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Nitrogen leaching in response to increased nitrogen inputs in subtropical monsoon forests in southern China

Yunting Fang [a,b,*], Per Gundersen [b], Jiangming Mo [a], Weixing Zhu [c]

[a] *South China Botanical Garden, The Chinese Academy of Sciences, Xingke Road No. 723, Tianhe District, Guangzhou 510650, China*
[b] *Forest & Landscape Denmark, Faculty of Life Sciences, University of Copenhagen, Hørsholm Kongevej 11, DK-2970 Hørsholm, Denmark*
[c] *Department of Biological Sciences, State University of New York - Binghamton, Binghamton, NY 13902, USA*

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ABSTRACT

Dissolved inorganic nitrogen (DIN) (as ammonium nitrate) was applied monthly onto the forest floor of one old-growth forest (>400 years old, at levels of 50, 100 and 150 kg N ha^{-1} yr^{-1}) and two young forests (both about 70 years old, at levels of 50 and 100 kg N ha^{-1} yr^{-1}) over 3 years (2004–2006), to investigate how nitrogen (N) input influenced N leaching output, and if there were differences in N retention between the old-growth and the young forests in the subtropical monsoon region of southern China. The ambient throughfall inputs were 23–27 kg N ha^{-1} yr^{-1} in the young forests and 29–35 kg N ha^{-1} yr^{-1} in the old-growth forest. In the control plots without experimental N addition, a net N retention was observed in the young forests (on average 6–11 kg N ha^{-1} yr^{-1}), but a net N loss occurred in the old-growth forest (−13 kg N ha^{-1} yr^{-1}). Experimental N addition immediately increased DIN leaching in all three forests, with 25–66% of added N leached over the 3-year experiment. At the lowest level of N addition (50 kg N ha^{-1} yr^{-1}), the percentage N loss was higher in the old-growth forest (66% of added N) than in the two young forests (38% and 26%). However, at higher levels of N addition (100 and 150 kg N ha^{-1} yr^{-1}), the old-growth forest exhibited similar N losses (25–43%) to those in the young forests (28–43%). These results indicate that N retention is largely determined by the forest successional stages and the levels of N addition. Compared to most temperate forests studied in Europe and North America, N leaching loss in these seasonal monsoon subtropical forests occurred mainly in the rainy growing season, with measured N loss in leaching substantially higher under both ambient deposition and experimental N additions.

1. Introduction

Tropical and subtropical forests in southern China play a pivotal role in maintaining local climate conditions and regulating regional soil–atmospheric exchange of carbon (C) and nitrogen (N) (Fang et al., 2001; Zhou et al., 2006). Nitrogen deposition in precipitation greater than 10 kg N ha^{-1} yr^{-1}, the threshold above which N leaching often increases in temperate and boreal forests (e.g. MacDonald et al., 2002), has frequently been reported in areas of southern China associated with rapid economic growth (Zhou and Yan, 2001; Chen and Mulder, 2007). This widespread elevated atmospheric N deposition, which may have persisted for more than a decade in some parts of the region (Huang et al., 1994), is expected to change the C and N cycles in forest ecosystems. However, concerns over the impact of increased N deposition are still at the starting stage. Little is known if the currently deposited N would change forest N status, the fate of deposited N, and the rate of N leaching (Fang et al., 2006; Chen and Mulder, 2007).

Nitrogen retention varies widely among forest ecosystems, and the understanding of the patterns and mechanisms of N retention are central to our understanding of N cycling in ecosystems. Concerns over adverse effects of N deposition including soil acidification, nutrient imbalance and even forest decline have initiated many studies in temperate forests of North America and Europe in the last decades (Aber et al., 1989, 1998; Gundersen et al., 1998). Measurements along N deposition gradients and from experimental N manipulation studies showed that temperate forests exhibited high efficiency of N retention, which consequently could delay the occurrence of potential negative impacts induced by increased N deposition (Aber et al., 1998; Gundersen et al., 1998). In contrast to temperate forests, negative effects of elevated N deposition have been suggested to occur rapidly in

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[*] Corresponding author at: South China Botanical Garden, The Chinese Academy of Sciences, Xingke Road No. 723, Tianhe District, Guangzhou 510650, China. Tel.: +86 758 2621915; fax: +86 758 2623242.
E-mail address: fangyt@scbg.ac.cn (Y. Fang).

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tropical and subtropical forests (Matson et al., 1999; Chen and Mulder, 2007), because most forests under warm and humid climate tend to cycle N fast and have high N availability but low available phosphorus (P) and other nutrients (Vitousek, 1984). However, this general hypothesis has only been tested in Hawaiian wet tropical forests (Lohse and Matson, 2005) and is questioned by other researchers (Adams et al., 2004). Positioned near the Pacific Ocean to the east and the Indian Ocean to the south, southern China has a monsoon climate with a high abundance of heat, light, and water throughout the rainy growing season when a major fraction of the N deposition also occurs (Zhou and Yan, 2001; Chen and Mulder, 2007; Fang et al., 2008), and a distinct dry season in late autumn and winter. Elevated N deposition thus coincides with the most productive season in these forests and may be retained by biological processes. On the other hand, high water fluxes and favorable temperature may stimulate soil N transformations and increase N leaching. In this kind of seasonal subtropical forest, the balance between biological uptake, hydrologic flow rate and contact time likely determine the fate of deposited N in the rainy season, whereas deposited N is likely retained throughout the dry season where plants remain productive and flows are minimal (Fang et al., 2008).

Traditionally, N retention has been thought to be mainly controlled by biological N demand by plants and microbes. Nutrient retention theory predicts that the losses of essential and limiting nutrients such as N should increase as forest ecosystems approach a steady state, the point that output equals input (Vitousek and Reiners, 1975). Nitrogen saturation theory predicts that N leaching should increase in response to elevated N deposition, and the response depends largely on the initial N status (Aber et al., 1989, 1998; Gundersen et al., 1998). Thus both theories predict that there will be more N leaching from the older forest than the younger forest under elevated atmospheric N deposition, since the old-growth forests have lower N accretion and higher initial (inherent) N availability. However, recent evidence from N fertilization studies in temperate forests indicates that soils, rather than plants, are the dominant long-term sink for applied N, by inference, for N from atmospheric deposition (Nadelhoffer et al., 1999; also see Davidson et al., 2003). Several studies show that N losses from old-growth forests, or those in which overstory biomass accumulation has ceased, have been lower than predicted by the nutrient retention theory (Fisk et al., 2002). The results from the N addition experiments performed in Hawaiian wet tropical forests showed that both young and old forests responded immediately to elevated N input with increased NO_3^--N leaching following N addition, but with more NO_3^- losses in the younger forest despite its rapid biomass accumulation (Lohse and Matson, 2005). These studies highlight the importance of other factors in addition to plant uptake in regulating N leaching.

