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Low Nitrogen Retention in Soil and Litter under Conditions without Plants in a Subtropical Pine Plantation

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Abstract: Soil acts as a major sink for added nitrogen (N) in forests, but it remains unclear about the capacity of soil to immobilize N under conditions without plant roots and whether added N interacts with ecosystem N to affect N retention. We added $^{15}\text{NH}_4^{15}\text{NO}_3$ to *in situ* soil columns (with leaching) and leaf litter (without leaching) of two tree species in a subtropical *Pinus elliottii* plantation. Soil and litter were collected three or eight months after N addition to measure concentrations of indigenous and exogenous N. About 70% of exogenous N was retained in soil three months after N addition, of which 65.9% were in inorganic forms. Eight months after N addition, 16.0% of exogenous N was retained in soil and 9.8%–13.6% was immobilized in litter. N addition increased the mineral release and nitrification of soil indigenous N. Loss of litter indigenous N was also increased by N addition. Our results suggest that N deposition on lands with low root activities or low soil carbon (C)

contents may lead to increased N output due to low N immobilization. Moreover, the effects of added N on ecosystem indigenous N may decrease the capacity of soil and litter in N retention.

Keywords: ^{15}N tracer; added N interaction; N deposition; N addition; priming effect

1. Introduction

Human activities have greatly increased the inputs of atmospheric nitrogen (N) to the Earth's land surface globally through agricultural fertilization and combustion of fossil fuel [1–3]. Rates of N deposition have leveled off or stabilized in the US and Europe since the late 1980s or early 1990s [4], while they have been increasing continuously since the 1980s in China [5]. Because plant growth is limited by N availability in most terrestrial ecosystems [6–8], inputs from atmospheric deposition can increase carbon (C) storage by increasing primary production and C accumulation within plants [9,10]. However, increased N deposition can result in N saturation when it exceeds the biotic demand, leading to increased rates of N cycling and losses of nitrate, soil and water acidification, plant nutrient imbalances and even forest decline [11–13]. Therefore, the fate of deposited N in forest ecosystems and the capacity of ecosystems to store deposited N have been investigated extensively since the 1990s [14–16].

Soil has been suggested to be the major sink of deposited N in forests. A meta-analysis of ^{15}N tracer studies across global forests showed that the mean ^{15}N tracer recovery from soil (organic and mineral layer) was around 80% for short-term (<1 week) and 65% for long-term (3–18 months) periods following N addition [16]. ^{15}N -tracer studies in nine temperate forests receiving $0.4\text{--}5.8\text{ g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$ of N deposition showed that an average of 70% of applied tracers were recovered from soil after 1–3 years of N addition [17]. Microbial N immobilization and abiotic N assimilation in soil organic matter (SOM) have been proposed as the mechanism of N retention in soil [18–20]. Within just hours to days of being introduced to soil in reactive mineral forms, N is incorporated in soil organic molecules [21,22], with a substantial portion of 40% staying stable in SOM for at least one year [23]. However, plant assimilation of deposited N and the subsequent leaf or root litter turnover have also been suggested to be a major pathway of N retention in the forest soil [15,24]. Most previous studies were conducted under conditions with plant uptake and turnover occurring simultaneously with soil microbial N immobilization and abiotic N assimilation. The capacity of soil and litter in retaining deposited N under conditions without plant activities was not usually quantified and remains unclear.

The capacity of soil in N retention may be affected by the interaction of added N and soil indigenous N. Studies generally showed that N addition increases most of the measured fluxes, concentrations or pools of the bulk soil N, which include both indigenous N and added N [25–27]. However, there were cases of net soil N loss (N retention was minus) as evidenced by decreased soil N pool after N addition [27,28]. This suggests that added N might accelerate the cycling of soil indigenous N and lead to net N loss. However, few studies quantified effects of N addition on the behavior and cycling of the indigenous N in forests, although effects of fertilizer N on soil indigenous N have been intensively investigated in agricultural ecosystems [29–31]. Studies conducted in agroecosystems showed that N fertilization

generally releases more soil native N than un-fertilized treatments, and the phenomenon was termed priming effect or added N interaction [29].

To explore the capacity of soil and litter in N retention under conditions without plant activities and effects of added N on the behavior of native N in forests, we conducted an experiment in a slash pine (*Pinus elliottii* Engelm.) plantation. Pine plantations account for a substantial portion of total plantation area in subtropical China and slash pine is an important member of planted pines due to its fast growth characteristic [32]. We hypothesized that (1) exogenous N immobilization would be low in soil and litter, and most of the added N would be in inorganic forms because there is no plant uptake and no root-C-fueled microbial immobilization; (2) the exogenous N content in the soil profile would decrease rapidly due to low immobilization and exposure of inorganic N to leaching and gaseous loss; and (3) N addition would alter behaviors of indigenous N in soil and litter such as mineral release, ammonification and nitrification due to a stimulated effect of added N on native N availability [29]. To test the hypotheses, we added inorganic N at a rate similar to the ambient N deposition rate in a subtropical slash pine plantation to *in situ* soil columns and litter, in which $^{15}\text{NH}_4^{15}\text{NO}_3$ (99 atom %) was applied to partition the behaviors of exogenous N and indigenous N in soil and litter.

2. Materials and Methods

2.1. Site Description

The study was conducted in an evergreen slash pine (*Pinus elliottii*) plantation (26°44' N, 115°03' E, elevation 102 m) at Qianyanzhou Ecological Research Station in subtropical China. This even-aged pine plantation was established in 1985 on vegetation-degraded hill land, which had suffered severe soil erosion. There are also a few native *Liquidambar formosana* trees that have emerged spontaneously since plantation. The soil, weathered from red sandstone and mudstone, is common for subtropical China and classified as a Typic Dystrudepts by the soil taxonomy of United States Department of Agriculture. Soil texture was sandy loam with 68% sand and 15% clay. C and N concentrations in the upper 0–40 cm soil layer are shown in Table 1, and P concentration of the 0–20 cm soil layer was 0.02%. This area was characterized by a humid monsoon climate, with a mean air temperature of 17.9 °C, rainfall amounts of 1542 mm·year⁻¹ [33]. A seasonal drought usually occurs between July and December and about 30% of rainfall occurred in the dry season [34].

