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Nitrogen-15 signals of leaf-litter-soil continuum as a possible indicator of ecosystem nitrogen saturation by forest succession and N loads

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Abstract Understanding forest carbon cycling responses to atmospheric N deposition is critical to evaluating ecosystem N dynamics. The natural abundance of ^{15}N (δ^{15}N) has been suggested as an efficient and non-invasive tool to monitor N pools and fluxes. In this study, three successional forests in southern China were treated with four levels of N addition. In each treatment, we measured rates of soil N mineralization, nitrification, N_2O emission and inorganic N leaching as well as N concentration and δ^{15}N of leaves, litters and soils. We found that foliar N concentration and δ^{15}N were higher in the mature broadleaf forest than in the successional pine or mixed forests. Three-year continuous N addition did not change foliar N concentration, but significantly increased foliar δ^{15}N ($p < 0.05$). Also, N addition decreased the δ^{15}N of top soil in the N-poor pine and mixed forests and significantly increased that of organic and mineral soils in N-rich broadleaf forests ($p < 0.05$). In addition, the soil N_2O emission flux and inorganic N leaching rate increased with increasing N addition and were positively correlated with the ^{15}N enrichment factor ($\varepsilon_{p/s}$) of forest ecosystems. Our study indicates that δ^{15}N of leaf, litter and soil integrates various information on plant species, forest stand age, exogenous N input and soil N transformation and loss, which can be used to monitor N availability and N dynamics in forest ecosystems caused by increasing N deposition in the future.

Keywords N transformation · N loss · ^{15}N natural abundance successional stages · N addition · Subtropical China

Introduction

The species diversity, biogeochemical cycles, productivity and carbon (C) sequestration of forest ecosystems are largely regulated by nitrogen (N) dynamics via plant uptake, soil N transformation and loss (Perakis and Hedin 2002; Frey et al. 2004; Boeckx et al. 2005; Luyssaert et al. 2008). Chronic atmospheric N deposition can obviously alter N transformation and loss processes, such as mineralization (Gundersen et al. 1998; Chen and Högberg

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2006), nitrification (Fenn et al. 1998; Venterea et al. 2004), ammonia volatilization (Huygens et al. 2007) and N_2O emission (Venterea et al. 2003; Zhang et al. 2008a), as well as organic and inorganic N losses (McDowell et al. 2004; Fang et al. 2009a, b), and their responses depend on ecosystem N saturation status (Aber et al. 1998). These N transformation and loss processes strongly affect the natural abundance of ^{15}N ($\delta^{15}N$) in plants and soils (Högberg 1997). Thus, $\delta^{15}N$ has been suggested to be an efficient and non-invasive tool to assess and monitor ecosystem N dynamics (Emmett et al. 1998; Martinelli et al. 1999; Sah et al. 2006; Pardo et al. 2006; Houlton et al. 2007).

Soil N transformation processes tend to discriminate against heavier ^{15}N, where lighter ^{14}N is preferentially lost through NO_3^- leaching or emission of N-containing gases while the residual soil N pool becomes enriched in ^{15}N (Högberg 1997; Pardo et al. 2006; Pörtl et al. 2007). Compared with low-N ecosystems, N cycling in N-saturated ecosystems should be more open, with larger inputs and losses of N than internal N cycling (Koopmans et al. 1997; Martinelli et al. 1999; Sah et al. 2006). Plants take up N from soil and then allocates it to various tissures (e.g., leaves). Thus, foliar N content can indicate the amount of soil N available for plant growth, and $\delta^{15}N$ content integrates information about the effects of biotic and abiotic factors on ecosystem N cycling (Sah et al. 2006; Pörtl et al. 2007). Due to lower N accretion and higher initial N availability, N transformation and loss in N-rich, mature forests are more sensitive to N addition than those in N-poor, younger forests (Fang et al. 2009b, c). Therefore, we hypothesize that leaves and litter will be more enriched in ^{15}N in the N-rich, mature forest than in the N-poor, younger forest.

Subtropical forest ecosystems in southern China have obviously been affected by high atmospheric N deposition (Zhou et al. 2006; Mo et al. 2008). Anthropogenic reactive N in this region has increased from 14 Tg N yr^{-1} in 1961 to 68 Tg N yr^{-1} in 2000 (Zheng et al. 2002), and atmospheric N deposition ranges from 30 to 73 kg N ha^{-1} yr^{-1} in some of these forest stands (Mo et al. 2006). Based on a manipulative N addition experiment in the Dinghushan Natural Reserve, some studies showed that N addition could inhibit litter decomposition (Mo et al. 2007), soil respiration (Mo et al. 2008) and soil CH_4 uptake (Zhang et al. 2008b), but promoted N leaching (Fang et al. 2008, 2009b, c) and N_2O emission (Zhang et al. 2008a) after four-years of continuous N addition. However, the integrated responses of plant and soil N processes to N addition and the relationship between soil N cycling rates and ecosystem ^{15}N enrichment remain unclear. More studies are needed to fully understand the integrated effects of N deposition and forest development on N cycling using the $\delta^{15}N$ approach.

In this paper, we sampled leaves, litters and soils from three subtropical, successional forests subject to different N loads to measure their $\delta^{15}N$ values. The main objectives were to: (1) clarify the patterns of foliar, litter and soil N concentrations and their $\delta^{15}N$ values in different N treatments; (2) clarify the responses of soil N transformation and loss rates to N addition; and (3) investigate the relationship between ecosystem ^{15}N enrichment and soil N cycling rates.