To better understand the risks and consequences of N saturation in warm and humid climate, where seasonal tropical and subtropical forests are located, a N addition experiment was started in Dinghushan Biosphere Reserve (DHSBR), southern China in July 2003. The DHSBR includes an old-growth evergreen broadleaved forest and two younger forest types (Fang et al., 2006). Inorganic N at levels of 50–150 kg N ha^{-1} yr^{-1} was sprayed monthly on the forest floor to simulate predicted future increase in N deposition in the region (Zheng et al., 2002). We have previously reported the input and output budget of N (from 2004 to 2005) in these three forest ecosystems under ambient deposition condition (Fang et al., 2008). In this paper, we report N leaching response to experimental N additions in the first 3-year study (2004–2006), to investigate how experimentally added N influenced N output in leaching, and whether there were differences between the old-growth and the younger forests in N retention. The seasonal monsoon climate also gives us a rare opportunity to test plant controls on N retention vs. hydrologic controls on N loss, and to compare our results with those conducted in temperate forests.

2. Materials and methods

2.1. Site description

This study was conducted in the Dinghushan Biosphere Reserve in the middle part of Guangdong province, southern China (112°33′E and 23°10′N, Fig. 1). This reserve is 20 km east of Zhaoqing (330 thousand inhabitants), and about 90 km west of metropolitan Guangzhou (10 millions inhabitants). The climate is strongly seasonal, with 75% of the mean annual rainfall of 1927 mm falling from March to August (wet-warm season) and only 6% from December to February (dry-cool season) (Huang and Fan, 1982). Mean annual relative humidity is 80% and mean annual temperature is 21.0 °C, with average temperatures in the coolest month (January) and the warmest month (July) of 12.6 and 28.0 °C, respectively (Huang and Fan, 1982). Total N input in precipitation was 21–38 kg N ha^{-1} yr^{-1} in the 1990s (Huang et al., 1994; Zhou and Yan, 2001; Mo et al., 2002).

The old-growth forest is a regional climax type (monsoon evergreen broad-leaved forest) and has been protected for more than 400 years by monks in the nearby temples (Wang et al., 1982).

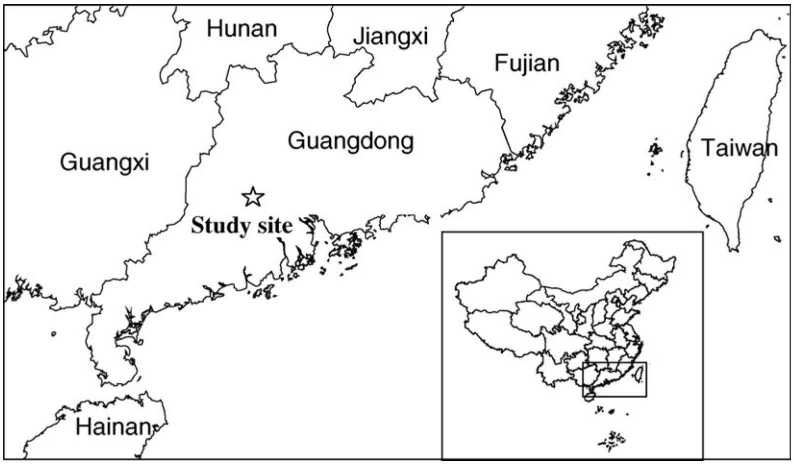

Fig. 1. Location of the study site (DHSBR) in Guangdong Province, southern China (112°33′E and 23°10′N).

The major species are *Castanopsis chinensis*, *Machilus chinensis*, *Schima superba*, *Cryptocarya chinensis*, *Syzygium rehderianum* in the canopy and sub-canopy layers. Both young forests originated from the 1930s clear-cut and subsequent pine plantation (Wang et al., 1982; Fang et al., 2006). The colonization from natural dispersal of regional broadleaf species has changed the plant composition in the mixed forest (major species are *Pinus massoniana*, *S. superba*, and *Castanopsis chinensis*), while the pine forest is still dominated by *P. massoniana* due to continuous human disturbances before 1990 (generally the harvesting of understory and litter, Mo et al., 1995). The old-growth forest had a basal area of almost twice (26.2 m^2 ha^{-1}) those in the pine and mixed forests (14.0 and 13.8 m^2 ha^{-1}), but less litter accumulation in the forest floor (8.9, 23 and 20 Mg ha^{-1} in the old-growth, pine and mixed forests, respectively; Fang et al., 2006). Basal area in the old-growth forest might be underestimated because particularly large trees were not included in our plots.

The three forests have similar elevation, slope aspect and degree. However, the topography is highly heterogeneous within each forest, with slopes ranging from 15° to 35°. The soil is lateritic red earth formed from sandstone (He et al., 1982). The soil depths vary with forest types. In the old-growth forest the soil depth ranges from 30 to 70 cm. The soil is about 40 cm deep in the mixed forest, and generally less than 40 cm in the pine forest. The old-growth forest had significantly higher concentrations of total C, N and P, but lower soil pH, C/N ratio and bulk density than the pine and mixed forests (Table 1). Soil conditions in the pine and mixed forests did not differ significantly (Table 1).

2.2. Experimental N treatment

Four N treatments, control, low-N, medium-N and high-N were established in the old-growth forest, and three N treatments (control, low-N, and medium-N) were established in both the pine and mixed forests in 2003. In each forest, three replicate plots were randomly selected for each level of treatment. There were total 9 plots in the pine forest, 9 plots in the mixed forest, and 12 plots in the old-growth forest. Each plot measures 10 m × 20 m, with about 10 m buffer strips around each plot. The overall size of each forest site within which the plots were nested was approximately 0.7 ha. Total applications were 0, 50, 100, 150 kg N ha^{-1} yr^{-1} for the control, low-N, medium-N and high-N treatments, respectively. Fertilizer additions of dissolved NH_4NO_3 began in July 2003 with equal monthly applications over the study period. For each N application, fertilizer was weighed, mixed with 20 L of water, and applied using a backpack sprayer below the canopy. Two passes were made across each plot to ensure an even distribution of fertilizer. The control plots received the same amount of water (20 L per plot each month) without additional N (Fang et al., 2006).

2.3. Nitrogen deposition and leaching loss

Precipitation, throughfall, surface runoff and soil solution were sampled in the reserve from January 2004 to December 2006. Bulk precipitation was collected in an open area using two glass funnels (15 cm in diameter), each connected to a 2.5 L sampling bottle with a black polypropylene tube. To avoid potential contamination from nearby construction activities, the precipitation collectors were moved to a more protected location in 2006, which may have underestimated precipitation N inputs in that year. To sample throughfall, five collectors made of split longitudinally PVC pipes (intercept area of 0.8 m^2 for each collector) were laid out randomly about 1.3 m above the ground in each forest. Each collector was connected to two 50 L interconnected sealed buckets (to avoid potential overflow) with polypropylene tubes. One of the collectors in the old-growth forest was destroyed by a falling tree during a August 2005 storm. The amount of stemflow has been found to be negligible (Huang et al., 1994) and was not included in this study.