Table 1. Initial C and N concentrations in soil and litter before N addition (mean ± standard error (se); $n = 8$ for soil and $n = 4$ for litter).

Soil or Litter	C (%)		N (%)		atom % ^{15}N of Total N		NO_3^- (mg N·kg ⁻¹)		NH_4^- (mg·N·kg ⁻¹)	
	Control N Addition		Control N Addition		Control N Addition		Control N Addition		Control N Addition	
0–5 cm soil	1.83 (0.2)	1.88 (0.2)	0.12 (0.0)	0.12 (0.0)	0.37 (0.0)	0.37 (0.0)	13.7 (2.8)	10.3 (2.1)	16.0 (1.7)	17.3 (4.3)
5–10 cm soil	0.82 (0.1)	0.82 (0.1)	0.07 (0.0)	0.07 (0.0)	0.37(0.0)	0.37 (0.0)	6.61 (0.8)	7.05 (1.1)	9.49 (0.6)	13.6 (3.3)
10–20 cm soil	0.61 (0.1)	0.60 (0.1)	0.06 (0.0)	0.06 (0.0)	0.37 (0.0)	0.37 (0.0)	4.92 (0.5)	4.92 (0.8)	7.82 (0.4)	7.78 (0.6)
20–40 cm soil	0.45 (0.1)	0.44 (0.0)	0.04 (0.0)	0.04 (0.0)	0.37 (0.0)	0.37 (0.0)	2.82 (0.4)	3.50 (0.5)	8.94 (2.7)	7.00 (1.0)
<i>P. elliottii</i> litter	51.2 (0.1)		0.41 (0.0)		0.36 (0.0)		7.9 (2.9)		18.5 (4.1)	
<i>L. formosana</i> litter	44.8 (0.2)		1.13 (0.0)		0.36 (0.0)		11.3 (4.6)		21.2 (4.3)	

2.2. Experimental Design

In late October 2012, eight pairs of intact soil columns (20 cm in diameter and 40 cm in depth) were established by digging away the soil around, and then the columns were fitted into a polyvinyl chloride (PVC) tube (20 cm in diameter and 60 cm in length), leaving 20 cm of the tubes above ground to prevent the lateral flow exchanges between within and outside soil columns. The litter layer (O_i) on the surface of soil columns was removed. Plants emerging in the columns during the experiment were pulled out days after appearance. Each pair consisted of two soil columns with a distance of 20–30 cm between them, and the pairs were about 2 m away from each other. For each paired columns, one was assigned as the control and the other was assigned as N addition treatment. There were eight replicated columns for the control and N addition treatment. We observed from the soil profiles that most roots were distributed in the upper 30 cm, and there were few fine roots below 40 cm in this plantation. Therefore we believe that root uptake of the added N from soil columns was excluded in our study. N addition occurred on two dates with equal amounts, the first on 21 October and the second on 21 November. Each N-addition soil column received $0.22 \text{ g}\cdot\text{N}$ ($7 \text{ g}\cdot\text{N}\cdot\text{m}^{-2}$) in the form of $^{15}\text{NH}_4^{15}\text{NO}_3$ (99 atom %), corresponding to $14 \text{ mg}\cdot\text{kg}^{-1}$ soil and 2.5% of total N in each 40 cm soil column. We added N by evenly dropping 10 ml of N solution on the soil surface with a dropper followed with 10 ml of de-ionized water. The application rate was comparable to the maximal N deposition rate ($6.2 \text{ g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$) in central Southern China (including our study area) in 2003, mostly in the form of wet deposition [35,36]. The control soil columns received the same amount of de-ionized water.

Leaf litter of *P. elliotii* was collected from the plantation and *L. formosana* litter was collected from a *L. formosana* plantation nearby. Leaf litter was collected with litter traps in the September and early October of 2012, air-dried for two weeks before use. Eight litterbags (20 cm \times 20 cm, 2 mm mesh) of *P. elliotii* and *L. formosana* litter, respectively, were constructed, with 18 g (dry-weight) intact litter in each bag. Initial C and N concentrations of *P. elliotii* litter and *L. formosana* litter are shown in Table 1. The quantity of litter in bags ($450 \text{ g}\cdot\text{m}^{-2}$) was slightly lower than the annual leaf litter production of this plantation, which is 550–609 $\text{g}\cdot\text{m}^{-2}$ (unpublished data). Two 1.5 m \times 1.5 m tree-free plots were established in the *P. elliotii* plantation, one as the control and the other the N addition treatment. We realized that one plot for each treatment posed an issue of pseudoreplication. Since no evidence has shown that N retention in litter is related to the soil beneath litter, the issue of pseudoreplication might have a minor effect on our results. Shrubs and grasses inside the plots were pulled out with roots. Edges of the plots were dug down to 40 cm depth and wrapped with a piece of iron sheet to prevent the root growth of plants outside plots. The iron sheets were kept 40 cm in soil depth and 20 cm aboveground to prevent the lateral flow exchanges between within and outside plots. Each plot was placed with four bags of *P. elliotii* and four bags of *L. formosana*, and there were four replicated litterbags for each treatment (control or N addition) of each litter species. N addition to litter occurred on the same dates as those for soil. Each litterbag in the N-treated plot received $0.28 \text{ g}\cdot\text{N}$ ($7 \text{ g}\cdot\text{N}\cdot\text{m}^{-2}$) with the same dropping method as that for N addition to soil. Each litterbag in the control plot received the same amount of de-ionized water. To estimate the mineral release of litter indigenous inorganic N, a shelter was placed 20 cm above each of the plots to prevent leaching. A volume of 75 L water (mimic 60 mm rainfall, the average amount of rainfall within two weeks) was added to each plot biweekly to keep the soil moist. Litterbags were taken out of plots before water addition and were placed back to plots after water

penetrated into the soil completely. We realize that the litter inside litterbags might receive less moisture than the surrounding environment, which might decrease microbial activities and N immobilization. However, litter moisture should be more constant in the litterbags than in the surrounding environment because the shelter (20 cm above litterbags) slowed down water evaporation and sheltered them from direct sunshine. The relatively more constant moisture in litterbags should favor microbial activities. Therefore, microbial activities might not be greatly affected by sheltering litterbags from rainfall and adding water to the soil beneath litterbags.

