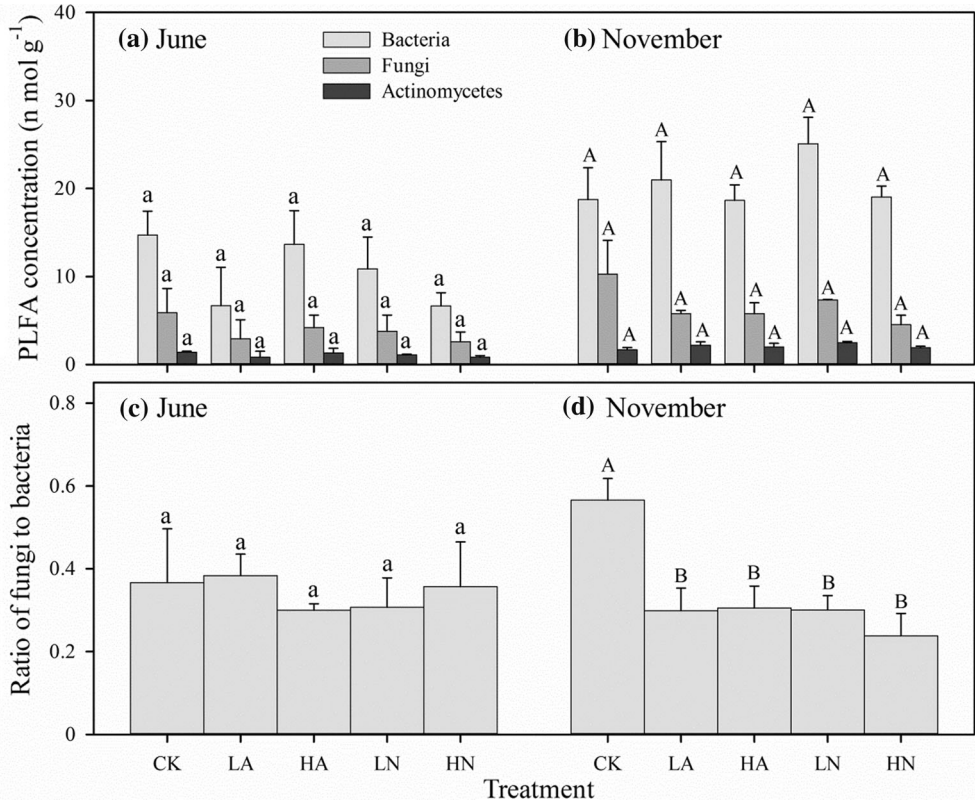

Fig. 2 Phospholipid fatty acid (PLFA) biomass (nmol g^{-1}) of bacteria, fungi, and actinobacteria and ratio of fungi to bacteria in control and N-addition plots in June (**a**, **c**) and November (**b**, **d**). *CK* control, *LA* low NH_4Cl, *HA* high NH_4Cl, *LN* low $NaNO_3$, *HN* high $NaNO_3$, respectively. Data are means + standard error (n = 3). Different letters indicate significant differences ($P < 0.05$) between treatments

Table 3 Relationships between N transformations (net N mineralization and nitrification rates) and microbial biomass (fungal, bacterial, actinobacterial, and total PLFA biomass) as well as between N transformations and soil pH after different forms of N (NH_4^+ vs. NO_3^-) were added

Variable	NH_4^+ addition				NO_3^- addition			
	Net N mineralization		Net nitrification		Net N mineralization		Net nitrification	
	R^2	P	R^2	P	R^2	P	R^2	P
Soil pH	0.527	0.041	0.582	0.029	0.166	0.359	0.331	0.137
Fungal PLFAs	0.442	− 0.018	0.332	0.038	0.317	0.057	0.369	0.038
Bacterial PLFAs	0.187	0.160	0.006	0.812	0.374	− 0.035	0.324	0.054
Actinobacterial PLFAs	0.257	0.092	0.037	0.548	0.570	− 0.005	0.387	0.031
Total PLFAs	0.283	0.075	0.044	0.513	0.418	− 0.023	0.382	0.032

Low (40 kg N ha^{-1} a^{-1}) and high (120 kg N ha^{-1} a^{-1}) rates of N treatments were pooled, and the control was precluded (n = 12)

Negative P values indicate a negative relationship between N transformation and the variable