Soil solution was collected from all plots (except in one of the medium-N plots in the old-growth forest due to its shallow soil and rocky substrate). Two replicate zero tension plastic tray lysimeters per plot were installed in April/May 2003, 3–4 months before the experiment (Fang et al., 2006). Each tray lysimeter was 25 cm × 30 cm with rims to help capture and channel the soil solution. Lysimeter pits were excavated with a shovel. A lysimeter installation tunnel was excavated from one side of the pits at 20 cm below the soil surface, and a tray lysimeter was inserted into the tunnel 10 cm from the pit edge and raised to the tunnel ceiling with soil. Each lysimeter was connected to a 5 L bottle using site slope to facilitate water flow and sampling. We chose 20 cm soil depth because previous studies showed that more than 70% of the fine root biomass (<5 mm) was distributed in the upper 20 cm soil in the mixed and old-growth forests (Wen et al., 1999). However, by this method N leaching was probably slightly overestimated, since a fraction of N was likely to be further retained in the deeper soil. Soil solution at 40 cm depth using a ceramic suction cup method was collected in 2004, but the collection was terminated due to technical difficulties. However, the measurements revealed that the difference in annual volume-weighted dissolved inorganic nitrogen (DIN) concentrations was minor between the two soil depths (Fang et al., 2008).

Since the plots are situated on steep slopes, one replicate plot of each treatment in the pine and old-growth forests had been delimited hydrologically in the winter of 2002, by installing plastic and concrete barriers to sample and quantify surface runoff. Soil solution and surface runoff were sampled twice a month (one prior to the N addition and another 15 days after the N addition, Fang et al., 2006) after N additions began.

Samples were filtered within 24–48 h of collection through 0.45 μm filters in the DHSBR laboratory, and then stored in plastic bottles at 4 °C until later chemical analysis. Concentrations of

Table 1

Characteristic of the mineral soil (0–10 cm) in the pine, mixed and old-growth forests at DHSBR in southern China

Parameter	Pine forest	Mixed forest	Old-growth forest	*P*
Bulk density (g cm^{-3})	1.16 (0.03)^{ab}	1.22 (0.03)^{a}	0.98 (0.06)^{b}	0.026
pH (H_2O)	4.04 (0.04)^{a}	3.95 (0.01)^{a}	3.83 (0.02)^{b}	0.003
Total C (%)	2.8 (0.3)^{b}	2.6 (0.3)^{b}	4.6 (0.2)^{a}	0.004
Total N (%)	0.11 (0.01)^{b}	0.10 (0.01)^{b}	0.19 (0.01)^{a}	0.001
C/N ratio	25 (1.1)^{ab}	28 (0.7)^{a}	22.1 (1.3)^{b}	0.05
Total P (%)	0.043 (0.003)^{b}	0.044 (0.004)^{b}	0.059 (0.003)^{a}	0.024
Extractable NH_4^+ (mg N kg^{-1})	3.2 (0.4)^{a}	3.0 (0.2)^{a}	2.0 (0.04)^{a}	0.027
Extractable NO_3^- (mg N kg^{-1})	2.5 (0.4)^{b}	2.8 (0.4)^{b}	11.7 (1.0)^{a}	<0.001

S.E. in parentheses, *n* = 3. For each parameter, means not sharing the same superscript letter were statistically different at *P*-value of 0.05. Data from Fang et al. (2006).

inorganic N (NH_4^+-N and NO_3^--N) was determined for all samples. NH_4^+-N was analyzed by the indophenol blue method followed by colorimetry, and NO_3^--N was analyzed after cadmium reduction to NO_2^--N, followed by sulfanilamide-NAD reaction (Liu et al., 1996). These concentrations were multiplied by the recorded water volume for the same sample date in the same plot, and then summed to determine monthly and annual N fluxes in the unit of kg N ha^{-1} for each plot.

2.4. Statistical analysis

For concentrations of inorganic N in soil solutions, for each forest type and in each of the sample year, we performed a repeated measurement ANOVA (RMANOVA) to identify the overall N treatment effects. One-way ANOVA with Tukey's HSD was performed to test the effect of N addition on annual N loss for each forest type, where N addition and control plots were randomly assigned. Paired *t*-test was used to compare the N concentrations and fluxes between the surface runoff and leaching collected at the 20 cm soil depth at the same site, and surface runoffs between the control pine plot and control old-growth plot through time. We performed correlation analysis to examine the relationships between different variables (e.g. N flux, N concentration, and water flux). Residuals were plotted to examine the equal variance assumptions for the ANOVA. Because forest type (old-growth, mixed, and pine forests) cannot be "truly replicated", we limited our statistical comparisons among forests mainly to the control plots. All analyses were conducted using SPSS 10.0 for Windows. Statistically significant differences were identified when *P*-values <0.05 unless otherwise stated.

3. Results

3.1. Nitrogen deposition in precipitation and throughfall

Annual precipitations were 1327, 1657, and 1850 mm in 2004, 2005 and 2006, respectively, which were lower than the long-term average precipitation rate of 1997 mm in the area (Zhou and Yan, 2001). The rain in all 3 years fell almost exclusively in the rainy season (March to August, 79–83% of the annual precipitation, Fig. 2). Deposition rates of inorganic N in precipitation were 34.2, 31.6, and 16.9 kg N ha^{-1} yr^{-1} in 2004, 2005, and 2006, respectively (Table 2). Lower N deposition in 2006 was probably due to the change of sampling location (see Section 2). Ammonium accounted for 47–68% of the inorganic N input in precipitation.

Throughfall amount varied from 1120 to 1560 mm, accounting for 74–86% of the precipitation amount. There was no significant difference in throughfall amount among three forest types. Inorganic N in throughfall ranged from 23.6 to 26.8 kg N ha^{-1} yr^{-1} -1 in the two young forests, and 29.1–35.4 kg N ha^{-1} yr^{-1} in the old-growth forest (Table 2). Throughfall N inputs were relatively constant in all three forests over the 3 years. In throughfall, NH_4^+-N comprised approximately half (41–58%) of the inorganic N, that percentage was slightly lower than those in precipitation.

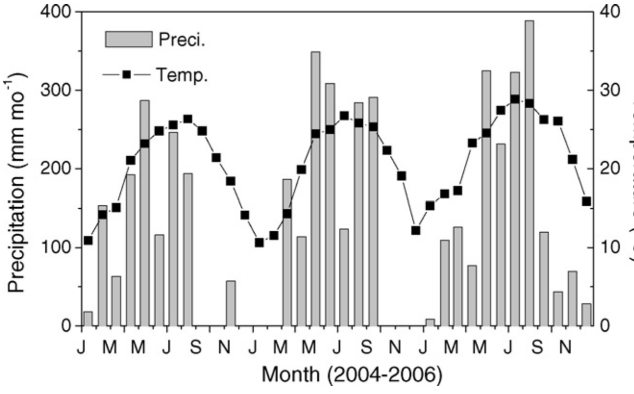

Fig. 2. Monthly precipitation and monthly mean air temperature at DHSBR, southern China, during this study period.