2.3. Sampling, Measurements and Calculations

Soil samples were taken three months and eight months after N addition (from the first application date) with a corer (3 cm in diameter). Holes after the first sampling were inserted with capped PVC tubes (3 cm in diameter) to prevent further disturbance. Each of the 20-cm-diameter soil columns was sampled at four soil layers: 0–5 cm, 5–10 cm, 10–20 cm and 20–40 cm. The sample of each soil layer was composed of three 3-cm-diameter soil cores. Soil samples of the 0–5 cm layer consisted of both organic (Oe + Oa, 5 millimeters thick) and mineral soil. All eight pairs of soil columns were sampled three months after N addition and only five pairs of soil columns were sampled eight months after N addition due to an accident that destroyed the other three pairs. In addition, two 3-cm-diameter soil cores were taken from each of the 20-cm-diameter soil columns to 40 cm depth before N addition to measure the initial soil C and N concentrations, and holes after sampling were filled with 3-cm-diameter soil cores taken from the outside of 20-cm-diameter columns. The filled holes were avoided from soil sampling by marking the center of each filled hole with a small stick. All litterbags were retrieved eight months after N addition for analysis.

Sub-samples of soil and litter were extracted with 2M (mol L⁻¹) KCl followed by filtration. The filtrate was measured for concentrations of total inorganic N (exogenous plus indigenous) with an auto analyzer (Bran Luebe AA3, Braunschweig, Germany). The ¹⁵N abundance (atom%) of NH₄⁺ and NO₃⁻ was determined by producing N₂O for isotopic analysis with an automated continuous-flow gas-phase isotope-ratio mass spectrometer (IRMS) (Thermo Fisher Finnigan MAT253, Waltham, MA, USA) [37,38]. For measuring ¹⁵N abundance of NH₄⁺, MgO was added to a sub-sample of the filtrate to release NH₃ from NH₄⁺, and the NH₃ was absorbed in diluted H₂SO₄ and transformed into (NH₄)₂SO₄. The (NH₄)₂SO₄ was then oxidized with NaOBr (with the molarity of NaOH adjusted to 10 M) to produce N₂O for isotopic analysis. For measuring ¹⁵N abundance of NO₃⁻, the NO₂⁻ in samples was removed by NH₂SO₃H and then NO₃⁻ in samples was reduced into NO₂⁻ and NH₂OH by Cd–Cu complex. The reaction between NO₂⁻ and NH₂OH produced N₂O for isotopic analysis. Sub-samples of oven-dried soil and litter were ground into powder for measurements of concentrations of total C and N using an elemental analyzer (Elementar Analysensysteme GmbH Vario EL, Hanau, Germany), and ¹⁵N abundance of total N with an isotope-ratio mass spectrometer (Thermo Fisher Finnigan MAT253, Waltham, MA, USA). The amplifiers in the IRMS (Finnigan MAT253) have a linear dynamic range of 50 voltage, and each channel can be equipped with computer switchable amplifier gain, implemented by switchable feedback resistors. When measuring highly enriched samples, the feedback resistor (and thus amplifier gain) of the ¹⁵N-ion collector was lowered by a factor of 10 to make the ion current signal detectable.

Concentrations of indigenous and exogenous N (NH_4^+ , NO_3^- or total N) in soil or litter from the N addition treatment were calculated according to mass balance equations:

$$C_{ind} + C_{exo} = C_{whole} \quad (1)$$

$$C_{ind} \times atom\%^{15}N_{control} + C_{exo} \times atom\%^{15}N_{exo} = C_{whole} \times atom\%^{15}N_{whole} \quad (2)$$

where C_{ind} , C_{exo} and C_{whole} are the concentrations of indigenous N, exogenous N and indigenous plus exogenous N, respectively; $atom\%^{15}N_{control}$ is the percent of ^{15}N in soil N or litter N of the control; $atom\%^{15}N_{exo}$ is the percent of ^{15}N in added N; $atom\%^{15}N_{whole}$ is the percent of ^{15}N in the whole soil N or litter N from the N-added treatment.

For the control, N remaining in litter was calculated using N concentrations and litter dry weights before and after experiment, because no exogenous N was added. For the N addition treatment, N remaining was calculated with two methods, which had different meanings: ^{15}N data were used to calculate the remaining of litter indigenous N according to the above-mentioned equations; N concentrations and litter dry weights before and after experiment were used to calculate the remaining of total initial N (indigenous plus exogenous).

2.4. Statistical Analyses

Differences of soil indigenous and total (indigenous plus exogenous) NO_3^- and NH_4^+ concentrations between the control and N addition treatment and among soil layers were determined by two-way analysis of variance (ANOVA). Differences of soil exogenous NO_3^- and NH_4^+ concentrations among soil layers were determined by one-way ANOVA. The data were tested for normality and homogeneity before ANOVA, and log or square-root transformations were applied when necessary. Differences of litter N remaining between the control and N addition treatment were compared by independent-samples *t*-tests at 0.05 level.