Materials and methods

Site descriptions

The study area (*ca.* 1133 ha) is located in the Dinghushan Natural Reserve in Guangdong Province, China (112°30′39″–112°33′41″ E, 23°09′21″– 23°11′30″ N). The region is characterized by a typical subtropical monsoon climate, with mean annual precipitation of 1927 mm, of which nearly 80% falls in the hot-humid season (April–September) and 20% in the cool-dry season (October–March). The mean annual temperature is 21.4°C, with the coldest and warmest monthly mean temperature of 12.6°C in January and 28.0°C in July, respectively. There are three major types of forests in the reserve: disturbed pine forest (hereafter referred to as pine forest), rehabilitated conifer and broadleaf mixed forest (hereafter referred to as mixed forest), and mature evergreen broadleaf forest (hereafter referred to as broadleaf forest), representing early-, mid-, and advanced-successional stages of vegetation, respectively (Peng and Wang 1995). The pine forest, dominated by *Pinus massoniana,* was originally planted in the 1930s and has suffered long-term human disturbances from litter and understory harvesting. The mixed forest has been free from human disturbances for about 70 years and developed from afforestation with gradual invasion of some pioneer broadleaf species through natural succession. Dominant species in the mixed forest include *Pinus massoniana*, *Schima superba*, *Castanopsis chinensis*, and *Craibiodendron kwangtungense*. The broadleaf forest is the regional climax vegetation and has been well protected from human disturbance for more than 400 years by Buddhist monks. Dominant species in the broadleaf forest include *Castanopsis chinensis*, *Cryptocarya chinensis*, *Cryptocarya concinna*, *Erythrophleum fordii*, and *Cyathea podophylla* (Mo et al. 2003). Soils in the above three study sites are classified as Lateritic Red Earth (Oxisol) formed from Devonian sandstone and shale (He et al. 1982), with variable depths (from 30 cm in the pine/mixed to more than 60 cm in the broadleaf forest). The thickness of the litter layer in the pine, mixed and broadleaf forests is 25.6 ± 2.1, 23.3 ± 1.8 and 8.9 ± 2.5 cm, respectively. The pine and mixed forests possess a well-developed O_{ae} layer, with thicknesses of 10 ± 0.6 and 8.8 ± 0.7 cm, respectively. However, the broadleaf forest lacks O_{ae} layer due to optimal temperature and moisture (Fang et al. 2009a). The other characteristics of surface soils in the three forests are provided in Table 1.

Experimental design

The chronic N addition experiment was composed of four treatments: control ($0\ g\ N\ m^{-2}\ yr^{-1}$), low-N ($5\ g\ N\ m^{-2}\ yr^{-1}$), medium-N ($10\ g\ N\ m^{-2}\ yr^{-1}$) and high-N ($15\ g\ N\ m^{-2}\ yr^{-1}$). However, only three treatments were established in the mixed and pine forests (control, low-N and medium-N). Thirty plots (9, 9, and 12 plots for the pine, the mixed, and the broadleaf forests, respectively) were randomly laid out in three forest stands and each was surrounded by a 10-m wide buffer strip. Plot size was 0.02 ha

Table 1 Stand characteristics and surface soil (0–20 cm) properties of three forests in Dinghushan Nature Reserve (mean, SE in parentheses, $n = 9$)[a]

Forest	Pine forest	Mixed forest	Broadleaf forest
Successional stage	Early	Medium	Advanced
Dominant species	*Pinus massoniana*	*Pinus massoniana,* *Schima superba*	*Castanopsis chinensis,* *Schima superba,* *Cryptocarya chinensis,* *Machilus chinensis,* *Syzygium rehderianum*
Mean tree height (m)	6.9	7.7	10.0
Mean diameter at breast height (cm)	17.5	14.2	18.5
Biomass ($Mg\ C\ ha^{-1}$)	40.6	116.2	147.8
Microbial biomass ($\times 10^6\ g^{-1}$ dry soil)	1.2	1.4	2.1
Fine root biomass in top soil ($Mg\ ha^{-1}$)	1.9 (1.1)	2.8 (1.1)	4.9 (3.0)
Litter input ($Mg\ C\ ha^{-1}\ yr^{-1}$)	1.8	4.3	4.2
Soil thickness (cm)	<40	30–60	>60
pH	4.02	3.92	3.8
Sand (2–0.05) (%)	39.2	36.8	24.8
Silt (0.05–0.002) (%)	26.5	29.4	34.7
Clay (<0.002) (%)	34.3	33.8	40.5
Soil moisture (%)	24.9 (1.1)	25.97 (0.91)	38.57 (1.19)
SOC ($Mg\ ha^{-1}$)	105.2	111.3	164.1
Total N ($kgN\ ha^{-1}$)	0.9 (0.1)	1.0 (0.1)	1.9 (0.1)
Soil C/N ratio	25 (1)	28 (1)	24 (1)
NO_3^--N ($kg\ ha^{-1}$)	2.8 (0.7)	3.0 (0.5)	8.4 (1.3)
NH_4^+-N ($kg\ ha^{-1}$)	3.6 (0.1)	2.7 (0.1)	2.1 (0.1)

[a] Data are from Tang et al. (2006) and Fang et al. (2008)

(20 m × 10 m). Applied fertilizer is NH_4NO_3 with mean $\delta^{15}N$ of -1.48 ± 0.62‰. Fertilizer was weighed (5.7, 11.4 and 16.1 kg for the low, medium, high treatment, respectively), mixed with 20 l of water, and applied to the plots below the canopy using a backpack sprayer (Mo et al. 2006). Control plots received an equivalent amount of water only. The solution was sprayed monthly at the same application rate over the entire year, beginning in July 2003 and continuing throughout the study period.

Sampling and analysis

In April 2006, current year leaves of *Castanopsis chinensis*, *Cryptocarya chinensi, Schima superba* (in the broadleaf forest), *Pinus massoniana* and *Schima superba* (in the mixed forest), and *Pinus massoniana* (in the pine forest) were cut with a pole pruner from the topmost whirls (one tree per species per plot). Samples of O-horizon soil, comprised of upper O_i and below $O_{ea,}$ were collected randomly with a trowel within a 5 cm radius. Mineral soil samples were taken from three depths (0–10, 10–20, 20–40 cm) using an auger (5 cm in diameter). At each depth, eight samples were collected and mixed together to represent one composite sample for that depth. Foliar and litter samples were oven-dried at 60°C. Mineral soils were air-dried at room temperatures and then sieved (2 mm mesh) to remove roots, gravel and stones. All samples were ground to a fine powder in a planetary mill and oven-dried at 70°C for 24 h before the analysis.