3.2. Nitrogen in leaching soil solution

The amount of soil solution collected at the 20 cm soil depth ranged from 351 to 823 mm, accounting for 31–53% of the corresponding throughfall. Soil solution flux was the highest in the old-growth forest and lowest in the mixed forest (on average 569, 440 and 680 mm in the pine, mixed and old-growth forests, respectively, $P < 0.05$). Both NH_4^+-N and NO_3^--N concentrations in the soil solution displayed a strong seasonality, with low values in the summer months, reflecting possibly both biological N demand and dilution by rainfall (Figs. 3 and 4). However, NH_4^+-N concentrations (annual averages ranging from 0.1 to 1.2 mg N L^{-1}, Fig. 3) were much lower than the NO_3^--N concentrations (annual averages ranging from 2.3 to 13.8 mg N L^{-1}, Fig. 4), even though N treatments added equal input of NH_4^+ and NO_3^- (2–6 times of the ambient throughfall input). Repeated measurement ANOVA in the control plots showed that three forests were not significantly different in NH_4^+-N concentrations, but NO_3^--N concentrations in the old-growth forest (annual means, 5.7–6.8 mg N L^{-1}) were 2–3 times higher than those in the two young forests (2.0–2.9 mg N L^{-1}, *P* from <0.001 to 0.028). The NO_3^--N concentrations between the two young forests were not statistically different.

Additions of inorganic N increased both NH_4^+-N and NO_3^--N concentrations in soil solutions in all three types of forests, but the increases in NH_4^+-N concentration was far smaller than those in NO_3^--N concentration. Statistically significant effects of N treatment on NH_4^+-N were observed only in the first two sample years in the pine forest (Fig. 3). The response of NO_3^--N depended on forest type. In the pine and old-growth forests, the treatment effect

Table 2
Annual fluxes of dissolved inorganic N (kg N ha^{-1} yr^{-1}) in the precipitation and throughfall in the pine, mixed and old-growth forests of DHSBR, southern China

	2004			2005			2006		
	NH_4^+-N	NO_3^--N	DIN	NH_4^+-N	NO_3^--N	DIN	NH_4^+-N	NO_3^--N	DIN
Precipitation[a]	23.2	10.9	34.2	17.8	13.8	31.6	8.1	8.8	16.9
Throughfall									
Pine forest	12.8 (0.6)^a	12.6 (1.0)^b	24.6 (1.4)^b	12.9 (0.7)^a	13.9 (0.6)^b	26.8 (1.3)^b	13.6 (0.8)^a	13.1 (1.0)^a	24.8 (1.4)^a
Mixed forest	15.2 (1.0)^a	11.6 (0.9)^b	26.1 (1.8)^b	11.2 (0.7)^a	11.5 (0.4)^c	22.8 (1.0)^b	12.6 (0.9)^a	12.9 (0.5)^a	23.6 (1.2)^a
Old-growth forest	16.5 (1.5)^a	18.9 (2.1)^a	35.4 (3.4)^a	14.2 (1.1)^a	17.4 (0.9)^a	31.6 (1.5)^a	12.0 (3.1)^a	15.3 (1.8)^a	29.1 (4.5)^a
P	0.104	**0.008**	**0.016**	**0.075**	<0.001	0.001	0.842	0.314	0.331

Note: S.E. in parentheses. For throughfall, $n = 5$, except in the old-growth forest in 2006, where $n = 4$ (see text). In each column, mean throughfall values not sharing the same superscript letter were statistically different between forests, $P < 0.05$. [a]Precipitation collectors were moved to a more protected location in 2006 due to nearby construction.

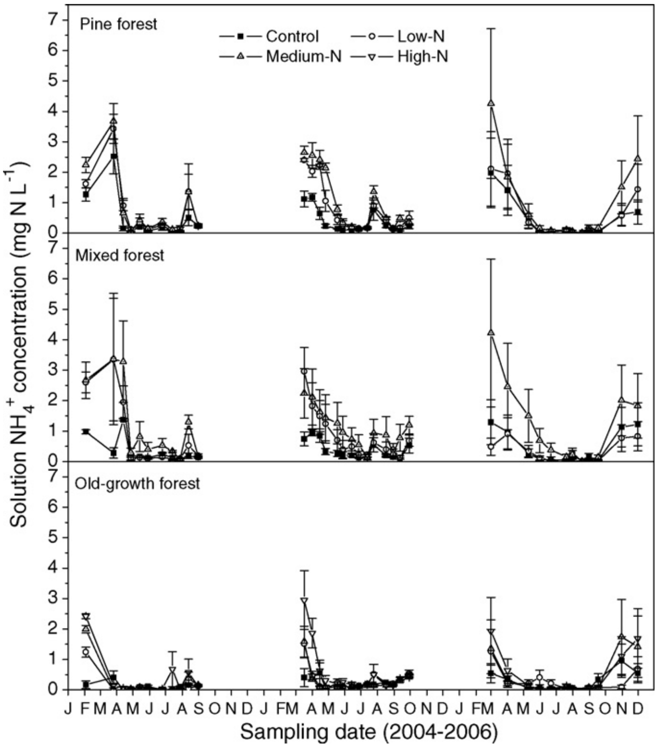

Fig. 3. Concentrations of ammonium (NH_4^+) N in the soil solution collected at the 20 cm depth from the pine, mixed and old-growth forests of DHSBR, southern China; means ($n = 3$ except in the medium-N treatment of the old-growth forest where $n = 2$, see text) with standard error bars. Fertilizer N has been added continuously on the monthly basis; there was no leaching solution during the dry season.

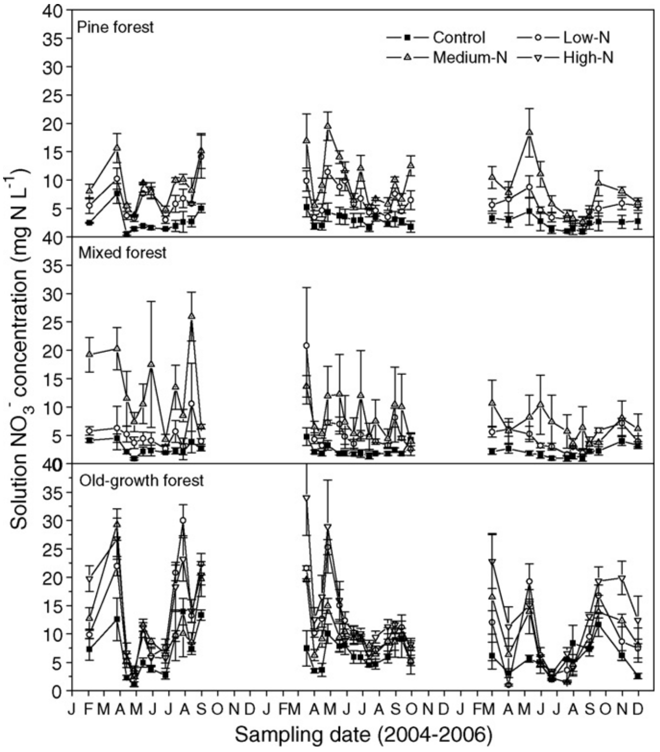

Fig. 4. Concentrations of nitrate (NO_3^-) N in the soil solution collected at the 20 cm depth from the pine, mixed and old-growth forests of DHSBR, southern China. Data arrangement same as in Fig. 3.

on NO_3^--N concentration was statistically significant in all 3 study years (RMANOVA, P from <0.001 to 0.013), whereas in the mixed forest it was significant ($P = 0.015$) only in 2004 (Fig. 4). Annual mean NO_3^--N concentrations in the N-addition plots was 1.5–5.1 times higher than in the controls.

