

RESEARCH ARTICLE

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Key Points:

- 42% of the atmospheric input of NO_3^- -N remains unprocessed and is leached as NO_3^- within half a year
- For NH_4^+ input, only 14% is leached, but after being nitrified
- N_2O emission varies seasonally with soil moisture and temperature and correlates significantly with the NO_3^- concentration in soil water

Supporting Information:

- Supporting Information S1

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In situ ^{15}N labeling experiment reveals different long-term responses to ammonium and nitrate inputs in N-saturated subtropical forest

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Abstract Chronically elevated deposition of reactive nitrogen (N), as ammonium (NH_4^+) and nitrate (NO_3^-), in subtropical forests with monsoonal climate has caused widespread N leaching in southern China. So far, little is known about the effect of further increases in N input and changes in the relative proportion of NH_4^+ and NO_3^- on turnover rate and fate of atmospheric N. Here we report a ^{15}N tracer experiment in Tieshanping (TSP) forest, SW China, conducted as part of a long-term N fertilization experiment, using NH_4NO_3 and NaNO_3 , where effects of a doubling of monthly N inputs were compared. In June 2012, the regular N fertilizers were replaced by their ^{15}N -labeled forms, viz., $^{15}\text{NH}_4\text{NO}_3$ and $\text{Na}^{15}\text{NO}_3$, as a single-dose addition. Mass balances of N for the initial 1.5 years following label addition showed that for both treatments, 70% to 80% of the annual N input was leached as NO_3^- , both at ambient and at double N input rates. This confirms the earlier reported extreme case of N saturation at TSP. The ^{15}N , added as $\text{Na}^{15}\text{NO}_3$, showed recoveries of about 74% in soil leachates, indicating that NO_3^- input at TSP is subject to a rapid and nearly quantitative loss through direct leaching as a mobile anion. By contrast, recoveries of ^{15}N in soil leachates of only 33% were found if added as $^{15}\text{NH}_4\text{NO}_3$. Much of the ^{15}N was immobilized in the soil and to a lesser extent in the vegetation. Thus, immobilization of fresh N input is significantly greater if added as NH_4^+ , than as NO_3^- .

1. Introduction

Accelerated use of energy and intensification of agriculture has increased the global emission rate of reactive nitrogen (N) from 15 Tg N yr^{-1} in 1860 to 187 Tg N yr^{-1} in 2005, and emission rates are predicted to reach 200 Tg N yr^{-1} in 2050 [Galloway *et al.*, 2008]. Nitrogen emissions are dominated by nitrogen oxides (NO_x ; mainly from the burning of fossil fuels and biomass) and ammonia (NH_3 ; mainly from crop and animal production in agriculture) [Galloway *et al.*, 2004; Van Vuuren *et al.*, 2011]. Increased N deposition, primarily as nitrate (NO_3^-) and ammonium (NH_4^+), has caused widespread concern over its impact on natural ecosystems. Since the 1980s, numerous studies in Europe [Bredemeier *et al.*, 1998; Emmett *et al.*, 1998; Gundersen *et al.*, 1998; Tietema *et al.*, 1998a] and in North America [McNulty *et al.*, 1990, 1991; Magill *et al.*, 2000; Aber and Magill, 2004] have documented N saturation in temperate and boreal forests, characterized by significant nitrate (NO_3^-) leaching. Nitrogen saturation may result in serious acidification and eutrophication of soil and water, emission of N_2O (a potent greenhouse gas and increasingly important for the destruction of stratospheric ozone [Ravishankara *et al.*, 2009]), and reduced forest growth and biodiversity [Aber *et al.*, 1989; Kreutzer *et al.*, 2009; Yuan *et al.*, 2011].

By 2010 China's emissions of NH_3 and NO_x had doubled and quadrupled, respectively, since the 1980s [Liu *et al.*, 2013], contributing more than 20% to the global N emission [Cui *et al.*, 2013]. Increasing amounts of atmospheric N deposition have been reported, particularly in eastern and southern China (commonly >3 g N m^{-2} yr^{-1}) [Lu and Tian, 2007; Zhao *et al.*, 2009; Du *et al.*, 2014], with increasing contributions of NO_x emission [Liu *et al.*, 2016; Xu *et al.*, 2015]. After a 30 year continuous increase, the emissions of NO_x have been reduced at the national scale in recent years, following the implementation of the *National NO_x Total Emission Control* in 2011. However, emissions of NH_3 have continued to increase because of technical and economic difficulties in controlling these emissions. Thus, the relative contribution of NH_4^+ to N deposition is expected to increase in the future.

In temperate and boreal forests, ^{15}N -labeled NH_4^+ and NO_3^- have been used to trace atmospheric N inputs [Magill *et al.*, 2000; Templer *et al.*, 2012]. These studies indicate that the quantitative importance of different N sinks is influenced by N input rate and N form [Preston and Mead, 1994; Koopmans *et al.*, 1996; Tietema *et al.*, 1998b; Nadelhoffer *et al.*, 1999; Zak *et al.*, 2004; Feng *et al.*, 2008]. In N-saturated forest ecosystems, the retention of NH_4^+ is larger than that of NO_3^- [Buchmann *et al.*, 1995, 1996; Preston and Mead, 1994; Emmett *et al.*, 1995; Moldan *et al.*, 2006], although some studies also report opposite patterns [Nadelhoffer *et al.*, 1999; Feng *et al.*, 2008; Sheng *et al.*, 2014].

In strongly acidic soils of N-saturated subtropical forests in SW China, receiving elevated atmospheric N inputs (about $5 \text{ g N m}^{-2} \text{ yr}^{-1}$), Huang *et al.* [2015] showed that there is little or no net N retention. Nearly 100% of the ambient N input was found to leach as NO_3^- , even though more than half enters as NH_4^+ -N. Furthermore, it was reported that experimental doubling of the N input (through monthly additions of either NH_4NO_3 or NaNO_3) resulted in an instantaneous doubling of NO_3^- -N leaching, irrespective of the form in which N was added [Huang *et al.*, 2015]. This may indicate that the added inorganic N, be it NH_4^+ or NO_3^- , largely bypasses the different soil N pools, particularly the ones with long turnover times, while NH_4^+ is efficiently nitrified to NO_3^- . Although autotrophic nitrification is not expected to be favored at low pH in soil [De Boer and Kowalchuk, 2001], significant nitrification rates have been found and were hypothesized to be due to heterotrophic nitrification [Zhang *et al.*, 2013; Yu *et al.*, 2017].

Recently, acidic soils of N-saturated forests have been reported to be quantitatively important sources of N_2O [Zhu *et al.*, 2013a; Yu *et al.*, 2017], primarily due to denitrification. Zhu *et al.* [2013a] reported that the emission of N_2O was largely explained by soil moisture content and soil temperature, while the concentration of NO_3^- in soil water, which was generally large (mean values $10\text{--}15 \text{ mg N L}^{-1}$), did not correlate with N_2O emission. The annual emission of N_2O in this N-saturated subtropical was found to be nearly 10% of the atmospheric N input.