$\delta^{15}N$ and N concentration of samples were determined simultaneously on an isotope ratio mass spectrometer (Finnigan MAT-253, Thermo Electron) coupled to an automatic, online elemental analyzer (Flash EA1112, Thermofinnigan). $\delta^{15}N$ was reported in per mil (‰) relative to atmospheric N_2. Standard deviation of 10 repeated samples was <0.2‰ for $\delta^{15}N$. Due to previous land management and soil age effects, the initial differences in $\delta^{15}N$ values of the soils and plants should be taken into account. An enrichment factor (ε) is usually defined as the difference in the ^{15}N abundance between the substrate and product (Emmett et al. 1998). In this study, we defined the enrichment factor ($\varepsilon_{p/s}$) as the difference in the ^{15}N abundance between the soil and foliage, i.e. $\varepsilon_{p/s} = \delta^{15}N_{foliage} - \delta^{15}N_{mineral\ soil}$, where $\delta^{15}N_{foliage}$ and $\delta^{15}N_{mineral\ soil}$ were averaged $\delta^{15}N$ values of the foliage and the mineral soil layer within the 40 cm depth, respectively.

Soil N transformation and loss

Soil N transformation (net N mineralization and nitrification) and soil N losses (soil inorganic N leaching and N_2O emission) were also investigated. The net N mineralization and net nitrification rates were measured in situ using the intact soil core incubation technique (Fang et al. 2009b). Four pairs of PVC tubes (15 cm in length × 7 cm in diameter) were inserted each month to the depth of 10 cm in each plot after excluding the organic layer. One pipe in each pair was capped with aluminum foil and left in place, and the other was removed and returned to the laboratory for immediate extraction of NO_3^- and NH_4^+ from the soil (within 12 h of collection) to determine initial inorganic-N stocks. After 30 days, the soil core left in the soil was collected and taken to the laboratory for inorganic-N extraction. Inorganic N concentration of each soil sample was extracted using 2 M KCl solution and the filtrate was used to determine NH_4^+ and NO_3^- concentrations on an autoanalyzer (Bran Luebbe, Germany). Net mineralization and nitrification rates were calculated as the difference in total inorganic N and NO_3^- concentrations before and after incubations in the field, respectively.

In each plot of the three forests, soil solution at 20 cm soil depth was collected using two replicated zero tension tray lysimeters (755 cm^2 per tray). Each lysimeter was connected to a 5 l bottle using the steep slope of the sites to facilitate sampling to determine the loss rate of total inorganic N (TIN), NH_4^+ and NO_3^- (Fang et al. 2008). Two zero tension lysimeters were installed at each plot in April 2003. Soil solutions from these two lysimeters were sampled and combined to one sample by the end of each month for determining NH_4^+ and NO_3^- concentrations using an autoanalyzer (Bran Luebbe, Germany). Inorganic N concentration in each plot was multiplied by the water volume collected by the zero tension lysimeters for the same period, and then summed up to determine the flux of N leaching in kg ha^{-1}.

Soil N_2O flux was measured using a static chamber and gas chromatography (Agilent 4890D, Agilent Co. USA) from October 2005 to September 2006

(Zhang et al. 2008a). A static chamber with a volume of about 20 l was inserted directly into the forest floor about 10 cm below the soil surface. Gas samples for N_2O flux measurements were taken once a week during the growing season (April–September) and once every other week during the rest time. The N_2O flux was measured from 9:00 to 11:30 AM, which was close to the diurnal mean of the flux (Tang et al. 2006). Gas samples (100 ml each) were collected at 10 min intervals using 100 ml plastic syringes. N_2O concentrations in the samples were analyzed on the gas chromatography in the laboratory within 24 h. N_2O flux was calculated from the linear regression of concentration versus time.

Statistical analysis

The differences of the foliar N concentration and the $\delta^{15}N$ content, and the rates of soil N transformation, inorganic N loss and N_2O emission among forest types and N addition treatments were tested using the two-way analysis of variance (ANOVA). Comparisons of the means were conducted using the Tukey's HSD test. Regression analysis was used to examine the relationships between foliar $\delta^{15}N$ and N concentration, and between ecosystem enrichment factor $\varepsilon_{p/s}$ and N cycling rates. All statistical analyses were conducted using the SAS software package (version 8.2). Statistical significant differences were set with p values < 0.05 unless stated otherwise.

Results

Foliar N concentration and $\delta^{15}N$

In the control plots, average foliar N concentration of the pine forest was 1.53 ± 0.09%, significantly lower than that (2.05 ± 0.08%) of the broadleaf forest ($p < 0.05$) (Fig. 1a). Foliar $\delta^{15}N$ of the three successional forests varied from −6.94‰ to −2.03‰. Average foliar $\delta^{15}N$ of the pine forest (−5.34 ± 0.82‰) was significantly more negative than that of the mixed (−3.39 ± 0.36‰) and broadleaf (−2.90 ± 0.32‰) forests ($p < 0.05$) (Fig. 1b). In the mixed forest, foliar N concentration of *Pinus massoniana* was significantly lower than that of *Schima superba* ($p < 0.05$) (Fig. 1a), but there was no difference in foliar $\delta^{15}N$ between them (Fig. 1b).