Under ambient condition, DIN losses in the leaching solution in the old-growth forest were 38.7–35.5 kg N ha^{-1} yr^{-1}, significantly higher ($P < 0.001$, two-way ANOVA using year and forest type as main factors) than those in the pine and mixed forests (8.9–20.3 kg N ha^{-1} yr^{-1}, Fig. 5). Additions of N increased DIN fluxes in all three forests, with the N treatment effect statistically significant in the pine forest in all 3 years, and in year 2004 in the mixed and old-growth forests (Fig. 5). Over the 3 years, average DIN loss in the pine forest increased from 15.8 kg N ha^{-1} yr^{-1} in the controls to 34.2 and 58.9 kg N ha^{-1} yr^{-1} in the low-N and medium-N treatments; in the mixed forest, average DIN loss increased from 9.8 to 22.4 and 38.0 kg N ha^{-1} yr^{-1}. In the old-growth forest, averaged DIN loss was 41.4 kg N ha^{-1} yr^{-1} in the controls, and increased to 71.3, 65.6 and 105.2 kg N ha^{-1} yr^{-1} in the N-addition plots (Fig. 5). Ammonium represented 1–17% of DIN export in soil solution, with lower percentages in the old-growth forests than in the two young forests, and lower percentages in the N-addition plots than in the control plots.

In all three forests, monthly variation of N leaching loss depended mainly on downward water movement (Fig. 6, only pine forest data were shown), not the N concentration. The correlation between monthly DIN fluxes and monthly water fluxes in soil solution were significant in all N treatments of the three forests across the 3-year study (r^2 from 0.53–0.78, P-values from <0.001 to 0.036, $n = 23$). High N losses coincided with the high downward

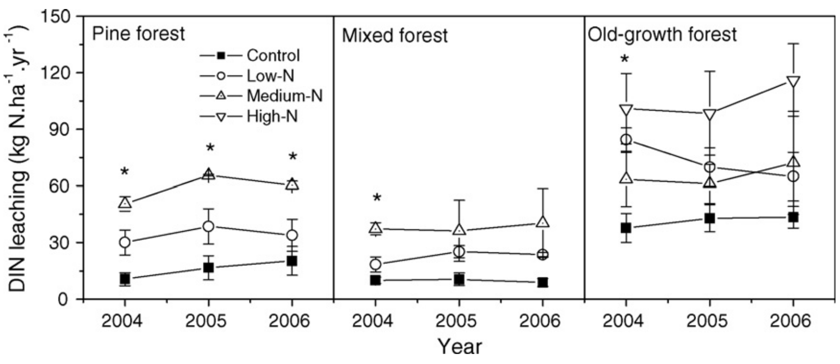

Fig. 5. Responses of annual leaching of dissolved inorganic nitrogen (DIN) in soil solution to N additions. Soil solutions were collected at 20 cm depth in the pine, mixed and old-growth forests of DHSBR, southern China. Means ($n = 3$ except in the medium-N treatment of the old-growth forest where $n = 2$, see text) with standard error bars; asterisk indicates significant treatment effect ($P < 0.05$) of N addition in the sample year.

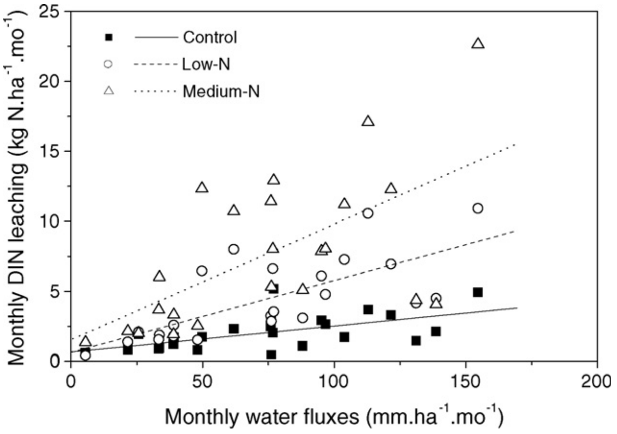

Fig. 6. The relationships between monthly fluxes of water and dissolved inorganic nitrogen (DIN) in the soil solution collected at 20 cm depth in the pine forest across the 3 years of sampling, grouped by N treatments. Each datum point is the mean of three replicated plots. $N = 23$ in each of the N treatments (some months had no leaching collection), $P = 0.003$, 0.000 and 0.002 for the control, low-N and medium-N treatments, respectively.

water movement from March to August, even though N concentrations were low in these months and plant N uptakes were presumably high (Figs. 2–4 and 6).

3.3. Nitrogen in surface runoff

Surface runoff accounted for 9% and 20% of the throughfall input in the pine (101–130 mm) and old-growth forests (225–312 mm), respectively. Under ambient condition, both NH_4^+-N ($P = 0.023$) and NO_3^--N ($P < 0.001$) concentrations in the surface runoff were significantly higher in the pine forest plot than in the old-growth forest plot (paired *t*-test across 33 samplings over 3 years). Average NH_4^+-N concentrations over this period were 1.0 and 0.7 mg N L^{-1} in the pine and old-growth forests, respectively, and average NO_3^--N concentrations were 2.1 and 1.5 mg N L^{-1}, respectively. Neither NH_4^+-N nor NO_3^--N concentrations were significantly altered by N additions during the study (data not shown). In the control plots, N losses via surface runoff were 3.2–4.3 in the pine, and 3.3–3.8 kg N ha^{-1} yr^{-1} in the old-growth forests (data not shown), and thus much lower than the corresponding N loss in soil solution reported above. This suggested that most of the leaching losses moved downward in soil solution, not through surface runoff. Three years of N additions did not significantly affect N export in surface runoff; in the N-addition plots N losses were 2.8–5.6 and 4.2–5.9 kg N ha^{-1} yr^{-1} in the pine and old-growth forests, respectively. Ammonium comprised 18–40% of inorganic N in surface runoff, that percentage was lower than those in the precipitation, but higher than those in soil solution 20 cm below the soil surface (data not shown).