So far, only few long-term in situ studies of N saturation, including N mass balances, have been reported for well-drained tropical [e.g., Fang *et al.*, 2009; Lu *et al.*, 2010, 2014; Gurmessa *et al.*, 2016] and subtropical forests [Larssen *et al.*, 2011; Zhu *et al.*, 2013a; Huang *et al.*, 2015]. Even fewer studies address the effect of increased N input rates, the dominant N sinks, and the fate of the different forms of added N (NH_4^+ and NO_3^-). Such information is of paramount importance for the prediction of the effect of long-term changes in N deposition in subtropical forests, which become increasingly exposed to N deposition [Vet *et al.*, 2014].

Here we present a 1.5 year ^{15}N tracer study, conducted in conjunction with a 7 year N addition experiment in the N-saturated, subtropical forest at Tieshanping (TSP) in SW China [Huang *et al.*, 2015]. At selected plots, with either ambient or a long-term doubling of N inputs, we added one monthly dose of ^{15}N , either as $^{15}\text{NH}_4^+$ or as $^{15}\text{NO}_3^-$, to trace the fate of the added N during the subsequent 18 months. Detailed objectives were (1) to quantify the effect of ambient and long-term doubling of NH_4^+ and NO_3^- inputs on N leaching and accumulation in soil and vegetation by means of a single monthly addition of either $^{15}\text{NH}_4^+$ or $^{15}\text{NO}_3^-$ and (2) to quantify and source-partition the emitted N_2O from ambient and N-manipulated long-term research plots.

2. Methods

2.1. Site Description

The study was conducted in the Tieshanping Forest Park, located about 25 km northeast of Chongqing city, SW China ($106^\circ 41.24'\text{E}$, $29^\circ 37.42'\text{N}$), at an altitude of 450 m. With a subtropical monsoonal climate, this site receives a mean annual precipitation of 1105 mm and has a mean air temperature of 18.2°C (average from 1971 to 2000; data from Meteorological Station of Chongqing). More than 75% of the annual rainfall occurs in the wet season from April to September. Additional information on climatic differences between the (wet) summer and (dry) winter season can be found in Zhu *et al.* [2013a]. The landcover is classed as coniferous-broadleaf mixed forest dominated by Masson pine (*Pinus massoniana*). The stand is naturally regenerated, nonmanaged secondary forest after the original forest was cut in the early 1960s. The dominant soil type, locally called Yellow Earth, is a Haplic Acrisol [World Reference Base for Soil Resources, 2006], with a relatively thin organic surface layer (O horizon; $<2 \text{ cm}$) and low pH value ($3.7\text{--}4.1$ from the O horizon to the lower B horizon). Ambient N deposition, estimated from throughfall fluxes (2005–2011), is $5.4 \text{ g m}^{-2} \text{ yr}^{-1}$, with

contributions of NH_4^+ -N and NO_3^- -N being 60% and 40%, respectively. For detailed site information, especially soil and vegetation characteristics, see *Chen and Mulder [2007]* and *Huang et al. [2015]*.

2.2. N Fertilization and ^{15}N Tracer Application

The ^{15}N tracer experiment was carried out at the plots set up in the previous study [*Huang et al., 2015*]. In brief, nine neighboring plots of 14 m \times 14 m were set up in October 2004. To avoid edge effects, sample collection was carried out only in the central 10 m \times 10 m at each plot. The plots were divided among three treatments, namely, reference (REF; ambient N deposition), NH_4NO_3 application (AN), and NaNO_3 application (SN). All treatments and the references were in triplicate. In each month, from January 2005, 200 g NH_4NO_3 or 400 g NaNO_3 , dissolved in 10 L deionized water (amounting to only 0.6 mm yr^{-1} water input), was sprayed evenly on the plots using a backpack sprayer. The REF plots received an equivalent volume of deionized water. The annual N addition was 4.3 and 4.0 g $\text{m}^{-2} \text{yr}^{-1}$ at the AN and SN plots, respectively, similar to ambient N deposition rates [*Larssen et al., 2011*].

Only for the monthly addition on 24 June 2012, we used ^{15}N -labeled fertilizer (60 at. % ^{15}N) (Table S1 in the supporting information). The AN plots received a single dose of 0.10 g $^{15}\text{N} \text{m}^{-2}$ as $^{15}\text{NH}_4\text{NO}_3$ (0.33 g $\text{N} \text{m}^{-2}$ with 60 at. % ^{15}N as NH_4^+). The SN plots received 0.20 g $^{15}\text{N} \text{m}^{-2}$ as $\text{Na}^{15}\text{NO}_3$ (0.33 g $\text{N} \text{m}^{-2}$ with 60 at. % ^{15}N as NO_3^-). To also allow tracing ambient N deposition, a single, very small dose of ^{15}N was applied to two of the three REF plots on the same date. One of the reference plots (designated R_{AN}) received 0.015 g $^{15}\text{N} \text{m}^{-2}$ as $^{15}\text{NH}_4\text{NO}_3$ (0.05 g $\text{N} \text{m}^{-2}$ with 60 at. % of ^{15}N as NH_4^+), while one of the other reference plots (designated R_{SN}) received 0.03 g $^{15}\text{N} \text{m}^{-2}$ as $\text{Na}^{15}\text{NO}_3$ (0.05 g $\text{N} \text{m}^{-2}$ with 60 at. % of ^{15}N in NO_3^-). These small amounts of N were assumed to be too little (equivalent to about 1% of annual N deposition) to affect N fluxes in the system; Table S1). The third remaining REF plot (designated R_0) received an equivalent volume of deionized water.

2.3. Sampling and Chemical Analysis

From 2012 to 2014, throughfall, soil water, soil, and plants were sampled not only for the analysis of nitrogen content (especially NH_4^+ and NO_3^- concentrations in the water samples; as done by *Huang et al. [2015]*) but also for measurement of ^{15}N enrichment. The N_2O emission and its ^{15}N enrichment were also monitored in this study. The recoveries of ^{15}N in each pool, including soil, plants, and N_2O , together with the N mass balance, were calculated for different treatments to distinguish the fates of NH_4^+ and NO_3^- .

2.3.1. Throughfall and Soil Water

Five throughfall collectors were placed randomly under the forest canopy. At each plot three ceramic cup lysimeters (P80; Staatliche Porzellanmanufaktur, Berlin) were used to collect soil water in the mineral soil at 5, 15, and 30 cm depth. Throughfall and soil water were collected at weekly intervals, and bulked per plot and depth to 4-weekly samples, prior to analysis. All water samples were stored at 4°C prior to analysis, except those for $^{15}\text{NO}_3^-$ measurement which were frozen (-20°C).

After filtration, soil water and throughfall samples were analyzed for $^{15}\text{NO}_3^-$ at the Norwegian University of Life Sciences, using a modified denitrifier method [*Casciotti et al., 2002; Yu et al., 2016*]. In brief, NO_3^- was quantitatively converted to N_2O by *Pseudomonas aureofaciens* (ATCC 13985). Next, $^{15}\text{N}_2\text{O}$ was analyzed by continuous flow isotope ratio mass spectrometry (IRMS; Thermo Scientific DELTA V Advantage). In this study, the NH_4^+ concentration in soil pore water was extremely small and was mostly below detection limit (0.01 mg L^{-1}). Due to the small contribution of NH_4^+ to total inorganic N in solution, $^{15}\text{NH}_4^+$ was not determined. The natural abundance of $^{15}\text{NH}_4^+$ in throughfall was obtained from *Yu et al. [2013]* (Table S2).