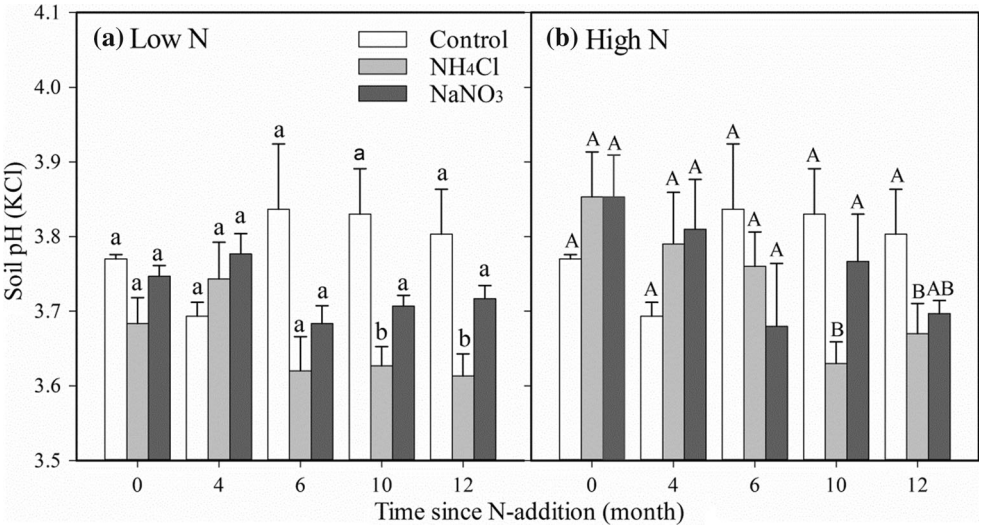

Fig. 3 Soil pH before and after low rates (**a**) and high rates (**b**) of N addition. Data are means + standard error (n = 3). Different letters indicate significant differences ($P < 0.05$) between treatments

nitrifiers in June and relatively favorable hydrothermal conditions in September (Figure S1).

Effects of N addition on net N mineralization and nitrification

N additions slightly reduced N mineralization throughout the year, but significantly inhibited nitrification in the AGS (Table 2; Fig. 1). This partially supports the first part of our second hypothesis that N additions would reduce net N mineralization and strongly supports the second part of the hypothesis that N additions would reduce nitrification. N transformations are mediated and influenced by the biomass, enzymatic activities, and functional group composition of the soil microorganisms (Wallenstein et al. 2006). In our study, N inputs slightly decreased the biomass of all microbial groups in June and of fungal groups in November (Fig. 2a, b). These decreases may, to some extent, be responsible for the slight declines in N mineralization (Fig. 1a).

Recent studies have revealed that humid subtropical acidic soils in China have low autotrophic and relatively high heterotrophic nitrification rates due to low soil pH (Zhang et al. 2011a, b, 2013). Heterotrophic nitrification is primarily carried out by fungi in the acidic forest soils of subtropical China (Zhu et al. 2013). Our study shows that the ratio of fungal to bacterial PLFAs decreased after the N additions (Fig. 2d), and the fungi PLFA had a positive relationship with nitrification (Table 3), which might partially explain the decreased nitrification rates (P = 0.031, Fig. 1b). In addition, accumulated NH_4^+ and NO_3^- in the soil due to the addition of NH_4^+ and NO_3^- (Table S2) may promote the reverse processes of N transformation, thereby suppressing the transformation of organic N to NH_4^+ (ammonification), the transformation of organic N to NO_3^-

(heterotrophic nitrification) and the oxidation of NH_4^+ to NO_3^- (autotrophic nitrification).

Unexpectedly, the inhibitory effect of N addition on net nitrification was much higher under low than high N rates ($P = 0.032$, Table 2; Fig. 1b). Relative to the low N rate, the high N rate is theoretically more capable of increasing the N to C ratio of soil organic matter or organic N (the base of heterotrophic nitrification), which may facilitate heterotrophic nitrification. This facilitation may, to some extent, offset the negative effects of high N rate on biomass and activities of some heterotrophic nitrifiers. Additionally, we found that NO_3^- contributed primarily to the stronger inhibitory effects of low N rate (Fig. 1b). In planted soils, NO_3^- generally has a greater stimulation on root-derived respiration (Gavrichkova and Kuzyakov 2008). A recent study based on our N-manipulative experiment shows that low level of NO_3^- input significantly increased soil CO_2 flux during the AGS, which is possibly due to the increased root autotrophic respiration (Wang et al. 2015). Hence, we speculate that low rates of N supply may stimulate autotrophic respiration and N uptake of roots, while adding N at high rate has a neutral or inhibitory effect on root-associated variables.