3.4. Total N leaching loss and N retention

To evaluate N losses from both downward leaching in soil solution and surface runoff, we added data from both fluxes to calculate the total N leaching loss and then estimated the overall ecosystem N retention over the 3-year study (Table 3). Because surface runoff was not collected for the mixed forest, we used data from the pine forest to estimate those in the mixed forest, since the floor litter amounts and slopes of the terrain were similar between the two forests.

Our results showed that in the control plots without experimental N addition, a net N retention was observed in the young forests (on average 6–11 kg N ha^{-1} yr^{-1}), but a net N loss occurred in the old-growth forest (−13 kg N ha^{-1} yr^{-1}, Table 3). In the N-addition plots, from 34% to 95% of the total N input was leached from the surface 20 cm of soil (Table 3). Considering the fate of experimentally added N, between 25% and 66% of the addition was found in the output. Both under ambient conditions and following experimental additions, higher leaching losses were found in the pine and old-growth forests than in the mixed forest (Table 3). Total leaching increased linearly with N input across the 10 treatment combinations in the three forests (total leaching = 0.46 total input + 10.3, $P = 0.004$, $r^2 = 0.66$, $n = 10$), but the percentage N retained also increased with N input ($P = 0.002$).

4. Discussion

4.1. Differences in the responses of N leaching among forests

Different types of forest ecosystems have different responses to increased N deposition, and the timing and magnitude of the response depends largely on initial nutrient pools and how close the system is to N saturation (Gundersen et al., 1998; Aber et al.,

Table 3

Average annual total N input (throughfall + experimental additions) and total leaching loss (downward leaching + surface runoff) from 2004 to 2006 in the pine, mixed and old-growth forests of DHSBR, southern China

Forest and N treatment	Total input (kg N ha^{-1} yr^{-1})	Leaching loss (kg N ha^{-1} yr^{-1})[a]	Leaching loss of total input (%)	Leaching loss of added N (%)[b]
Pine forest				
Control	25.4	19.5	77	
Low-N	75.4	38.5	51	38
Medium-N	125.4	62.8	50	43
Mixed forest				
Control	24.2	13.5	56	
Low-N	74.2	26.7	36	26
Medium-N	124.2	41.9	34	28
Old-growth forest				
Control	32.3	45.5	141	
Low-N	82.3	78.5	95	66
Medium-N	132.3	70.3	53	25
High-N	182.3	110.1	60	43

[a] Notice the total leaching loss reported here (soil solution collected at the 20 cm depth plus surface runoff) is different from that reported in Fig. 5 on soil solution alone.

[b] Calculated as the percentage of the increased N output due to N addition (i.e. output from N-treated plots minus that from the control plots) out of the experimental N input.

1998). Forest accumulates organic matter and N in both vegetation and soils, as the ecosystem develops. Old-growth forests can be expected to have larger N pools and higher inherent N availability than the young, successional forests (Vitousek and Reiners, 1975). Therefore, the old-growth forest should lose more N when being exposed to high-level N inputs than the younger forests.

Under ambient N deposition, our results appear to support this view. The old-growth forest had 2–5 times higher DIN loss in soil solution than in the two younger ones (Fig. 5). Greater N pools in soils and vegetation, and faster soil N turnover rates were observed in this old-growth forest. It was estimated that 1546 kg N ha^{-1} was contained in vegetation of the old-growth forest (Yu et al., 1998), but only a third of that (307 kg N ha^{-1}) in the pine forest (Mo et al., 2004). Correspondingly, litter N production was 8 times greater in the old-growth forest (128 kg N ha^{-1} yr^{-1}, Yu et al., 1998) than in the pine forest (16 kg N ha^{-1} yr^{-1}, Mo et al., 2004). Faster N cycling and greater N fluxes in the old-growth forest were also reflected by the higher foliage N and litter N contents (Mo et al., 1995, 2000; Yu et al., 1998) and faster litter decomposition rates (Mo et al., 2006). In the control plots, the total N in the 0–10 cm mineral soils was calculated (from data of Table 1) to be 46% higher in the old-growth forest (1862 kg N ha^{-1}) than in the pine forests (1276 kg N ha^{-1}). Although there was no significant difference in extractable NH_4^+-N between the two forests, extractable NO_3^--N concentration in the old-growth forest was 3–4 times higher at most sampling dates (Table 1). The mixed forest is likely to have similar N turnover rates and pools as the pine forest since they share similar soil and stand characteristics (Table 1).

Under experimental N additions, we observed that the rates of N leaching loss were also higher in the old-growth forest (53–95% of the total inputs) than in the young forests (34–51%, Table 3). However, when considering leaching as the percentage of experimentally added N, the N leaching in the medium- and high-N treatments of the old-growth forest (25–43% of added N) was in the same range of the low and medium N treatments of the two younger forests (26–43%, Table 3). It implies that additional 75–85 kg N ha^{-1} yr^{-1} may have been retained in these old-growth forest plots if other pathways of N losses such as DON or gaseous N exports are not considered (see more below), which were comparable to, or higher than, the 37–72 N ha^{-1} yr^{-1} calculated retention in the two younger forests. These results indicate that N retention is largely determined by the forest successional stages and the levels of N addition. Under ambient N input and low N treatment, the old-growth forest exhibits larger N loss than the younger forests, whereas the difference in N loss among forests becomes smaller when they both received high doses of N input. However, as forest types are not truly replicated, further research may be required to address forest difference in response to N addition.

4.2. Comparisons with N addition experiments in temperate forests

Forests in tropical and subtropical areas have been proposed to have a lower retention capacity to increased N inputs (thereby greater N leaching loss) than those in temperate zone (Matson et al., 1999). Throughfall N inputs were measured at 23–35 kg N ha^{-1} yr^{-1} in the present study, of which 14–46 kg N ha^{-1} yr^{-1} was exported by leaching (Table 3). These N leaching losses fit an empirical regression as a function of throughfall N deposition that has been noted for European forests, where deposition exceeds 25 kg N ha^{-1} yr^{-1}, leaching is consistently high and sites are N-saturated (Dise and Wright, 1995). Our study forests seem to have even higher N loss when compared with most forests receiving long-term comparable fertilizer N input in Europe and North America (Table 4). In these temperate forests, when N additions were 25–50 kg N ha^{-1} yr^{-1}, N leaching losses ranged from 0.7 to 20 kg N ha^{-1} yr^{-1} (but mostly were lower than 10 kg N ha^{-1} yr^{-1}, Table 4). Such losses were mostly lower than those from our forests even under ambient N input (13.5–45.5 kg N ha^{-1} yr^{-1}, Table 3). Leaching losses comprised 0–57% of added N in these temperate forests (Table 4), in comparison with 25–66% in our subtropical forests (Table 3).