2.3.2. Soil

In early November (at the end of the growing season) of each year during 2011–2013 (viz., once before ^{15}N addition and twice after), five bulked soil samples (consisting of five subsamples each) were randomly taken at each plot, from each layer. The samples from three mineral soil horizons, S1 (0–10 cm), S2 (10–20 cm), and S3 (20–30 cm), were taken using a cylindrical soil auger (5 cm in diameter). Samples from the organic surface horizons, consisting of the litter layer (LF) and the H/A horizon, were collected separately.

Soil samples were transported to Tsinghua University, air-dried, sieved (<2 mm), and analyzed for total nitrogen (TN; using the Kjeldahl method) and ^{15}N (using elemental analyzer interfaced to continuous flow isotope ratio mass spectrometer, EA-IRMS; Thermo Scientific DELTA V Advantage).

2.3.3. Plants

The diameter at breast height (DBH) of all Masson pines located in the central 10 m × 10 m of each plot were measured in early November of 2011, 2012, and 2013. Needles and tree rings of Masson pine, as well as aboveground parts of four major species of herbaceous ground vegetation (*Miscanthus sinensis*, *Lopatherum gracile*, *Dicranopteris pedata*, and *Dryopteris fuscipe*; contributing more than 90% of the total biomass of the ground vegetation), and fine roots (0–30 cm depth) were also sampled annually. From the two highest pine trees, located close to the center of each plot, tree ring samples were collected by a corer, and three branches were cut randomly from the top of the tree crown. Current-year and last-year needles were collected separately. In general, few last-year needles and no prior-to-last-year needles were present.

Roots were sampled annually from in-growth cores at each plot 1 year after deployment. Around one of the two tallest Masson pines in each plot, three directions were selected for core deployment. Using a soil auger, three holes (5 cm in diameter and 30 cm in depth) were made in the soil at 0.5, 1.0, and 1.5 m from the tree in each of three directions. Each of the three holes was equipped with a silica sand-filled stocking (1.00 mm of mesh), which was left in the soil for 1 year. Upon removal from the soil, the sand in each core was washed with tap water, and carefully sieved until only roots remained. The collected fine roots were separated per individual soil layer (i.e., 0–10 cm, 10–20 cm, and 20–30 cm, respectively). Fine roots (diameter < 2 mm) from the three cores along the same directional line were combined as one sample.

Four major species of herbaceous ground vegetation (*Miscanthus sinensis*, *Lopatherum gracile*, *Dicranopteris pedata*, and *Dryopteris fuscipe*) were sampled annually in five permanent 1 m × 1 m squares, randomly distributed in the central 10 m × 10 m of each plot. The entire aboveground biomass of each herbaceous species was harvested for biomass weighing and chemical analysis.

All plant samples were dried at 70°C for at least 48 h and finely milled before analysis. The plant samples were analyzed for total nitrogen (TN; using the Kjeldahl method) and ¹⁵N (by EA-IRMS) at Tsinghua University.

2.3.4. N₂O Emission

Fluxes of N₂O were measured manually using static chambers [Zhu *et al.*, 2013a] in each season from summer 2012 to autumn 2013. At each plot, three zinc-coated iron chambers (30 cm in diameter and 30 cm in height) were deployed. Gas samples were taken from a sampling port at the top of the chambers at 0, 15, 30, 60, and 120 min after deployment. Samples were collected by a 100 mL plastic syringe and transferred immediately to preevacuated 100 mL gas bags. Since ¹⁵N₂O emissions may be large shortly after fertilization, N₂O emissions were sampled once every day during the first week after tracer application. In the subsequent seasons, emitted N₂O was sampled at least 3 days per season (twice per day), including days following rainstorms when the N₂O flux is expected to be elevated [Zhu *et al.*, 2013a].

All gas samples were analyzed at Tsinghua University. The N₂O concentration was analyzed by a gas chromatograph (Model 6890A, Agilent) equipped with electron capture detector. The N₂O flux (μg N m⁻² h⁻¹) was calculated based on the increase in N₂O concentration over time, multiplied by the chamber volume, divided by the covered area of the chamber, and corrected for temperature. To determine the increase in N₂O concentration, the slope of a linear fit of the concentration data against time was calculated. The ¹⁵N at. % of emitted N₂O was determined based on the Keeling plot approach [Yakir and Sternberg, 2000].

2.4. Calculations

2.4.1. ¹⁵N Abundance

The ¹⁵N abundance of any N pool was presented as the atom percentage of total N:

$$^{15}\text{N at. \%} = R_{\text{sample}} / (1 + R_{\text{sample}}) * 100\% \quad (1)$$

where R_{sample} is the ratio of ¹⁵N to ¹⁴N in the sample. The excess ¹⁵N at. % of a pool was calculated by subtracting the background ¹⁵N at. %, which was derived from the values measured before ¹⁵N tracer addition in June 2012.

The ¹⁵N recovery in each pool was calculated according to Nadelhoffer *et al.* [2004]:

$$\%^{15}\text{N}_{\text{rec},i} = m_i * ^{15}\text{N}_{\text{exc},i} \text{ at. \%} / m_{\text{tot}} \quad (2)$$

where $\%^{15}\text{N}_{\text{rec},i}$ is ¹⁵N recovery (%) in N pool i , $^{15}\text{N}_{\text{exc},i}$ at. % is the excess ¹⁵N at. % of N pool i , m_i is the N pool size (g N m⁻²), and m_{tot} is the total amount of added ¹⁵N (g N m⁻²).

Table 1. Fluxes of Water and N_r in 2012 and 2013 for Different Treatments

	Throughfall Water Flux (mm yr ⁻¹)	Soil Water Flux (mm yr ⁻¹)	N Input (Deposition + Fertilizer) (g N m ⁻² yr ⁻¹)			N leaching ^a (at 30 cm Soil Depth) (g N m ⁻² yr ⁻¹)			N-Leaching/N-Input (%)
			NH ₄ ⁺ -N	NO ₃ ⁻ -N	N _r	NH ₄ ⁺ -N	NO ₃ ⁻ -N	N _r	
2012									
REF ^b	978	367	2.6	2.7	5.3	0.0A	3.5A	3.5A	67
AN	978	367	4.7	4.8	9.5	0.1A	6.9B	7.1B	74
SN	978	367	2.6	6.9	9.5	0.1A	7.5C	7.6C	80
2013									
REF	750	295	2.5	2.5	5.0	0.0A	4.2A	4.2A	84
AN	750	295	4.6	4.6	9.2	0.1A	7.7B	7.8B	85
SN	750	295	2.5	6.7	9.2	0.0A	7.5B	7.5B	82

^aThe different capital letters indicate significant difference in cumulative N leaching between treatments ($p \leq 0.05$, RMANOVA).

^bThe assessment of total N fluxes in N input and leaching at the reference (REF) plots is based on fluxes at the R₀, R_{AN}, and R_{SN} plots (see section 2). The N fluxes at the latter two plots are affected only marginally by the single labeled N addition in June 2012 (+1%; see also Table S1).

Data on N content in each pool are summarized in Table S3. The annual production of biomass in trunks, branches, and needles of Masson pine were calculated based on measured DBH according to *Zhang et al.* [2006].