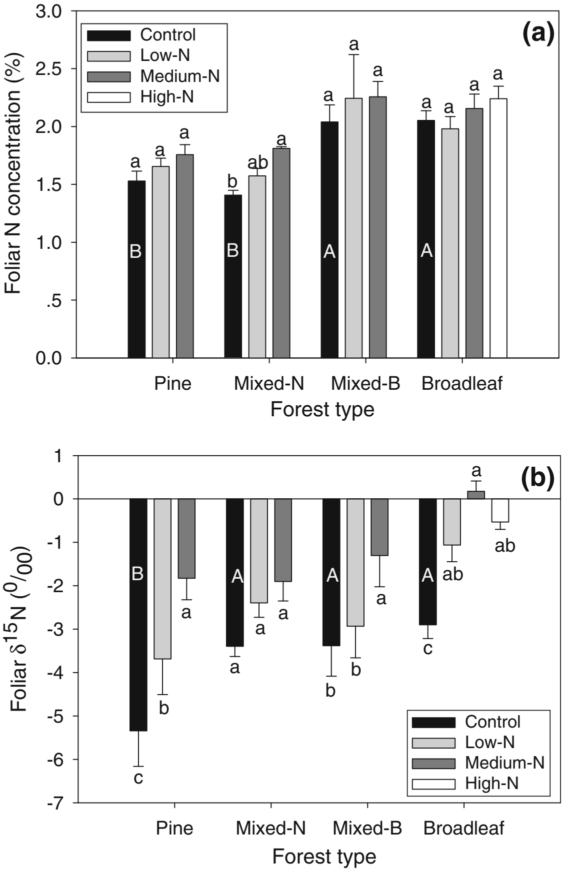

Fig. 1 Variation in foliar N concentration and ^{15}N abundance among different dominant species and N treatments. (Mixed-N and Mixed-B referred to needle and broadleaf species in the mixed forest. The different capital and lowercase letters indicate significant differences among plant species and among N treatments, respectively)

N addition did not change foliar N content in the three forest stands (Fig. 1a). Except for *Pinus massoniana* in the mixed forest, N addition significantly increased foliar $\delta^{15}N$ value ($p < 0.05$). In addition, there was a significant positive relationship between the foliar $\delta^{15}N$ and N concentration ($R^2 = 0.53$, $p < 0.01$) (Fig. 2), suggesting that leaves with high N concentration tend to have high $\delta^{15}N$ values.

Soil N concentration and $\delta^{15}N$

In the control plots, the mean N concentration in the three forest soils decreased from the upper O horizons (0.97 − 1.58%) to the lower mineral soil horizons (0.05 − 0.20%). However, soils at the three forest sites showed lower $\delta^{15}N$ values (−1.71 to +0.28%)

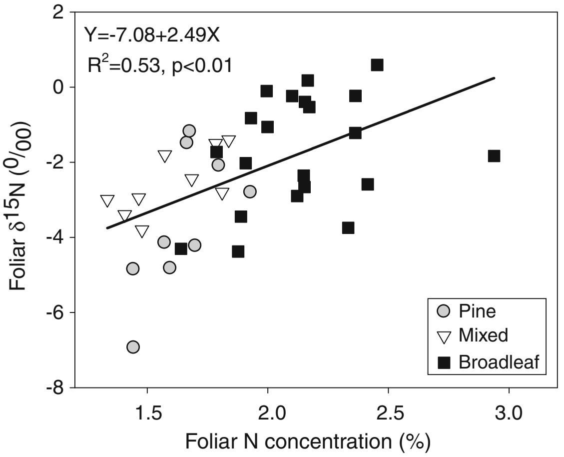

Fig. 2 The relationship between foliar N concentration and ^{15}N abundance

in the O-horizon and increased up to +2.12 to +4.07‰ in the lower mineral soil depths (Fig. 3). Average soil N concentration of each soil layer in the mixed forest was significantly lower than that in the broadleaf forest ($p < 0.05$) (Table 2). The δ^{15}N of each soil layer in the pine forest was slightly higher than those in the mixed and broadleaf forests. However, only the difference of δ^{15}N content in the upper 10 cm soil layer was significant among the three forest types ($p < 0.05$) (Table 2).

N addition did not increase total N concentration of each layer in the three forests, but greatly changed the soil δ^{15}N (Fig. 3). The δ^{15}N of the O_i layer tended to be increased due to N addition, and its difference among N treatments in the broadleaf forest was significant ($p < 0.05$) (Fig. 3). In the N-poor pine and mixed forests, surface soils (O_{ea} and 0-10 cm layers) were depleted in ^{15}N with increasing N addition rate (Fig. 3a, b). However, in the N-rich broadleaf forest, the soil below the 10 cm layer was significantly enriched in ^{15}N ($p < 0.05$) (Fig. 3c).

Soil N transformation and loss

Net N mineralization and nitrification rates in the control plots in the mixed forest were 3.9 and 1.1 kg N ha^{-1} month^{-1}, respectively, being lower than