The reasons for high N leaching loss observed in our forests are complex. First of all, abundant and intense precipitation in the rainy season reduces the contact time of deposited N with the soil, thus contributing to high N leaching in our forests. In this study, monthly DIN fluxes in soil solution were strongly regulated by water fluxes not only in the control plots but also in the N-treated plots (Fig. 6). Thus the N added would move rapidly with the enhanced water flux regardless of whether or not soil available N exceeds the biotic N requirement. Such hydrologic enhancement of N loss, surpassing biological N retention in the growing season, is likely a unique feature of the subtropical seasonal monsoon forests we have studied. In addition, the first few rains in the rainy season could also flush out the N accumulated during the dry season (Figs. 3 and 4). Secondly, the small organic matter pool in our forest soils may explain in part the low N retention capacity if SOM is the main sink for fertilizer N (see more below). The carbon storage in our soils were shown to be lower than the average forests in China (Fang et al., 2003) and also generally lower than those in European and North American temperate forests (Gundersen et al., 1998; Magill et al., 2004). Finally, the extremely high ambient atmospheric N deposition of 22–39 kg N ha^{-1} yr^{-1} in the last 15 years (Huang et al., 1994; Mo et al., 2002, Table 2), alone and/or in combination with other pollutants, is likely to have diminished the capacity of these forests to retain incoming N. Decrease in plant N demand has already been found in the old-growth forest (Zhang et al., 2002; Guan et al., 2004), as well as a net N loss in the upper 20 soils (Table 3). The ability to sequester additional N might also have been reduced in the two younger forests, because considerable DIN leaching losses had already occurred in the control plots without additional N input (Fig. 5). Compared to forests in many other parts of the world, these subtropical monsoon forests in southern China could be particularly vulnerable to continued N pollutant input predicted in the near future.

4.3. The fate of added N in our study forests

In addition to inorganic N leaching, a fraction of deposited N may be lost via organic N leaching or via gaseous emission, which are not included in the above calculation. Dissolved organic N has been shown to be an important component in overall N fluxes, including in N-saturated forests (McDowell et al., 2004; Pregitzer et al., 2004). Measurement of DON in soil solution in 2005 suggested that DON fluxes could be as high as 6.5–16.9 kg N ha^{-1} yr^{-1}, and that number increased 30–160% further after N additions (Fang, 2006). Increased DON loss driven by experimental N additions ranged from 4 to 13 kg N ha^{-1} yr^{-1}, which comprised 4–17% of their corresponding annual DIN applications (Table 5). This indicates the relative importance of DON as a sink of experimentally added N. However, the old-growth forest was not significantly different in the increased DON loss than the pine forest, although they both had more DON loss than the mixed forest (Table 5).

Ammonia volatilization losses are expected to be minimal in our sites since soils are strongly acidic (pH 3.8–4.1, Table 1). Other nitrogen gases N_2, N_2O and NO could make up an important N loss. However, the emissions of these gases are also shown to be low under acidic conditions and the importance of N_2O increases over N_2 as the soil pH decreases (Simek and Cooper, 2002). In some acid

Table 4
Total N input (throughfall + experimental additions) and leaching loss in the chronic N addition experiments in European and Northeastern American temperate forests

Sites	N treatment	Form of added N	Treatment time (yr)	Total input ($kg\ N\ ha^{-1}\ yr^{-1}$)	Leaching loss ($kg\ N\ ha^{-1}\ yr^{-1}$)	Leaching loss of total input (%)	Leaching loss of added N (%)	Sources
Europe								
Stråsan, Sweden	Control				0.1			Andersson et al. (2001)
	+35	NH_4NO_3	30		0.4–1.2		1–3	
Gårdsjön, Sweden	Control			15	0.6	4		Moldan et al. (2006)
	+40	NH_4NO_3	13	55	4.2	8	9	
Skogaby, Sweden	Control			18	0.1	1		Bergholm et al. (2003)
	+100	NH_4NO_3	11	118	28.5	24	28	
Klosterhede, Denmark	Control			18	0.3	2		Gundersen (1998)
	+35	NH_4NO_3	4	53	4.2	8	11	
Aber, UK	Control			14	18	129		Emmett et al. (1998)
	+35	NH_4NO_3	5	49	20	41	6	
	+35	HNO_3		49	40	82	57	
	+75			89	60	67	27	
Alptal, Switzerland	Control			18	4	22		Hagedorn et al. (2001)
	+30	NH_4NO_3	3	48	8	17	13	
Northeastern America								
Harvard Hardwood	Control			8	0.3	4		Magill et al. (2004)
	+50	NH_4NO_3	15	58	1	2	1	
	+150			158	50	32	33	
Harvard Pine	Control			8	0.3	4		Magill et al. (2004)
	+50	NH_4NO_3	15	58	17	29	33	
	+150			158	80	51	53	
Bear Brooks, watershed	Control			8	0.3	4		Jefts et al. (2004)
	+25.2	$(NH_4)_2SO_4$	13	33.2	7.3	22	28	
Bear Brooks, plot level[a]	Control			5	0.3	6		Magill et al. (1996)
	+25	HNO_3	4	30	1	3	3	
	+45			50	4.5	9	9	
Fernow	Control			15	5.8	39		Adams et al. (1997)
	+35	$(NH_4)_2SO_4$	5	50	9.1	26	9	
Michigan Gradient[b]	Control			9.4	2	21		Pregitzer et al. (2004)
	+30	$NaNO_3$	8	39.4	12	30	33	
Hubbard Brook	Control							Christ et al. (1995)
	+40	$(NH_4)_2SO_4$	2				0	
	+160						0	
	+540						3.6	

[a] means over the study period.
[b] means of the four sites.

forest soils the emissions were almost entirely as N_2O (Wolf and Brumme, 2003). In our acidic study sites, we assume N_2O to dominate over N_2 in denitrification. The annual rates of soil N_2O release were from 2.2–2.8 $kg\ N\ ha^{-1}\ yr^{-1}$ in the control plots (Zhang et al., 2008). Although N treatments significantly increased N_2O emission in the pine and old-growth forests (but not in the mixed forest), the average fertilization-derived N_2O emission was only 0.2–1.2 $kg\ N\ ha^{-1}\ yr^{-1}$ or 0.4–2% of the experimental N additions (Table 5). These results suggest that N_2O is of minor importance in these subtropical monsoon forests in response to elevated N input.

Production of NO can occur during both nitrification and denitrification, and could be important in seasonally dry forests like the ones we have studied, particularly during wet and dry transition time (Davidson et al., 1993). Several researchers reported that NO was a more important gaseous N emitted from N-saturated forest than N_2O, with emission rate of NO being 10 times higher than that of N_2O (Hall and Matson, 2003; Venterea et al., 2003). For instance, annual NO emissions estimated from monthly sampling accounted for 3.0–3.7% of N inputs in the high-N treatment plots and 8.3% of inputs in the low-N plots at the temperate Harvard Forest site (Venterea et al., 2003). Soil NO emission was considered high under ambient N deposition at our study site (4.0–6.9 $kg\ N\ ha^{-1}\ yr^{-1}$ in the pine and old-growth forests in 2005, Li et al., 2007). However, the contribution of soil NO emission to the overall N loss under experimental N input in our forests needs to be further evaluated.