2.4.2. ¹⁵N Leaching

The leaching of ¹⁵N was calculated using the concentration and excess ¹⁵N at. % of NO₃⁻-N in soil water below the root zone (30 cm depth), and the soil water flux estimated on the basis of the sodium (Na⁺) balance at the SN plots. Recently, *Huang et al.* [2015] showed that this is a reasonable assumption. In brief, the ratio of monthly soil water flux to throughfall water flux was assumed to equal the ratio of the Na⁺ concentration in the input (from throughfall and NaNO₃ fertilizer) to that in soil water. The basic assumption of this method is that there is no net change in the pool of dissolved Na⁺ in soil water over a 1 year period and that mineral weathering does not produce significant quantities of dissolved Na⁺. As the NH₄⁺ concentration in soil water was negligible, we did not include ¹⁵NH₄⁺ to calculate ¹⁵N loss by leaching.

2.5. Statistical Analysis

The NO₃⁻ fluxes in soil water and ¹⁵NO₃⁻ recovery of the AN and SN treatments were analyzed by repeated measures analysis of variance (RMANOVA) with 4-weekly values as the within-subjects factor and fertilization treatment as the between-subjects factor. In addition, posthoc analysis using one-way ANOVA was applied to analyze differences in vegetation parameters among treatments in each year. Differences were considered significant at the $p \leq 0.05$ level. The ¹⁵NO₃⁻ recovery for the R_{AN} and R_{SN} treatments were not replicated and could not be used to test for significant differences between treatments.

3. Results

3.1. Fluxes of Inorganic N at Ambient and Increased Levels of N Input

The soil water flux at 30 cm depth, draining from the root zone of the research plots at TSP, ranged from 37% to 39% of the throughfall flux in 2012 and 2013, respectively (Table 1 and Figure S1 in the supporting information). These values are indicative for relatively dry years and are slightly smaller than the averages reported for the period of 2005–2011 (43% ± 6%) [*Huang et al.*, 2015]. The N input in throughfall were 5.3 and 5.0 g N m⁻² yr⁻¹ in 2012 and 2013, respectively, 50% of which was as NH₄⁺ (Table 1 and Figure S2). The total N deposition rates were similar to the long-term average for 2005–2011 (5.3 g N m⁻² yr⁻¹) [*Huang et al.*, 2015]. However, the relative contribution of NH₄⁺ was smaller than in previous years (about 60%) [*Huang et al.*, 2015], confirming the recent trend toward increasing contributions of NO₃⁻ [*Liu et al.*, 2016; *Xu et al.*, 2015]. The AN and SN treatments, which received equal amounts of additional N fertilizer, either as NH₄NO₃ or as NaNO₃, had total N inputs of 9.5 and 9.2 g m⁻² yr⁻¹ in 2012 and 2013, respectively (Table 1). These inputs were almost the same as for the long-term experimental treatments during 2005–2011 [*Huang et al.*, 2015]. At the R_{AN} and R_{SN} plots, the single small dose of ¹⁵N-labeled fertilizer, added in June 2012, increased the annual N load only marginally (+1%) from the ambient level (Table S1).

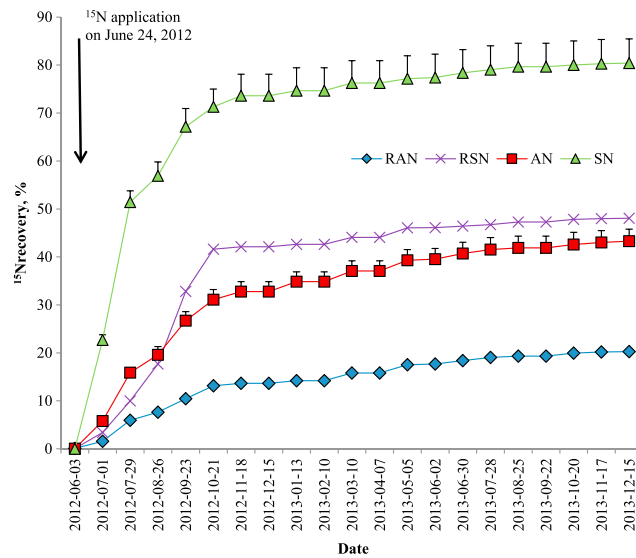


Figure 1. Cumulative ¹⁵N recovery as dissolved N_r in soil water leachate (at 30 cm soil depth) for different treatments. The error bars for AN and SN indicate standard deviations. Values for AN and SN differ significantly between treatments ($p \leq 0.05$, RMANOVA). Data for R_{AN} and R_{SN} treatments are indicative only, as they were not replicated.

At all plots, including AN and R_{AN}, the N in soil leachate was dominated by NO₃⁻, whereas NH₄⁺ contributed little (<2%; Table 1). The leaching rate of NO₃⁻ at the reference plots (REF) in 2012 and 2013 were 3.5 and 4.2 g N m⁻² yr⁻¹, respectively, which was significantly smaller than the long-term average (5.5 g N m⁻² yr⁻¹) reported for 2005–2011 [Huang *et al.*, 2015]. This indicates an important N sink, which may be associated with relatively dry years, when NO₃⁻ fluxes tend to be relatively small, as opposed to larger fluxes in wetter years [Huang *et al.*, 2015]. Also for the AN and SN treatments, the annual leaching rate of NO₃⁻ in 2012 and 2013 (6.9 to 7.7 g N m⁻² yr⁻¹) were significantly smaller than the average for 2005–2011 (9.4–9.9 g N m⁻² yr⁻¹) [Huang *et al.*, 2015]. As observed for the long-term data (2005–2011), the N leaching rates at the AN and SN plots were significantly greater than at the REF plots ($p \leq 0.05$, RMANOVA; Figure S2). The N leaching rates below the root zone varied between 67% and 85% of the summed annual N input by throughfall and fertilizer in 2012 and 2013, for all treatments (Table 1 and Figure S2).

3.2. ¹⁵N Recovery in Soil Water Leachates

The ¹⁵N at. % of NO₃⁻ in soil water at the 30 cm depth for the SN treatment peaked (12%) within the first week after ¹⁵N addition (Figure S3). For the R_{SN} treatment with a smaller dose of ¹⁵NO₃⁻, the increase in ¹⁵N at. % of NO₃⁻ in soil water was more gradual and the peak values (~2%) were not reached until about 2 months after application. For the ¹⁵NH₄⁺ treatments (AN and R_{AN}), we also observed significant levels of ¹⁵NO₃⁻ in soil water leachates, although the ¹⁵N at. % of NO₃⁻ did not show well-defined peaks but rather extended out over the 2012 growing season, never exceeding 2% (AN > R_{AN}). From the relatively dry winter season of 2012–2013 onward, the ¹⁵N at. % of NO₃⁻ was small and did not differ significantly between SN and AN (Figure S3). A similar tendency was observed for R_{SN} relative to R_{AN}.