3. Results

3.1. Soil N Retention and Behaviors of Soil Exogenous and Indigenous N

About $4.90 \text{ g}\cdot\text{m}^{-2}$ of exogenous N (70.0% of added N) was retained in the 0–40 cm soil layers three months after N addition (Table 2) under conditions with leaching, and the value decreased to $1.11 \text{ g}\cdot\text{m}^{-2}$ (16.0% of added N) after eight months (Table 2). The 0–5 cm layer (including Oe + Oa layer) received the largest portion (40%–50%) of the retained exogenous N and the 20–40 cm layer received the second largest portion (~30%) (Tables 2 and 3). Among the retained exogenous N in soil three months after N addition, 65.9% was in inorganic forms (Table 2). The retained inorganic exogenous N decreased to 10% (from $3.23 \text{ g}\cdot\text{m}^{-2}$ to $0.30 \text{ g}\cdot\text{m}^{-2}$) while organic exogenous N decreased to 48% (from $1.67 \text{ g}\cdot\text{m}^{-2}$ to $0.80 \text{ g}\cdot\text{m}^{-2}$) over the following five months (Tables 2 and 3).

Table 2. Contents and ^{15}N abundances in soil N pools of the N addition treatment three months after N addition (mean \pm standard error (se); $n = 8$).

Soil Layer	Total N ($\text{g}\cdot\text{m}^{-2}$)	Total NH_4^+ ($\text{g}\cdot\text{m}^{-2}$)	Total NO_3^- ($\text{g}\cdot\text{m}^{-2}$)	Atom% ^{15}N of Total N	Atom% ^{15}N of Total NH_4^+	Atom% ^{15}N of Total NO_3^-	Exogenous N ($\text{g}\cdot\text{m}^{-2}$)	Exogenous NH_4^+ ($\text{g}\cdot\text{m}^{-2}$)	Exogenous NO_3^- ($\text{g}\cdot\text{m}^{-2}$)	Exogenous Organic N ($\text{g}\cdot\text{m}^{-2}$)	Exogenous ($\text{NH}_4^++\text{NO}_3^-$)/Exogenous N (%)
0–5 cm	61.4 (2.9)	2.00 (0.2)	0.22 (0.0)	3.51 (0.3)	60.1 (2.0)	19.2 (2.6)	1.96 (0.2)	1.23 (0.13)	0.04 (0.01)	0.69 (0.1)	64.8 (4.4)
5–10 cm	47.0 (2.2)	1.76 (0.1)	0.26 (0.0)	1.91 (0.2)	27.1 (3.6)	15.7 (5.1)	0.74 (0.2)	0.23 (0.06)	0.05 (0.02)	0.46 (0.1)	37.8 (3.4)
10–20 cm	62.7 (4.5)	1.43 (0.1)	0.67 (0.1)	1.67 (0.2)	11.9 (1.7)	40.9 (3.2)	0.78 (0.1)	0.18 (0.04)	0.28 (0.04)	0.32 (0.0)	59.0 (5.6)
20–40 cm	100.4 (4.0)	1.85 (0.1)	1.80 (0.1)	1.81 (0.1)	4.91 (0.8)	62.0 (2.3)	1.42 (0.1)	0.09 (0.00)	1.13 (0.11)	0.20 (0.0)	85.9 (2.3)
Whole 0–40 cm	271 (11)	7.04 (0.4)	2.95 (0.2)				4.90 (0.3)	1.73 (0.13)	1.5 (0.10)	1.67 (0.2)	65.9 (2.4)

Table 3. Contents and ^{15}N abundances in soil N pools of the N addition treatment eight months after N addition (mean \pm standard error (se); $n = 5$).

Soil Layer	Total N ($\text{g}\cdot\text{m}^{-2}$)	Total NH_4^+ ($\text{g}\cdot\text{m}^{-2}$)	Total NO_3^- ($\text{g}\cdot\text{m}^{-2}$)	Atom% ^{15}N of Total N	Atom% ^{15}N of Total NH_4^+	Atom% ^{15}N of Total NO_3^-	Exogenous N ($\text{g}\cdot\text{m}^{-2}$)	Exogenous NH_4^+ ($\text{g}\cdot\text{m}^{-2}$)	Exogenous NO_3^- ($\text{g}\cdot\text{m}^{-2}$)	Exogenous Organic N ($\text{g}\cdot\text{m}^{-2}$)	Exogenous ($\text{NH}_4^++\text{NO}_3^-$)/Exogenous N (%)
0–5 cm	56.7 (3.8)	0.18 (0.0)	0.012 (0.00)	1.33 (0.0)	1.05 (0.1)	6.47 (1.0)	0.54 (0.0)	0.001 (0.00)	0.008 (0.00)	0.53 (0.0)	1.6 (0.6)
5–10 cm	46.6 (3.6)	0.19 (0.0)	0.019 (0.00)	0.68 (0.0)	1.07 (0.2)	8.24 (1.5)	0.15 (0.0)	0.001 (0.00)	0.017 (0.01)	0.13 (0.0)	11.9 (3.8)
10–20 cm	69.6 (4.1)	0.38 (0.0)	0.045 (0.00)	0.54 (0.0)	0.76 (0.0)	10.6 (2.0)	0.12 (0.0)	0.002 (0.00)	0.049 (0.01)	0.07 (0.0)	41.8 (8.7)
20–40 cm	112.2 (9.8)	1.21 (0.1)	0.123 (0.03)	0.63 (0.1)	1.27 (0.3)	16.8 (3.2)	0.30 (0.1)	0.006 (0.00)	0.216 (0.05)	0.07 (0.0)	73.9 (4.3)
Whole 0–40 cm	285 (17)	1.96 (0.2)	0.199 (0.03)				1.11 (0.2)	0.010 (0.00)	0.288 (0.05)	0.80 (0.0)	26.9 (6.9)

Concentrations of soil inorganic N did not differ between treatments before N addition (Table 1). Three months after N addition, soil total (indigenous plus exogenous) NO_3^- and NH_4^+ were significantly higher in the N addition treatment than in the control ($p < 0.001$; Figure 1a,b). Eight months after N addition, the concentration of soil total NO_3^- was significantly higher in the N addition treatment ($p < 0.01$) but soil total NH_4^+ was significantly lower in the N addition treatment than in the control ($p < 0.001$) (Figure 1c,d).