When the losses of DON and N_2O-N are included in the budget calculation (Table 5), the rates of N loss as added N increase to 56% and 57% in the low- and medium-N treatments of the pine forest, and to 36% and 32% in the mixed forest, respectively. In the old-growth forest, the rates of N loss increase to 82%, 31% and 53% in the low-N, medium-N, and high-N plots, respectively. Nevertheless, this detailed N budget (including DON and N_2O-N) does not change the pattern of N loss and N retention among forests, and suggests that the old-growth forest has still retained a substantial amount of added N in the medium- and high-N treatments (69–71 $kg\ N\ ha^{-1}\ yr^{-1}$), as those in both younger forests (43–68 $kg\ N\ ha^{-1}\ yr^{-1}$, Table 5).

Table 5

Estimated distribution of experimentally added N (kg N ha^{-1} yr^{-1}) in the pine, mixed and old-growth forests of DHSBR, southern China; runoff, soil solution, and soil extractable N and microbial N are calculated as the difference between N-addition plots and the control plots

	Pine		Mixed		Old-growth		
	Low-N	Medium-N	Low-N	Medium-N	Low-N	Medium-N	High-N
N addition	50	100	50	100	50	100	150
Surface runoff DIN[a]	1	0	1	0	1	1	1
Solution DIN[a]	18	43	13	28	32	24	64
Surface runoff DON[b]	0	0	0	0	0	−1	1
Solution DON[b]	9	13	4	4	7	6	12
N_2O[c]	0	1	0	0	1	1	1
N retention[d]	22	43	32	68	9	69	71
ESIN[e]	1	2	0	1	0	1	1
ESON[f]	0	3	1	1	3	3	6
Microbial N[f]	2	1	1	1	−1	0	1
Plant, SOM and ionexchange site[g]	19	38	30	65	8	65	63

[a] Means over this study period (2004–2006).

[b] DON fluxes were measured in 2005 (Fang, 2006).

[c] N_2O emissions from soils were monitored during the period from September 2005 to August 2006 (Zhang et al., 2008).

[d] N retention calculated as N input minus all outputs.

[e] ESIN, extractable soil inorganic N, mean of five sampling dates in the upper 10 cm soil layer in 2004 and 2005 (Fang, 2006).

[f] Extractable soil organic N (ESON) and microbial N was measured for the upper 10 cm soil layer collected in December 2004 (17 months after the first N addition, Fang, 2006).

[g] Calculated as the difference between the retained N and the accumulation in extractable N and microbial N.

Since the old-growth forest was at an advanced stage in the N saturation continuum and a net N loss was observed even under ambient deposition condition, why did it still retain experimentally added N at the same magnitude as younger forests? Part of the added N may remain in the organic horizon or form new microbial organic N, but our measurements suggested that neither soil extractable N nor microbial biomass N was the main sink for retained N (Table 5). Thus, a large portion of the retained N (8–65 kg N ha^{-1} yr^{-1}, Table 5) must have ended up in plant biomass or complex soil organic matter.

In the young forest, the tissue N concentration was relatively low (1.3% in the needles, Mo et al., 1995), thus the plant had the potential to retain added N. We do not think the same process occurring in the old-growth forest; tissue N concentration was already very high (1.3–2.5% in leaves, Mo et al., 2000) and we have observed reduced tree growth during the 3-year study (after 3 years of N additions, the absolute basal-area increment was 19–25% lower in the N-addition plots than in the control plots, Y. Fang et al., unpublished data). The mechanisms for N retention have yet to be elucidated, but we suggest that the existing large coarse woody debris (CWD) and soil organic matter (SOM) are main sinks for added N in this forest. The old-growth forest has smaller carbon storage in the organic horizon (3.6 Mg C ha^{-1}) than in the pine forest (12.5 Mg C ha^{-1}), but much greater carbon storage in CWD (13.2 Mg C ha^{-1}) and mineral soils (0–20 cm, 44.6 Mg C ha^{-1}) than the pine forest (0.1 and 23.0 Mg C ha^{-1}, respectively, Fang et al., 2003). Soil organic matter has long been considered as a major sink for deposited N in temperate forests (Nadelhoffer et al., 1999), and both biotic immobilization and abiotic incorporation into SOM have been demonstrated to occur at significant rates under high N inputs (Aber et al., 1998; Davidson et al., 2003; Magill et al., 2004; Colman et al., 2007). In addition, anion adsorption may also be important in retaining N inputs in some forest soils (Lohse and Matson, 2005). Taken together, considerable amounts of experimental N input could have been retained in soils in all three forests as is observed in many temperate forests. More research is needed on the fate and stability of the N retained and the mechanisms of N retention, especially in the old-growth forest where a net loss is already happening under ambient N input.

The N retention in our forests may have been underestimated. First of all, N leaching in soil solution collected at the 20 cm depth is less than that eventually leached from the systems, since a fraction of N is likely to be further retained in the deeper soil. Another concern is that soil solution collected using zero tension lysimeters may not have recovered all the downward leaching. However, the collecting efficiency can be improved by increasing tray area and using rims, as we did in this study. Our results showed that the amount of soil solution collected at the 20 cm depth ranged from 351 to 823 mm, accounting for 31–53% of the corresponding throughfall. Together with surface runoff, these two pathways explained 50–73% of the throughfall input in the pine and old-growth forests, which are reasonable estimates for the region. In 2006, we also analyzed the concentration of chloride ion (Cl^-) for all water samples, and found 45–76% of Cl^- in throughfall input was lost via surface runoff plus leaching of soil solution, which indicate an underestimation. However, the short-term measurement (1 year) of Cl^- may not be enough to achieve a balanced budget due to “carry over” effect from year to year and possible biological accumulations (Lovett et al., 2005).

5. Conclusions

In this study, dissolved NH_4NO_3 was applied monthly onto the forest floor of three subtropical monsoon forests in southern China over 3 years, to investigate the influence of increased deposition on N leaching in warm and humid climate. Our results showed that experimental N addition immediately increased DIN leaching in all three forests, with 25–66% of added N leached over the 3-year experiment. But the N loss response is largely determined by the forest successional stages and the levels of N addition. Under low N treatment, the old-growth forest exhibits larger N loss than the younger forests, whereas the difference among forests becomes smaller when they both received high doses of N. Compared to most temperate forests studied in Europe and North America, N leaching loss in our forests occurred mainly in the rainy growing season, with measured N loss in leaching substantially higher under both ambient deposition and experimental N additions. In the present study, the detailed N budget including the losses of DON and N_2O-N does not change the pattern of N loss and N retention among forests, and suggests that all three forests still retained a substantial amount of added N in ecosystems (9–71 kg N ha^{-1} yr^{-1}) regardless of large concurrent N loss.

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