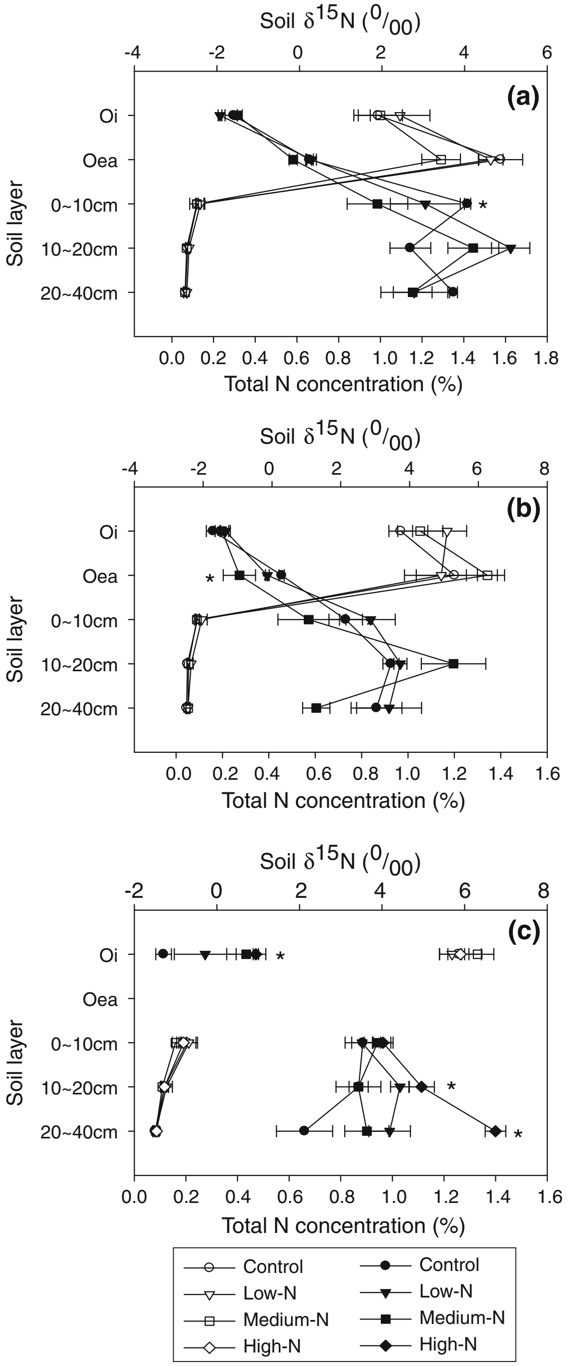

Fig. 3 Variation in soil N concentration (*open symbols*) and δ^{15}N (*solid symbols*) with soil depth in different N treatment plots and forests; **a** the pine forest, **b** the mixed forest, and **c** the broadleaf forest. Asterisks (*) indicates significant differences among treatments at the level of $p < 0.05$

Table 2 Variance analysis for soil N concentration and δ^{15}N in control plots

Soil layers	Forests	δ^{15}N (‰)			N (%)		
		Mean (SE)[a]	*F*	*p*	Mean (se)[a]	*F*	*p*
O_i	Pine	−1.59 (0.21)a	1.17	0.37	0.99 (0.12)b	4.33	0.03
	Mixed	−1.71 (0.20)a			0.97 (0.05)b		
	Broadleaf	−1.29 (0.19)a			1.26 (0.04)a		
0–10 cm	Pine	4.07 (0.08)a	6.96	0.03	0.12 (0.04)ab	3.41	0.04
	Mixed	2.14 (0.49)b			0.09 (0.01)b		
	Broadleaf	3.54 (0.43)a			0.20 (0.04)a		
10–20 cm	Pine	2.69 (0.50)a	1.63	0.27	0.08 (0.01)b	11.45	0.005
	Mixed	3.46 (0.24)a			0.05 (0.003)b		
	Broadleaf	3.43 (0.23)a			0.12 (0.02)a		
20–40 cm	Pine	3.73 (0.09)a	1.92	0.23	0.07 (0.01)ab	5.51	0.02
	Mixed	3.04 (0.74)a			0.05 (0.01)b		
	Broadleaf	2.12 (0.68)a			0.08 (0.01)a		

[a] Different lowercase letters indicate significant differences among forest types

those in the pine (8.2 and 7.8 kg N ha^{-1} $month^{-1}$) and broadleaf forests (6.7 and 6.1 kg N ha^{-1} $month^{-1}$) (Fig. 4a, b). The losses of total inorganic N (TIN) and of NO_3^--N in the pine and mixed forests ranged from 10.56 to 16.65 kg N ha^{-1} yr^{-1} and from 9.5 to 15.5 kg N ha^{-1} yr^{-1}, respectively, which were significantly less than those of the broadleaf forest (42.96 kg N ha^{-1} yr^{-1} for the TIN loss and 41.7 kg N ha^{-1} yr^{-1} for the NO_3^--N loss) ($p < 0.05$) (Fig. 4c, d). However, there was no significant difference in NH_4^+-N loss among three forest sites (Fig. 4e). Additionally, there was a significant difference in the soil N_2O emission among the three forests ($p < 0.05$) (Fig. 4f).

Except in the broadleaf forest, N addition had no influence on net N mineralization and nitrification rates (Fig. 4a, b). However, it significantly increased the loss of TIN, NO_3^--N and NH_4^+-N ($p < 0.05$) (Fig. 4c–e), and the magnitude of the increase depended on N addition levels and N availability at the sites. Based on the N input to the forests via atmospheric N deposition (39 kg N ha^{-1} yr^{-1}) and N addition, the fraction of TIN lost from the total N input ranged from 35.0% to 44.4% for different N treatment plots in the pine forest and from 22.2% to 25.8% in the mixed forest, which was significantly lower than that in the broadleaf forest (41.4% to 90.3%) ($p < 0.05$). Additionally, N addition significantly promoted soil N_2O emission from the soils in the pine and broadleaf forests ($p < 0.05$) (Fig. 4f).

Relationship between the ^{15}N enrichment factor and N cycling rates

Under natural conditions, the enrichment factor, $\varepsilon_{p/s}$, in the three forests ranged from -8.25 ± 1.37‰ to -4.97 ± 0.83‰ (Fig. 5). $\varepsilon_{p/s}$ in the pine forest was significantly less than that of the mixed and broadleaf forests ($p < 0.05$) (Fig. 5c). $\varepsilon_{p/s}$ was positively correlated with both the N addition level and N_2O flux (Fig. 5a). Also, $\varepsilon_{p/s}$ was positively correlated with soil NO_3^- rather than NH_4^+-N loss (Fig. 5b). However, we did not find a significant relationship between forest $\varepsilon_{p/s}$ and the nitrification/mineralization ratio (Fig. 5c). If we divided forests (or plants) into N-poor (needle) and N-rich (broadleaf) types, a marginal positive and negative correlation between forest $\varepsilon_{p/s}$ and nitrification/mineralization ratios were observed at the level of 0.1, respectively (Fig. 5c). Moreover, the correlation was higher between $\varepsilon_{p/s}$ and soil N_2O flux ($R^2 = 0.64$, $p < 0.01$) than between $\varepsilon_{p/s}$ and soil NO_3^--N loss ($R^2 = 0.43$, $p < 0.05$) (Fig. 5).