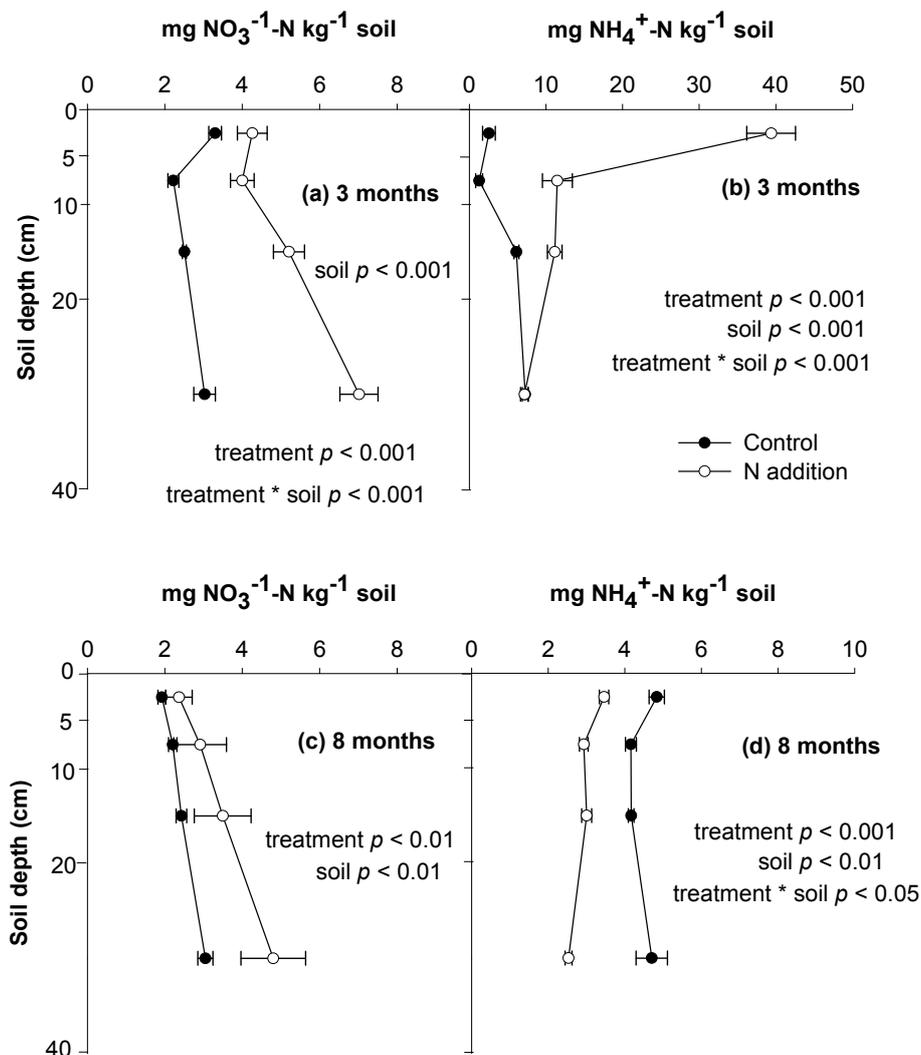


Figure 1. (a) Concentration of soil total (indigenous + exogenous) NO_3^- (mean \pm standard error (se); $n = 8$) three months after N addition. (b) Concentration of soil total (indigenous + exogenous) NH_4^+ (mean \pm se; $n = 8$) three months after N addition. (c) Concentration of soil total (indigenous + exogenous) NO_3^- (mean \pm se; $n = 5$) eight months after N addition; (d) Concentration of soil total (indigenous + exogenous) NH_4^+ (mean \pm se; $n = 5$) eight months after N addition. Data are graphed at the mid-point of our soil sampling depths.

Soil exogenous NH_4^+ and NO_3^- showed different patterns of distribution with soil depth. Three months after N addition, the concentrations of exogenous NO_3^- increased with soil depth, whereas the concentrations of exogenous NH_4^+ decreased with soil depth ($p < 0.01$; Figure 2a). Eight months after N

addition, the concentrations of soil exogenous NO_3^- still showed an increasing pattern with soil depth ($p < 0.01$), whereas concentrations of NH_4^+ was close to zero and did not differ among soil layers (Figure 2b).

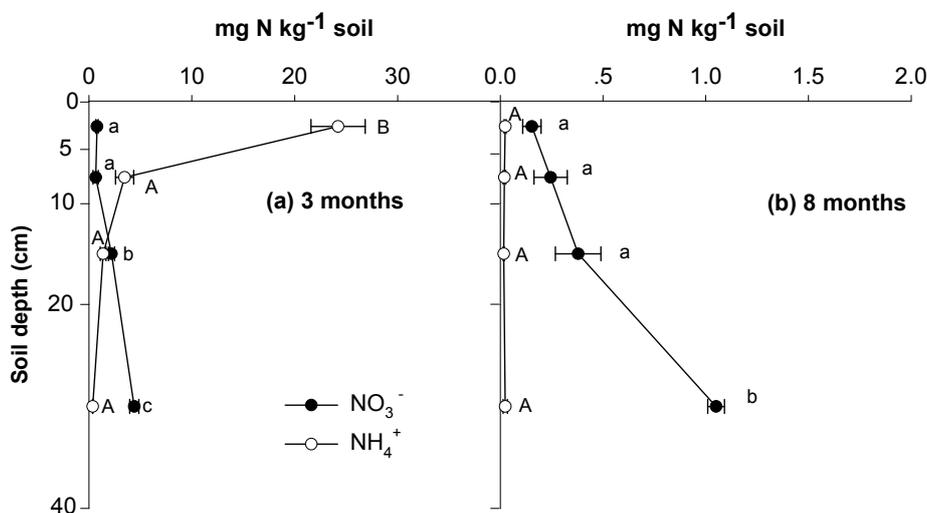


Figure 2. (a) Concentrations of soil exogenous NO_3^- and NH_4^+ (mean \pm standard error (se); $n = 8$) three months after N addition. (b) Concentrations of soil exogenous NO_3^- and NH_4^+ (mean \pm se; $n = 5$) eight months after N addition. Note: Differences of NO_3^- among soil layers are indicated by different lowercase letters. Differences of NH_4^+ among soil layers are indicated by different uppercase letters. Data are graphed at the mid-point of our soil sampling depths.