The cumulative ¹⁵N recovery in soil leachates (at 30 cm depth) in the first week after ¹⁵N application was largest for SN (22%) and smaller for the other treatments (1.6%, 3.4%, and 5.8% at the R_{AN}, R_{SN}, and AN plots, respectively; Figure 1). After four more weeks (by 29 July 2012), the cumulative ¹⁵N recovery in soil leachates increased to 6.0%, 10%, 16%, and 51% at the R_{AN}, R_{SN}, AN, and SN plots, respectively. The relatively large ¹⁵N recovery in leachates in the initial months after ¹⁵N application, in particular at SN plots, was associated with intense summer rainfall (Figure S1). By November 2012, the cumulative ¹⁵N recovery in soil leachates reached a plateau in all treatments (Figure 1). Due to the sharp decrease in soil water flux (Figure S1) and the decline in ¹⁵NO₃⁻ signal in winter 2012 (Figure S3), the increment of ¹⁵N recovery in soil leachates became small in the dormant season (Figure 1). However, even the summer monsoon of 2013 did not cause a major increase in ¹⁵N recovery in soil leachates. In contrast to 2012, the growing season of 2013 showed a similar, albeit small,

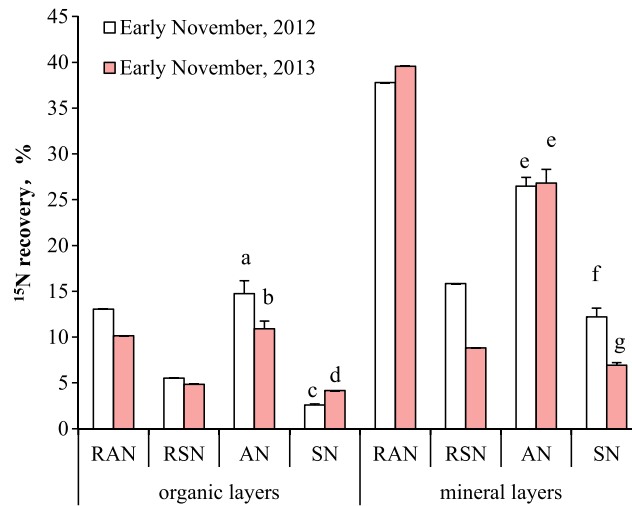


Figure 2. ¹⁵N recovery in different soil layers for different treatments in early November of 2012 and 2013, respectively. The error bars show standard deviations. The different letters above the bars indicate significant difference between treatments and years ($p \leq 0.05$, one-way ANOVA). Data for R_{AN} and R_{SN} treatments are indicative only, as they were not replicated.

increment in ¹⁵N recovery for both of the AN and SN treatments. By November 2013, the cumulative ¹⁵N recovery in leachates was significantly greater for SN (80%) than AN treatments (43%) ($p \leq 0.05$; Figure 1). The ¹⁵N recovery in soil leachates for the R_{SN} (48%) and R_{AN} treatments (20%) were about half of those found for the SN and AN treatments.

3.3. ¹⁵N Recovery in Soil

In early November 2012, about 4.5 months after ¹⁵N tracer application, the ¹⁵N at. % of bulk soil had increased significantly for the AN and SN treatments, particularly in the organic horizons (LF and H/A; Figure S4). The ¹⁵N at. % in the LF and H/A layers was significantly greater for the AN treatment than the SN treatment ($p \leq 0.05$), despite

the fact that the ¹⁵N dose in the AN treatment was only half of that in the SN treatments (Table S1). Also, R_{AN} showed this tendency, with increased ¹⁵N at. % levels compared to R_{SN} , but this was found only in the LF layer, not in the underlying H/A horizon and mineral soil. From the winter of 2012 onward, the ¹⁵N at. % of soil for the AN treatment decreased significantly in the LF horizon ($p \leq 0.05$; Figure S4) but not in the deeper soil layers. By contrast, for the SN treatments, the ¹⁵N at. % values increased significantly in the LF and H/A horizons ($p \leq 0.05$).

A major fraction of the applied ¹⁵N was recovered in the bulk soil. In early November 2012, when ¹⁵N levels in soil water had returned to relatively low values (Figure S3), the total ¹⁵N recovery in bulk soil for the AN treatment (42%) was significantly greater than for the SN treatment (16%) ($p \leq 0.05$; Figure 2). Similarly, the ¹⁵N recovery in soils at the R_{AN} plot (52%) was greater than at the R_{SN} plot (22%). There was no significant change in the total soil pool of ¹⁵N between 2012 and 2013.

3.4. ¹⁵N Recovery in Trees and Ground Vegetation

In November 2012, the ¹⁵N at. % values in aboveground tree biomass, ground vegetation, and fine roots in each soil layer (except those in the top mineral soil layer) were significantly greater for the SN treatment than for the AN treatment ($p \leq 0.05$; Figure S5). The difference had become quite small in November 2013, when the ¹⁵N at. % in the plant components dropped significantly for both treatments ($p \leq 0.05$).

In November 2012, the ¹⁵N recovery in aboveground tree biomass, ground vegetation, and fine roots was modest, ranging from 0.6% to 8.2% (Figure 3). In general, ¹⁵N recovery in plant components was larger for the AN treatment than that for the SN treatment ($p \leq 0.05$). Most ¹⁵N was recovered in aboveground tree biomass. A similar tendency was observed for the R_{AN} and R_{SN} treatments, with greater values for the former. In 2013, there was a significant decline in ¹⁵N recovery, in particular for the ground vegetation and the roots, which returned to near-natural abundance levels. While the decline in ¹⁵N recovery of tree biomass was moderate, but significant at the AN and SN plots, no decline was found at the R_{AN} and R_{SN} plots.

3.5. ¹⁵N Recovery in N₂O Emission

The average annual N₂O fluxes in the REF treatments (R_{AN} , R_{SN} , and R_0) were 0.21 g N m⁻² yr⁻¹ (both in 2012 and 2013; Figure 4a). These fluxes were about half of those measured for the AN and SN treatments under long-term fertilization and of those reported earlier at TSP by Zhu *et al.* [2013a] during 2009–2010. The N₂O fluxes for the SN treatment (0.45 g m⁻² yr⁻¹) were significantly greater than those for the AN treatment (0.36 g m⁻² yr⁻¹) ($p \leq 0.05$; Figure 4a).

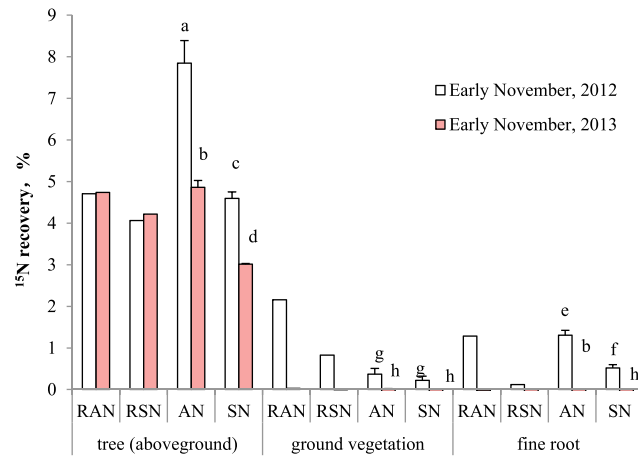


Figure 3. ¹⁵N recovery in plants and roots for different treatments in early November, 2012 and 2013. The error bars show standard deviations. The different letters above bars indicate significant difference between treatments and years ($p \leq 0.05$, one-way ANOVA). Data for R_{AN} and R_{SN} treatments are indicative only, as they were not replicated.