Discussion

Effects of N addition on N cycling

Under natural condition, both soil N transformation and loss rates in the broadleaf forest were higher than those in the pine and mixed forests (Fig. 4). Soil N

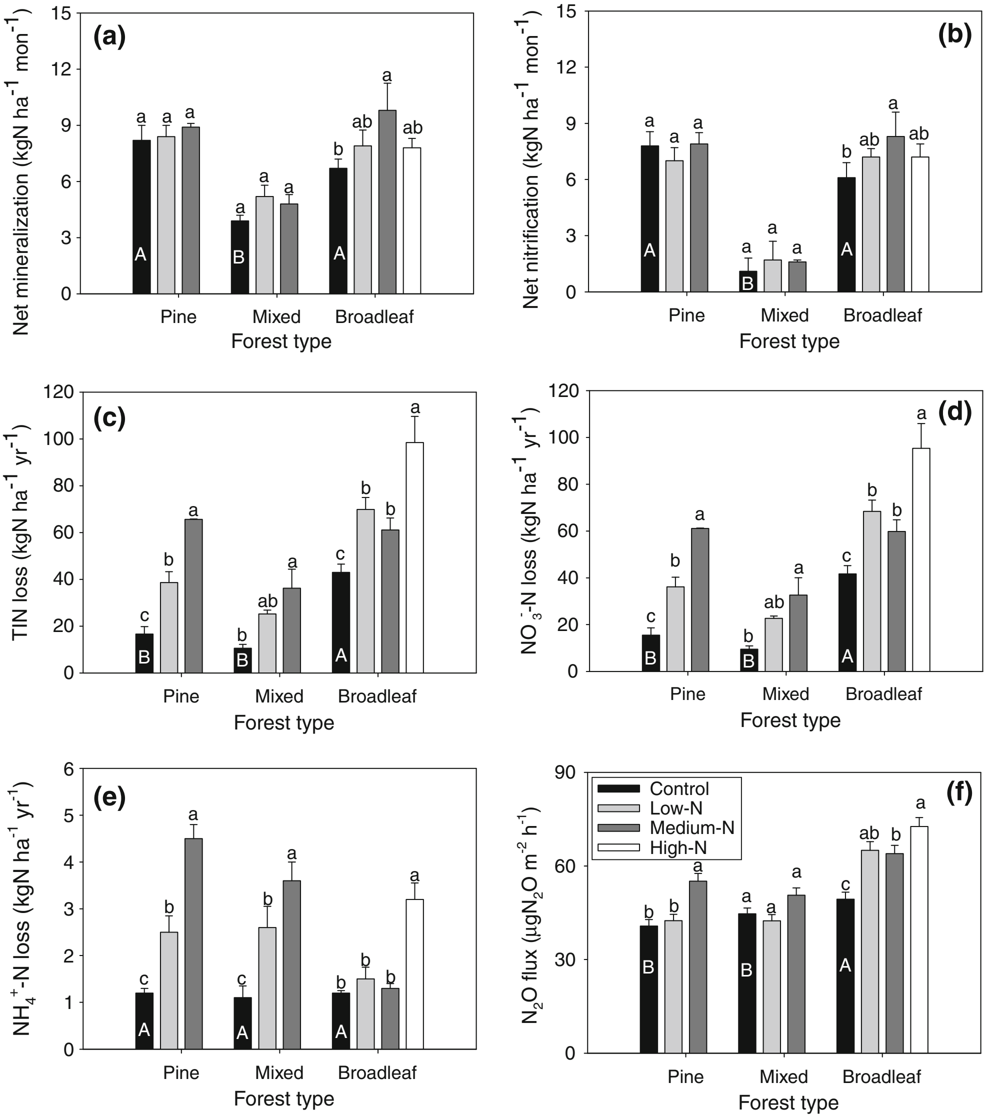

Fig. 4 Variation in soil N transformation and loss rates among different N treatments and forests (different capital and lowercase letters indicate significant differences among forest types under natural conditions and among N treatments, respectively)

transformation and loss are determined by many factors such as soil temperature (Fang et al. 2004), soil moisture (McDowell et al. 2004), available N (Adams et al. 2004), and litter and soil organic matter quality (e.g., C/N) (Arunachalam et al. 1998; MacDonald et al. 2002; Mo et al. 2003), etc. When soil temperature, soil moisture and other environmental factors are constant, C/N ratios of substrates are dominant in controlling soil N mineralization and nitrification (Adams et al. 2004; Fang et al. 2004). Some studies suggest that litter C/N ratio is negatively correlated to net N mineralization rate (Gundersen et al. 1998; MacDonald et al. 2002). In the N-poor pine and mixed forests, microbial activity is limited by N due to high C/N ratios of substrates, where mineral N of the soils tend to be immobilized, and vice versa in the broadleaf forest with low C/N ratios (Table 1, Fang et al. 2004). Soil nitrification and NO_3^--N loss will increase with the decrease in C/N ratios of soil substrates when they are less than 25 (Adams et al. 2004). Our data on soil C/N ratios in the three forests also support this conclusion (Table 1). In addition, the