Contrasting effects of N form on microbial groups and pH and thus nitrification

Soil pH was more markedly reduced by ammonium-based than nitrate-based N inputs (Fig. 3), concurring with with previous studies that ammonium-based N contributed greatly to soil acidification (Matson et al. 1999). Microorganisms are considered to present differential sensitivities to acidity (Fierer et al. 2009). In our study, microbial groups exhibited close relationships with net nitrification as nitrated-based N was added (Table 3). However, only fungal groups presented a strong relationship with net nitrification ($P = 0.038$, Table 3) when ammonium-based N was applied. Hence, relative to other microbial groups, fungal groups play a dominant role in soil N transformations when ammonium-based N is applied. This finding indicates that soil N transformation is mediated by compositional shifts in microbial groups due to inorganic N additions, and in particular, that this mediation is also strongly N-form dependent.

Reduced soil pH may affect N transformations by altering soil chemical and biological properties (Fu et al. 1987). Accumulating evidence shows that soil pH is positively related to nitrification (Cheng et al. 2011; Persson and Wiren 1995). For instance, Nugroho et al. (2007) found that the low nitrification rates in acidic soils of Scots pine (*Pinus sylvestris* L.) forest are due to low pH. We observed a significantly positive relationship between soil pH and net nitrification rate ($P = 0.029$, Table 3) with ammonium-based rather than nitrate-based N inputs. Consequently, different forms of N inputs may exert contrasting influences on soil pH and therefore on soil N transformations.

Despite varying responses of microbial groups and soil pH to different forms of N, the N form effects on nitrification were only marginally significant ($P = 0.054$, Table 2; Fig. 1b). The reduced pH in our study occurred only 10 months after ammonium-based N was added (Fig. 3). Two factors may be responsible for this observation. First, the N-retention mechanism in the acidic forest soils, which can efficiently immobilize inorganic N ions (NH_4^+ and NO_3^-) into organic N pool, reduces the release of hydrogen ions (H^+) (Zhang et al. 2013). Second, the seasonal drought from early July to late October (Wen et al. 2010) may reduce the risk of leaching loss of NO_3^-. Therefore, the lag effect of soil acidification may contribute to explaining the lack of contrasting effects of the N form (Fig. 1).

Heterotrophic nitrification is the dominant mode of nitrification in humid subtropical acidic soils in China (Zhang et al. 2011a, b, 2013) and primarily carried out by fungi (Zhu et al. 2013). Given the decreased ratio of fungi to bacteria, enhanced acidification, and inhibited nitrification after N addition, our findings imply that enhanced N deposition on the subtropical acidic soils may promote the accumulation of soil organic matter (the basis of heterotrophic nitrification), increase the ratio of NH_4^+ to NO_3^-, and decelerate N cycling. All these effects may therefore influence plant growth and plantation productivity via altering soil N supply and plant uptake. Soil N transformations are extremely complex and are affected by multiple factors. Our results based on a year-long manipulative experiment provide only a "snapshot" of short-term soil N transformations in response to N additions. Further studies are therefore needed to examine the long-term effects of N deposition on soil N cycling.

Conclusions

In our field N-manipulation experiment, net N mineralization and nitrification were higher during the active growing season (AGS) than during the non-active growing season (NAGS). Both ammonium- and nitrate-based N inputs significantly reduced net nitrification rates during the AGS. Ammonium-based N inputs exerted larger effects on microbial functional groups and on soil pH than did nitrate-based N inputs. The inhibitory effects on soil N transformations are better explained by changes in the fungal groups and soil pH when ammonium-based N was applied. However, bacteria and/or actinobacteria groups contributed more than fungal groups to the inhibitory effects when nitrate-based N was applied. In summary,

accelerated soil acidification (abiotic factor) together with compositional shifts in microbial groups (biotic factor) was largely responsible for the inhibited nitrification by N addition. These results may contribute to mechanistically understanding soil N cycling in the context of N loading.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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