In the first week after ¹⁵N application (24 June 2012), the ¹⁵N at. % of emitted N₂O increased from natural abundance to 0.9%, 2.3%, 1.8%, and 7.7% for the R_{AN}, R_{SN}, AN, and SN treatments, respectively (Figure S6). Values were significantly larger for the SN treatment than the AN treatment ($p \leq 0.05$, one-way ANOVA), and the same trend was found for the R_{SN} and R_{AN} treatments. The ¹⁵N at. % of N₂O returned to near-background levels in October 2012 (16 weeks after ¹⁵N application). Emitted N₂O accounted for only a small fraction of the recovered ¹⁵N. During the first half year following ¹⁵N application, greater cumulative ¹⁵N recovery rates in N₂O were found for the SN treatment (~5%) than for the AN treatment (~2%) (Figure 4b). A similar trend was found for the R_{SN} and R_{AN} treatments.

3.6. Total ¹⁵N Recovery and Implications for N Leaching and Immobilization

The total recovery of ¹⁵N was close to 100% for the SN treatment in November 2012, more than 4 months after ¹⁵N application (Figure 5). For the AN treatment, the total ¹⁵N recovery was slightly less than 90% in 2012 and did not increase significantly in 2013. Considerably, smaller total ¹⁵N recoveries were found at the REF plots (74% to 76%), possibly due to the uncertainty associated with small application rates of ¹⁵N.

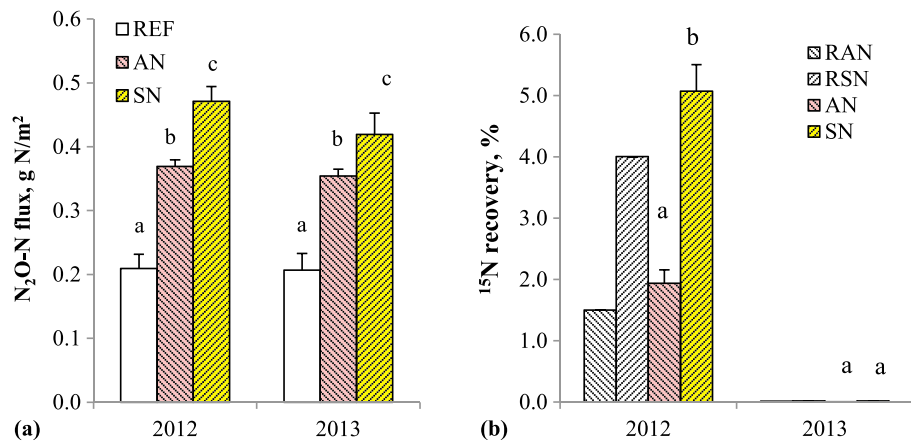


Figure 4. (a) Average annual N₂O-N fluxes for the different treatments, where REF is based on data from the R₀, R_{AN}, and R_{SN} plots. The error bars are standard deviations. Figure 4b. ¹⁵N recovery in emitted N₂O-N, where R_{AN} and R_{SN} data are based on values for one single plot each (viz. no replication) in 2012 and 2013. The error bars for AN and SN show standard deviations. The different letters above bars indicate significant difference between treatments ($p \leq 0.05$, one-way ANOVA).

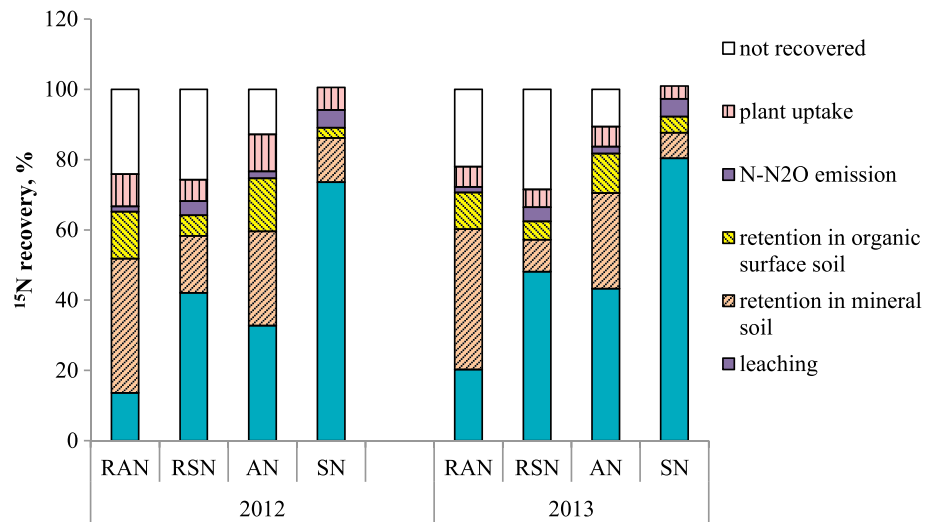


Figure 5. Cumulative ¹⁵N recoveries for different treatments from 24 June 2012 to early November 2012 and to early November 2013, respectively.

The distribution of ¹⁵N recoveries among the different sinks changed little in the second year (2013; Figure 5). In general, there was continued “bleeding” of ¹⁵N, as NO₃⁻, but this occurred at a small, yet similar, rate at the AN and SN plots (Figure 1). Most of this continued ¹⁵N leaching was derived from the ¹⁵N pools in soil (LF layer) and vegetation (Figures S4 and S5). For example, in 2013, no ¹⁵N remained in ground vegetation and fine roots, while its content significantly decreased in pine needles (Figure S5). For R_{AN} and R_{SN}, the bleeding of ¹⁵N was smaller but similar for the two treatments (Figure S1).

Based on the ¹⁵N recovery data in soil water in 2012 (Figure 5), we estimated direct leaching rates of NO₃⁻-N derived from the input of fresh N (NO₃⁻ and NH₄⁺) in 2012 (Table 2). The calculated rates amounted to 43%, 72%, and 78% of the measured total NO₃⁻-N leaching rates at REF, AN, and SN plots, respectively. Assuming that the N stock in vegetation is in steady state and the production of N₂ by denitrification in hill slope soils is negligible [Zhu *et al.*, 2013b; Yu *et al.*, 2016], we can estimate net mineralization rate of previously stored (unlabeled) N in the ecosystem by subtracting calculated direct leaching rates of N, freshly added in 2012, from the sum of the measured leaching of total inorganic N and the emission rate of N₂O-N (Table 2). Taking into account the uncertainty in the calculated rates, the increase in N input from 5.3 g m⁻² yr⁻¹ to

Table 2. Rate of N Transformation and Leaching and Immobilization (g N m⁻² yr⁻¹), Based on ¹⁵N Recovery, Total Annual N Fluxes and N Balance in 2012

	2012					
	REF		AN		SN	
	NH ₄ ⁺	NO ₃ ⁻	NH ₄ ⁺	NO ₃ ⁻	NH ₄ ⁺	NO ₃ ⁻
N input rate ^a	2.6	2.7	4.7	4.8	2.6	6.9
Actual N leaching rate ^a	0.0	3.5	0.1	6.9	0.1	7.5
N leaching rate from fresh N input ^b		0.4 + 1.1		1.6 + 3.6		0.4 + 5.1
N leaching rate from mineralized N ^c		2.2		2.4		2.6
Contribution of fresh N to leaching (%)		43%		72%		78%
Apparent soil N immobilization rate ^d	1.3	0.6	2.0	0.8	1.1	1.1

^aAccording to Table 1 (g N m⁻² yr⁻¹).