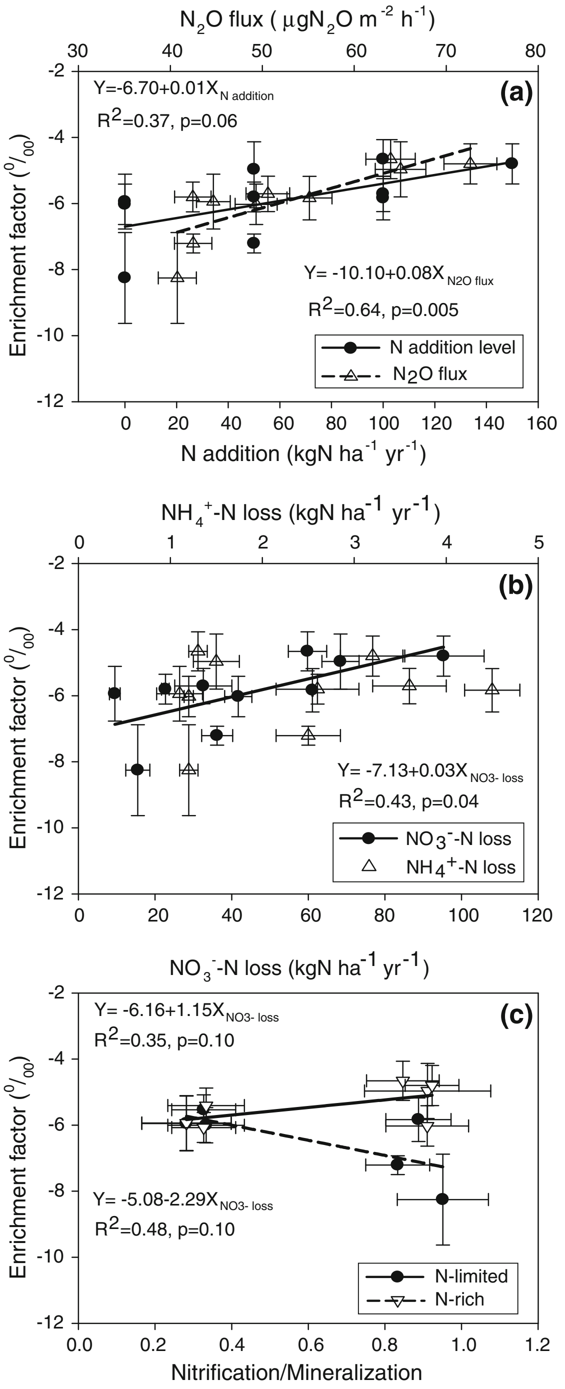

Fig. 5 Relationships between ^{15}N enrichment factor $\varepsilon_{p/s}$ and N cycling rates

higher TIN and NO_3^--N losses in the broadleaf forest than those in the pine and mixed forests could also be attributed to higher N input from throughfall and litter fall. Inorganic N input via throughfall and litterfall in the broadleaf forest was 106 kg N ha^{-1} yr^{-1}, much higher than those in the pine (53 kg N ha^{-1} yr^{-1}) and mixed (56 kg N ha^{-1} yr^{-1}) forests, respectively (Fang et al. 2008). Meanwhile, higher soil available N generally lead to higher soil N_2O emission in the mature forest (Venterea et al. 2004; Davidson et al. 2004).

Different types of forest ecosystems have different responses of soil N transformation and loss rates to the increase in N deposition, and the timing and magnitudes of the responses largely depend on initial nutrient pools and how close the system is to N saturation (Gundersen et al. 1998; Aber et al. 1998). Forests accumulate organic matter and N in both vegetation and soils, as the ecosystem develops. Mature forests can be expected to have larger N pools and higher inherent N availability than the young, successional forests (Vitousek and Reiners 1975). Based on long-term N addition experiments, soils, rather than plants, are the dominant long-term N sink from atmospheric deposition (Nadelhoffer et al. 1999; Davidson et al. 2003; Lohse and Matson 2005). Since the mature forest has lower N accretion and higher initial N availability (Venterea et al. 2003; Merino et al. 2004), both soil N nitrification and mineralization rates are higher in the mature forest than in the younger forests (Magill et al. 2000; Zhang et al. 2008a), which accordingly results in more N leaching and N_2O emission from the broadleaf forest under elevated atmospheric N deposition (Zhang et al. 2008a).

Effects of plant species and forest succession on foliar δ^{15}N

Foliar δ^{15}N can function as a sensitive indicator of soil N status, affected by plant species, forest stand age and land use history (Nadelhoffer et al. 1996; Emmett et al. 1998; Sah et al. 2006). In general, broadleaves are more ^{15}N enriched than conifer needles (Martinelli et al. 1999; Eshetu 2004; Sah et al. 2006), which was verified by our result that broadleaves in the mixed and broadleaf forests had a higher δ^{15}N than needles in the pine forest. However, in the same mixed forests, the difference of foliar δ^{15}N between coniferous (*Pinus massoniana*) and broadleaved species (*Schima superba*) is not significant (Fig. 1b). In contrast, foliar

δ^{15}N of the same tree species (*Pinus massoniana*) changed more enriched from the pine forest at low successional stage to the mixed forest at middle successional stage (Fig. 1b). Moreover, foliar δ^{15}N increased from the pine to the mixed forest and finally to the broadleaf forest when not considering tree species. These results indicate that foliar δ^{15}N increases with the increase of forest successional stage or forest stand age, which is consistent with those reported by other researchers (Chadwick et al. 1999; Selmants and Hart 2008). Therefore, both tree species and forest succession determine the pattern of foliar δ^{15}N.

Effects of applied N fertilizer on the δ^{15}N of foliage and top soil

Patterns of foliar and top soil δ^{15}N depend on the amount of applied fertilizer N (Choi et al. 2002; Watzka et al. 2006; Yun and Ro 2009). On one hand, net accumulation of ^{15}N-depleted fertilizer N leaves more distinct imprints in soil. On the other hand, fertilizer-induced stimulation of isotope fractionating processes leads to N losses depleted in ^{15}N. In the N-poor pine and mixed forests, it was particularly evident for plots receiving mineral fertilizer where direct fertilizer immobilization in soil would lead to a decrease in top soil δ^{15}N with the increasing fertilization rate (Fig. 3a, b). However, contrasting trends were observed in the broadleaf forest, where N isotope discriminating processes could be dominant (Fig. 3c).