Concentrations of soil indigenous NH_4^+ and NO_3^- were significantly higher in the N addition treatment than the control three months after N addition ($p < 0.05$ or $p < 0.001$; Figure 3a,b). The difference was especially marked for NH_4^+ , being four times higher in the N addition treatment than the control (Figure 3b). The concentration of soil indigenous $\text{NH}_4^+ + \text{NO}_3^-$ did not differ between treatments eight months after N addition, but NH_4^+ and NO_3^- showed the opposite trends. Soil indigenous NO_3^- tended to be higher in the N addition treatment ($p = 0.055$; Figure 3c), but indigenous NH_4^+ was significantly lower in the N addition treatment than the control ($p < 0.001$; Figure 3d).

3.2. Litter N Retention and Behaviors of Litter Indigenous and Exogenous N

Eight months after decomposition, no net N loss was found in the control of both litter species under conditions without leaching, but substantial portions of initial N (indigenous or indigenous plus exogenous) were lost in the N addition treatment (Table 4). About 23.0% and 20.7% of the exogenous N remained in *P. elliotii* and *L. formosana* litter, respectively, eight months after N addition (Table 4). Among the exogenous N remaining in litter, half was organic (immobilized), with 13.6% and 9.8% of added N immobilized in *P. elliotii* litter and *L. formosana* litter, respectively (Table 4). Litter indigenous NH_4^+ and NO_3^- in *P. elliotii* and *L. formosana* litter was 21 and 13 times higher, respectively, in the N addition treatment than in the control ($p < 0.001$; Table 4).

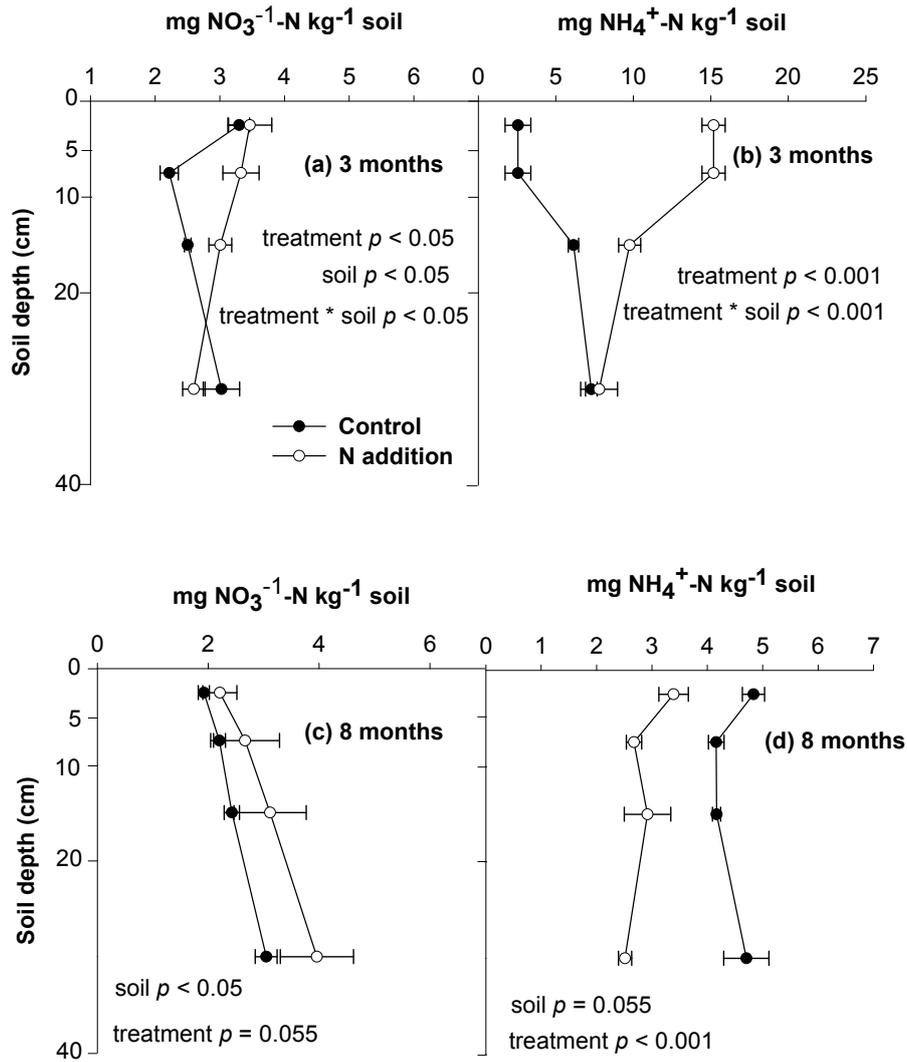


Figure 3. (a) Concentration of soil indigenous NO₃⁻ (mean ± standard error (se); $n = 8$) three months after N addition. (b) Concentration of soil indigenous NH₄⁺ (mean ± se; $n = 8$) three months after N addition. (c) Concentration of soil indigenous NO₃⁻ (mean ± se; $n = 5$) eight months after N addition. (d) Concentration of soil indigenous NH₄⁺ (mean ± se; $n = 5$) eight months after N addition. Data are graphed at the mid-point of our soil sampling depths.