^bCalculation method for REF plots (g N m⁻² yr⁻¹): (NH₄⁺-N input * ¹⁵N recovery (%) in leachates at R_{AN} + NO₃⁻-N input * ¹⁵N recovery (%) in leachates at R_{SN})/100. Calculation method for AN and SN plots: (NH₄⁺-N input * ¹⁵N recovery (%) in leachates at AN + NO₃⁻-N input * ¹⁵N recovery (%) in leachates at SN)/100.

^cDifference between actual N leaching and leaching from fresh N input (g N m⁻² yr⁻¹).

^dCalculation method for REF plots (g N m⁻² yr⁻¹): (NH₄⁺-N input * ¹⁵N recovery (%) in soils at R_{AN} + NO₃⁻-N input * ¹⁵N recovery (%) in soils at R_{SN})/100. Calculation method for AN and SN plots: (NH₄⁺-N input * ¹⁵N recovery (%) in soils at AN + NO₃⁻-N input * ¹⁵N recovery (%) in soils at SN)/100.

$9.5 \text{ g m}^{-2} \text{ yr}^{-1}$, through the addition of NH_4NO_3 (AN treatment) or NaNO_3 (SN treatment), had little effect on N mineralization rate (about $2.2\text{--}2.6 \text{ g m}^{-2} \text{ yr}^{-1}$; Table 2). By contrast, the N immobilization rate increased with increasing N input, in particular if added as NH_4^+ (Table 2 and Figure 5). For the AN treatment, the N immobilization rate was slightly greater than the mineralization rate, indicating increased N retention under elevated NH_4^+ deposition.

4. Discussion

4.1. Fate of N Input

In early November 2012, nearly half a year after ^{15}N application, the total recovery of ^{15}N ranged from 88 to 101% (Figure 5) for the AN and SN treatments, respectively, whereas for R_{AN} and R_{SN} this was 76% and 74%, respectively. These values are similar to those reported for temperate and boreal forests [Tietema *et al.*, 1998b; Schleppi *et al.*, 1999; Nadelhoffer *et al.*, 2004; Wessel *et al.*, 2013] and for subtropical forest in South China [Sheng *et al.*, 2014].

For the SN treatment, ^{15}N , added as NO_3^- , was predominantly recovered as leached NO_3^- (74%, 6 months after label addition), while ^{15}N addition as NH_4^+ (the AN treatment) resulted in a much smaller ^{15}N fraction recovered in the leachates (33%; Figure 5), also as NO_3^- . For the R_{SN} and R_{AN} treatments, ^{15}N leaching contributed significantly less (42% and 14%, respectively) to total recovery. The N leaching rates were far greater than reported for a tropical forest at Dinghushan under similar ambient N deposition (15%) [Gurmesa *et al.*, 2016]. Immobilization of ^{15}N in the soil was the second most important N sink at TSP, showing greater values if the label was added as NH_4^+ (42% at the AN plots and 51% at the R_{AN} plot) than if added as NO_3^- (16% at the SN plots and 22% at the R_{SN} plot; Figure 5). A similar retention of $^{15}\text{NH}_4$ in the soil has been reported in several earlier studies [Emmett *et al.*, 1995; Buchmann *et al.*, 1996; Feng *et al.*, 2008; Sheng *et al.*, 2014]. This may be due to sorption of NH_4^+ to soil clays and organic matter [Tietema *et al.*, 1998b; Kjønaas and Wright, 2007] or to net N immobilization in microbial biomass [Tahovska *et al.*, 2013]. At TSP, most of the $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ retention occurred in the mineral soil and less in the organic layers (Figure 5). This is in contrast to temperate forests, where relatively large ^{15}N immobilization was reported for organic layers (11–65%) compared to the mineral soil (1–39%) [Buchmann *et al.*, 1996; Koopmans *et al.*, 1996; Tietema *et al.*, 1998b; Schleppi *et al.*, 1999; Nadelhoffer *et al.*, 2004; Wessel *et al.*, 2013]. Probably, the warm-humid monsoonal climate at TSP facilitates relatively fast turnover of litter, resulting in a thin organic layer having a relatively small capacity to accumulate N [Yu *et al.*, 2017]. The limited capacity of the organic horizon in monsoonal, subtropical forest to immobilize ^{15}N is in line with the findings from a ^{15}N tracer experiment conducted in the tropical forest at Dinghushan, South China [Gurmesa *et al.*, 2016].

For all treatments, much of the $^{15}\text{NO}_3^-$ leaching occurred in the initial weeks after fertilization (Figure 1), in the midst of the growing season, facilitated by intensive rainstorms (Figure S1). The rapid leaching of added ^{15}N as NO_3^- , which also has been reported earlier for TSP [Yu *et al.*, 2017], reinforces the extreme case of N saturation at this site [Huang *et al.*, 2015].

The recovery of ^{15}N in plant biomass, about 4.5 months after its application, was modest ranging from 6% (for the SN treatment) to 11% (for the AN treatment). This was considerably smaller than previously reported values for temperate forests (10–45%), at N input rates of $1\text{--}9 \text{ g N m}^{-2} \text{ yr}^{-1}$ [Nadelhoffer *et al.*, 2004; Schleppi *et al.*, 1999; Tietema *et al.*, 1998b; Wessel *et al.*, 2013; Buchmann *et al.*, 1996; Koopmans *et al.*, 1996; Zak *et al.*, 2004]. Probably, the minor net assimilation of N at TSP is related to poor forest growth, which has been attributed to soil acidification and phosphorus (P) limitation [Huang *et al.*, 2015; Wang *et al.*, 2007]. The significantly larger ^{15}N recovery in plant biomass for the $^{15}\text{NH}_4^+$ treatments compared to $^{15}\text{NO}_3^-$ (Figure 3) suggests the preference for NH_4^+ uptake [Preston and Mead, 1994; Stark and Hart, 1997]. This preference may be species-dependent as suggested by Nadelhoffer *et al.* [1999], who observed a larger uptake of $^{15}\text{NO}_3^-$ than $^{15}\text{NH}_4^+$ by trees in oak and red pine forests.

The cumulative recovery of ^{15}N in emitted N_2O was significantly greater for the SN and R_{SN} treatments (about 5%) than for the AN and R_{AN} treatments (2%). The ^{15}N at. % of emitted N_2O from all treatments showed a significant 1:1 relationship relative to $^{15}\text{NO}_3^-$ in soil water at 5 cm soil depth ($p \leq 0.05$; Figure 6a), confirming the importance of denitrification as the dominant N_2O source, as previously shown for TSP by Zhu *et al.* [2013b] and Yu *et al.* [2017]. However, by contrast to Zhu *et al.* [2013a], we found not only soil moisture