In the literature, the effects of ^{15}N-depleted fertilizer addition on foliar δ^{15}N are not consistent. For example, some studies suggest that application of ^{15}N-depleted urea results in a decrease in foliar δ^{15}N (Aoyama and Kumakura 2001; Zhao et al. 2002). However, our result showed that N addition caused foliage enrichment in ^{15}N in the three forests (Fig. 1b), as has been shown in other studies (e.g., Malhi et al. 1997; Choi et al. 2002; Watzka et al. 2006). These conflicting results suggest that foliar δ^{15}N is affected by various factors, such as fractionations during and after N uptake by plants, form of N used (e.g., NH_4^+, NO_3^-, and organic N), N source, and soil depth of N uptake (Nadelhoffer et al. 1996). If fertilizer N is directly taken up by plants, foliar δ^{15}N should reflect the δ^{15}N signature of the fertilizer. However, the uptake efficiency of fertilizer-N is generally limited due to N loss and mineralization-immobilization turnover (Choi et al. 2002), and the increased contribution of indigenous soil-N to total plant-N with N addition (Kuzyakov et al. 2000). Therefore, the variations in foliar δ^{15}N in the present study could be attributed to the different availability of soil- and fertilizer-N for plant uptake in the various successional forests.

N isotope fractionation stimulating by N addition

N addition resulted in ^{15}N enrichment in the soil at the N-rich broadleaf forest, probably resulting from a few sources. First, inorganic N input increased rates of nitrification and nitrate leaching, which results in ^{15}N-depleted nitrate and ^{15}N-enriched ammonium (Choi et al. 2005). Soil nitrate and ammonium have different fates: most of the ^{15}N-enriched ammonium is retained on the soil exchange complex and is available for plant uptake due to high soil clay and soil organic matter content, but much of the ^{15}N-depleted nitrate is leached from the ecosystem (Pardo et al. 2002, 2006). Thus, the net effect of nitrification is to enrich the δ^{15}N of the soil and of the plant available NH_4^+ pool. Second, consecutive processes such as denitrification may also operate. An alternative explanation is that high N_2O losses (partly by nitrifying partly by denitrifying microbes) would (1) lead to losses of ^{15}N-depleted gaseous products leaving nitrate (and ammonium) ^{15}N-enriched, (2) lead to whole plant ^{15}N enrichment because the whole TIN pool becomes ^{15}N enriched, whether plants take up ammonium or nitrate. Our data also showed that soil N_2O emission had a greater contribution to ecosystem enrichment than nitrate export, accounting for 64% of its variation (Fig. 5a, b). Besides liquid and gaseous loss of N, ^{15}N-enriched litter fall in high N treatment plots in the broadleaf forest can also cause top soil ^{15}N enrichment compared with low N treatment plots.

However, in the N-limited forests (especially in the mixed forest), rates of N transformation and loss were relatively lower (Fig. 4a–f), which could result in soil NO_3^- more depleted in ^{15}N (Martinelli et al. 1999). During the early period after N addition, as a result of microbial N transformation (from NH_4^+ to NO_3^-), plants take up more ^{15}N-depleted NO_3^-, resulting in foliage depleted in ^{15}N relative to the soil (Högberg 1997). The lower abundance of ^{15}N in the upper soil horizons mainly results from ^{15}N-depleted litter fall. Because more than 60% of the added N was retained in the ecosystem to enhance microbial and aboveground plant biomass (Fang et al. 2008), there could be no excess ^{15}N-depleted NO_3^- to affect the pattern of δ^{15}N in subsurface soils in N-limited forests. However, with increased N accumulation, less C is available for fungi, resulting in an increased N dissimilation and export (either to the plant or into the soil environment), thus causing higher δ^{15}N values in the O_i layer (Dijkstra et al. 2008).

The ecosystem enrichment factor ($\varepsilon_{p/s}$) in the N-rich broadleaf forest was higher than the other two N-limited forests, which is partly attributed to soil texture and N retention. The relatively high N and clay content of the broadleaf forest soil (Table 1) has been more susceptible to N addition, resulting in more open N cycling and ^{15}N enrichment of both soil and vegetation (Emmett et al. 1998; Zhang et al. 2008a; Fang et al. 2008). An alternative internal mechanism could be that higher cation exchange capacity in the broadleaf soil would enhance the capability of the exchange complex to discriminate against ^{14}N, which would be preferentially lost from the profile (Martinelli et al. 1999). On the contrary, sandy soil in the pine forest was poor in conserving soil organic matter (SOM) that is enriched in ^{15}N. Lower SOM could also decrease immobilization of NH_4^+ relative to NO_3^- (Table 1), leading to an even lower $\varepsilon_{p/s}$ in the pine and mixed forests (Fig. 5). Martinelli et al. (1999) also reported that δ^{15}N values of white sandy soil in N-poor tropical forests were more negative even under conditions of high air temperature and N deposition.

Conclusions

This study examined the effects of N addition on N dynamics of subtropical forests at different successional stages based on a manipulative N addition experiment. Under natural condition, both soil N transformation and loss rates in the broadleaf forest were higher than in the pine and mixed forests, partly attributed to higher available N content and lower C/N ratios of soil substrates. The responses of soil N transformation and loss to the increasing N deposition were more sensitive in the N-rich broadleaf forest than in the N-poor pine and mixed forests, largely correlated with higher initial N availability and lower N retention in soil. This result also suggests that the responses of soil N transformations and losses to N deposition in the subtropical forests in southern China largely depend on the status of soil N and forest types. Both plant species and forest succession stages dominated the variation of foliar δ^{15}N, indicating the difference of soil N availability for plant uptake in the various forests. The type and accumulation of applied fertilizer, availability and source of soil N, ^{15}N-enriched litter fall, and isotope fractionation accompanying N transformation and losses influenced the patterns of δ^{15}N in the leaf-litter-soil continuum. Continuous 3-year N addition did not significantly increase foliar N concentration, but significantly increased foliar ^{15}N natural abundances of trees. This result suggest that ^{15}N natural abundance could be a more sensitive indicator than total N content in response to N addition. Moreover, it is the soil N losses especially NO_3^- leaching and N-containing gases emission that dominate the ^{15}N enrichment of N-rich subtropical forests in China.

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