Table 4. Contents and ¹⁵N abundances in litter N pools at the initiation of N addition and eight months after N addition (mean ± standard error (se); n = 4).

Litter Species	Treatments	Initial	Initial	Total N Remaining		Atom%	Exogenous N		Indigenous N	Exogenous N		NH ₄ ⁺	Atom	Atom	Exogenous	Exogenous	Indigenous	Indigenous		
		Exogenous	Indigenous	(g·m ⁻²)	(g·m ⁻²)	¹⁵ N of	Remaining	Remaining	Remaining	Immobilization	(g·m ⁻²)	of		of					NO ₃ ⁻	% ¹⁵ N
		N (g·m ⁻²)	N (g·m ⁻²)	(g·m ⁻²) (%)	(g·m ⁻²) (%)	Total N Remaining	(g·m ⁻²) (%)	(g·m ⁻²)	NH ₄ ⁺	NO ₃ ⁻	NH ₄ ⁺ (g·m ⁻²)	NO ₃ ⁻ (g·m ⁻²)	(%)							
<i>P. elliotii</i>	Control	0	1.84	1.85 (0.0)	101 (1.9) a *	0.36 (0.0)		1.85 (0.0) a	101 (1.9) a			0.005 (0.00)	0.001 (0.00)	0.36 (0.0)	0.36 (0.0)			4.56 (0.78) a	0.53 (0.09) a	
	N addition	7.00	1.84	3.02 (0.1)	34.2 (1.7) b	54.9 (2.2)	1.67 (0.1)	23.0 (1.5)	1.35 (0.0) b	73.4 (3.0) b	0.95 (0.1)	13.6 (1.6)	0.59 (0.05)	0.24 (0.04)	84.3 (1.6)	92.4 (1.0)	0.496 (0.04)	0.226 (0.04)	43.2 (2.7)	585 (55) b
<i>L. formosana</i>	Control	0	5.08	5.33 (0.1)	105 (2.1) a	0.36 (0.0)		5.33 (0.1) a	105 (2.1) a			0.010 (0.00)	0.001 (0.00)	0.36 (0.0)	0.36 (0.0)			10.38 (1.21) a	1.30 (0.13) a	
	N addition	7.00	5.08	5.36 (0.3)	44.4 (2.5) b	28.3 (2.2)	1.50 (0.1)	20.7 (1.1)	3.86 (0.2) b	76.0 (5.1) b	0.69 (0.12)	9.8 (1.7)	0.48 (0.08)	0.48 (0.10)	70.3 (1.8)	96.8 (0.6)	0.340 (0.06)	0.472 (0.11)	54.1 (8.1)	478 (77) b

*: Different lowercase letters in the same column of the same species denote significant difference between treatments at 0.05 level. Exogenous N immobilization indicates the portion of exogenous N present in organic forms.

4. Discussion

4.1. Low N Retention in the Soil Profile and Litter

As expected in the second hypothesis, exogenous N in the 0–40 cm soil profile decreased steeply from 100% of addition at the initiation to 70% three months after addition and further to 16% eight months after N addition (Table 3) under conditions with leaching. About 21%–23% of the exogenous N was retained in litter eight months after N addition under conditions without leaching (Table 2), suggesting that the added N experienced strong gaseous loss on the surface of litter. We believe that only the immobilized organic portion (9.8%–13.6%) of exogenous N could be retained in litter under field conditions with leaching, because the inorganic portion could be readily leached out from litter and subsequently leached through soil. Similarly, Micks *et al.* [39] showed that litter retained only 6%–7% of N tracers over two years under elevated N inputs ($5 \text{ g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$) in the field. Therefore, the N retention rate in litter plus soil was estimated as 28% eight months after N addition in our study. Except for the extremely low ^{15}N recovery (2.54%–7%) found in Zak *et al.* [15] and Zogg *et al.* [21], the N retention rate we observed was at the lower end of the reported range of 20%–87% in previous N addition ($5\text{--}22.4 \text{ g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$) studies using ^{15}N tracers with experimental durations ranging from 1 year to 10 years [17,24,40–44].

The low soil N retention rate we observed might be mainly due to the absence of plants in soil columns. Under conditions without plant uptake and root-C-driven microbial N immobilization, a large fraction of added N existed in inorganic forms (Table 2) as expected in the first hypothesis and was exposed to leaching and gaseous loss. The plant-soil-microbial interactions have been suggested to play an important role in soil N retention [15,45], because carbon inputs from roots may enhance soil microbial N immobilization [46]. Retention of deposited inorganic N was dominated by rapid immobilization and turnover through microbial biomass in the short term (weeks), and transfer from microbial biomass into plant pools and subsequently incorporated into soil through leaf and root litter in the long term (months to years) [45]. In this study, intact soil columns without plants were used and we added a considerable amount of N at the beginning of the experiment. Soil columns were also used to measure soil N retention rate in subtropical forests in Huang *et al.* [47] and Pan *et al.* [48], and only 57%–84% of ^{15}N tracer was found one month after ^{15}N tracer addition, suggesting a rapid loss of ^{15}N tracer from these soil columns.

The low soil N retention rate we observed should also be due in part to the thin litter (around 1 cm) and soil organic layer (millimeters of Oe + Oa layer), because litter and soil organic layer have been shown to be an important sink of deposited N in temperate and boreal forests [49,50]. Furthermore, the relatively low soil C concentration (1.8% at 0–5 cm) in our study might also be a reason, because larger soil C pools may promote greater soil N storage by C-fueled microbial N immobilization and abiotic reactions between mineral N and aromatic compounds [23]. Abiotic immobilization of nitrate has been proposed as a mechanism of soil N retention in some forests [19,20], but the contribution of abiotic immobilization to soil N retention in our study could not be determined under our experimental settings.