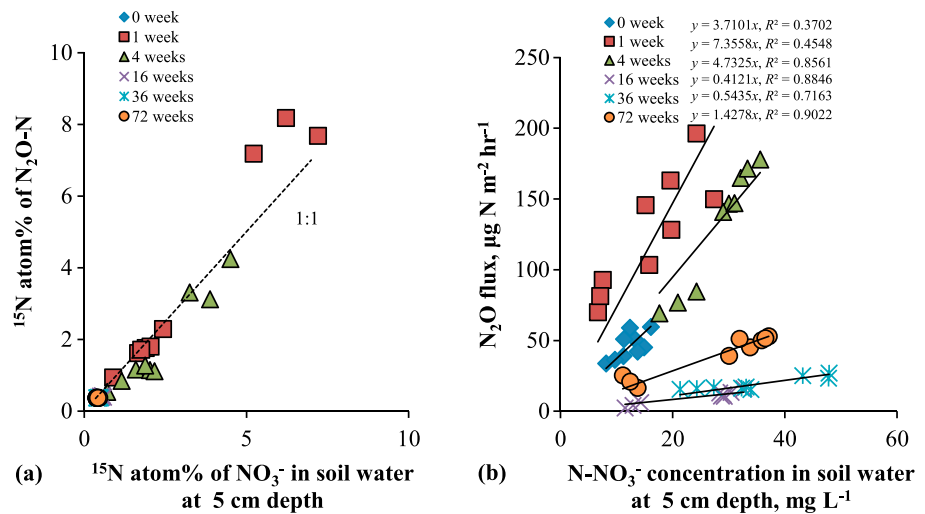


Figure 6. (a) Relationship between ^{15}N at. % of $\text{N}_2\text{O}-\text{N}$ and ^{15}N at. % of NO_3^- in soil water at 5 cm depth for all treatments. (b) Relationship between N_2O flux and NO_3^- -N concentration in soil water at 5 cm depth for all treatments.

and soil temperature but also the NO_3^- concentration in soil water at 5 cm depth, to be an important explanatory variable for N_2O emission, as shown by their significant positive relationship ($p \leq 0.05$; Figure 6b). In accordance with Zhu *et al.* [2013a], N_2O emission has a clear seasonality with greater flux values (up to $200 \mu\text{g N m}^{-2} \text{h}^{-1}$) in the warmer and wetter summer months (0, 1, and 4 weeks after ^{15}N addition on 24 June 2012) and smaller values in the colder and drier months (16, 36, and 72 weeks after 24 June 2012; Figure 6b).

4.2. N Balance and N Turnover Rates

Based on ^{15}N tracer recovery, we estimated the leaching of freshly added N, within half a year after its application, to be 72% and 78% of the total N leaching for the AN and SN treatments, respectively (Table 2). This proportion was larger than for REF (43%), indicating that a doubling of N input leads to rapid leaching of most of the added N fertilizer (86% and 95% of the added N fertilizer leached during the first half year of its application at AN and SN, respectively). At the same time, a relatively large proportion of freshly added N to the REF plots was recovered in the soil (Figure 5). The large leaching rate of $^{15}\text{NO}_3^-$ for the SN treatment suggests that atmo-genic NO_3^- is quickly leached, indicating that it behaves like an inert ion, without being processed in the ecosystem.

The leaching of NH_4^+ , even at the AN plots where extra NH_4^+ was added, was negligible, and a significant proportion (33%; Figure 5) of the added $^{15}\text{NH}_4^+$ was effectively nitrified and leached as $^{15}\text{NO}_3^-$ in soil water within half a year after fertilizer application. Simultaneously, 42% of the ^{15}N label was immobilized in the soil. In addition, minor fractions were assimilated by the vegetation and lost as N_2O and possibly some N_2 , due to denitrification. As indicated by the N mass balance (Table 1) [Huang *et al.*, 2015], ^{15}N immobilized in the system is close to being balanced by leaching of “old” ^{14}N .

Based on ^{15}N recovery in soils, we found that the apparent immobilization rate of NH_4^+-N was close to 50%, whereas this was about 20% for $\text{NO}_3^- -\text{N}$. By contrast, the N mineralization rate seemed little affected by input N flux or N form ($2.2\text{--}2.6 \text{ g N m}^{-2} \text{ yr}^{-1}$ for all treatments; Table 2). This differs from observations in the N-saturated tropical forest at Dinghushan, where a long-term N manipulation ($150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) experiment showed that the net mineralization rate decreased with elevated NH_4^+ input. This was attributed to reduced microbial activities by aggravated C-limitation under extremely high N level [Chen *et al.*, 2016]. In particular, for the AN treatment, the N immobilization rate was greater than the mineralization rate (Table 2), indicating minor net accumulation of N in the soil accounting for about 5% of the ambient annual N input.

The emission of N_2O in the relatively dry summer of 2012 was small compared to previously reported fluxes for the wet summers of 2009 and 2010 [Zhu *et al.*, 2013a]. The relatively small N_2O flux in the dry summer of

2012 is as expected, because denitrification is the predominant N production process at TSP (Figure 6a). A doubling of the input of N resulted in a doubling of the N_2O emission rate, in particular for the SN treatment. This is directly related to the strong increase in NO_3^- concentration for the SN treatment. The increase in NO_3^- concentration, and thus the production of N_2O , is slightly less for the AN treatment, due to the immobilization of NH_4^+ (Table 2).

4.3. Ecosystem Response to Further Increases in N Deposition

The TSP site has been N saturated for more than a decade [Chen *et al.*, 2004]. At ambient N deposition levels (about $5 \text{ g m}^{-2} \text{ yr}^{-1}$), the contribution of direct N leaching of freshly added N within 1 year was 43% at the REF plots, while the remainder derived largely from N mineralization (Table 2). N mineralization was roughly balanced by immobilization of N in soils. The limited capacity of the soils at TSP to immobilize N (maximum 36% of N input at the REF plots, according to Table 2) in comparison with rates reported for other sites (e.g., around 30% at four NITREX sites in Europe with similar N deposition as TSP) [Tietema *et al.*, 1998b] may be due to the small pool of soil organic matter at TSP [Huang *et al.*, 2015]. In addition, the N retention capacity of the vegetation is relatively small at TSP, due to limited forest growth [Wang *et al.*, 2007; Huang *et al.*, 2015]. Indeed, the ^{15}N recovery in plant biomass at the end of the first growing season was below 10%, whereas this was larger than 20% at Dinghushan [Gurmesa *et al.*, 2016]. Doubling of N deposition at the N-saturated forest at TSP resulted in a near-complete leaching of the added N, particularly if added as NO_3^- . In addition, this caused a doubling of the N_2O emission, as well as a strong increase in soil acidification and mobilization of aluminum [Huang *et al.*, 2015].

5. Conclusions

The ^{15}N labeling experiment confirms the extreme case of N saturation in the Tieshanping subtropical forest in Chongqing, Southwest China. Nitrogen input was nearly quantitatively converted (about 80%) to NO_3^- -N in soil water leachates, with slightly, but significantly less leaching if N is added as NH_4^+ rather than NO_3^- . In addition, our data indicate that N immobilization in soil is greater if added as NH_4^+ than as NO_3^- . Acid forest soils, low in organic matter, like those at TSP have little capacity to immobilize NO_3^- , so that it largely leaches as a mobile anion. With respect to NH_4^+ , two main processes occur. One fraction of NH_4^+ , increasing from about 15% at ambient conditions to about 35% at twice the ambient input, is nitrified and leached. A second fraction is immobilized in the soil, while at the same time native soil N is mineralized and nitrified at a rate of about $2 \text{ g m}^{-2} \text{ yr}^{-1}$. The future increase in the contribution of NH_4^+ to N deposition may thus increase N immobilization.

Acknowledgments

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