Strong leaching should be the primary driver of N loss in this subtropical area with an annual rainfall amount of 1542 mm. The soil N_2O emission for this plantation was estimated at $0.93 \text{ kg N}_2\text{O ha}^{-1}\cdot\text{year}^{-1}$ (*i.e.*, $59 \text{ mg}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$), and N addition of $4 \text{ g}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$ and $12 \text{ g}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$ increased the N_2O emission by 403% and 762%, respectively [51]. Calculated by the linear relation between N_2O emission

and N addition rate found in Wang *et al.* [51], the N loss through N₂O emission in our study (N addition of 7 g·m⁻²·year⁻¹) was estimated as 0.08 g·N·m⁻² and 0.2 g·N·m⁻² within three months and eight months, respectively. The estimated N loss through N₂O emission contributed only 3.8% and 3.4% of the unaccounted exogenous N of 2.10 g·m⁻² and 5.89 g·m⁻² three and eight months after N addition, respectively (Tables 2 and 3). This suggests that most of the unaccounted exogenous N was lost by leaching and N₂ emission. Strong leaching was evidenced by the contrast in patterns of exogenous NH₄⁺ and NO₃⁻ distribution with soil depth (Figure 2). The concentration of soil exogenous NH₄⁺ decreased with soil depth because NH₄NO₃ was added on the soil surface and NH₄⁺ has a poor mobility in soil. However, the concentration of exogenous NO₃⁻ increased with soil depth, which was due to solution leaching because NO₃⁻ has a strong mobility with water flow [1]. A large portion of soil NH₄⁺ might have been transformed into NO₃⁻ and lost by leaching due to enhanced nitrification by N addition. This was confirmed by the enhanced nitrification by N addition (Figures 1c and 3c) and the near zero exogenous NH₄⁺ eight months after N addition in spite of a poor mobility (Figure 2b). This result supports the view that tropical and subtropical soils display little delay in nitrification in response to N additions [52]. Therefore, N deposition in tropical or subtropical areas in forms of either NO₃⁻ or NH₄⁺ may have similar effects on N leaching loss because added NH₄⁺ is nitrified after addition and exposed to leaching. Consistent with our results, hydrologically driven leaching was also considered as an important reason for N loss in a subtropical forest in China [28].

Our results suggest that in subtropical areas with strong leaching, N deposition on barren or vegetation degraded lands with low plant activities and low soil C contents may lead to increased N output due to low N immobilization in soil and litter, and result in environmental consequences such as air NO_x pollution, water acidification and eutrophication.

4.2. N Addition Altered the Behaviors of Indigenous N in Soil and Litter

N addition stimulated the mineral release of soil and litter indigenous N. This was confirmed by the higher concentrations of soil and litter indigenous NO₃⁻ and NH₄⁺ in the N addition treatment than in the control (Figure 3; Table 4). Our result was consistent with previous studies, which showed a priming effect (stimulating effect) of added N on the availability of soil native N [30,53]. An apparent priming effect could be induced by pool substitution or displacement reactions [29,54]. Pool substitution is the process by which added N stands proxy for native N that would otherwise have been removed from that pool [29]. Microbial immobilization and denitrification of added inorganic N instead of the exiting pool of soil indigenous inorganic N are the typical forms of pool substitution. Displacement reaction refers to any process involving the displacement of soil N from a “bound” pool by exogenous N (e.g., displacement of NH₄⁺ fixed in clay minerals by added NH₄⁺) [29]. A real priming effect could be induced by increased mineralization of soil organic N by inorganic N addition [29]. Since gross N mineralization was not measured in this study, it remains unclear whether the stimulated mineral release of soil and litter indigenous N was induced by a real or apparent priming effect.

N addition altered the relative intensity of ammonification and nitrification of indigenous N and total N, as expected in the third hypothesis. Concentrations of soil total and indigenous NH₄⁺ were lower in the N addition treatment than in the control eight months after N addition ($p < 0.001$; Figures 1d and 3d). However, soil total and indigenous NO₃⁻ were higher in the N addition treatment ($p < 0.01$ or $p = 0.055$;

Figures 1c and 3c). This indicates that soil nitrification was enhanced by N addition to a greater extent than ammonification, leading to a depletion of NH_4^+ [55]. Enhanced nitrification is expected to increase the potential of N loss through denitrification and leaching because NO_3^- is the substrate of denitrification and is more mobile than NH_4^+ [1,52]. Indeed, increased loss of litter indigenous N by N addition has occurred in our study as evidenced by the lower remaining rate of litter indigenous N in the N addition treatment than in the control ($p < 0.05$; Table 4).

5. Conclusions

By applying ^{15}N labeled inorganic N at a rate similar to ambient N deposition to intact soil columns and litter, we evaluated the capacity of soil and litter in N retention without plants. Moreover, we partitioned exogenous and indigenous N to examine the interactions between added N and ecosystem intrinsic N. We showed that most of the exogenous N added to soil existed in inorganic forms three months after N addition, and the exogenous inorganic N experienced a much faster loss than exogenous organic N over the following five months. Only 28% of the added N was retained in litter plus 0–40 cm soil profile eight months after N addition, which was possibly due to low N immobilization and thus exposure to leaching and gaseous loss in our study. Our results suggest that N deposition on barren or vegetation degraded lands with low plant activities and low soil C contents may lead to increased N output due to low N immobilization in soil and litter. Furthermore, N addition stimulated the mineral release and nitrification of indigenous N in soil and litter, further increasing the potential of N loss and decreasing the capacity of soil and litter in N retention. Therefore, vegetation status and interactions between added N and ecosystem intrinsic N should be taken into consideration in further studies in evaluating soil N retention.

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Author Contributions

Yanmei Xiong, Xingliang Xu and Dali Guo conceived and designed the experiment. Yanmei Xiong carried out the experiment and data analysis. Yanmei Xiong, Xingliang Xu and Fusheng Chen prepared the manuscript. Hui Zeng and Huimin Wang contributed materials and analysis tools.

Conflicts of Interest

The authors declare no conflict of interest.

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