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Nitrogen deposition and forest nitrogen cycling along an urban–rural transect in southern China

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Abstract

There is increasing concern over the impact of atmospheric nitrogen (N) deposition on forest ecosystems in the tropical and subtropical areas. In this study, we quantified atmospheric N deposition and revealed current plant and soil N status in 14 forests along a 150 km urban to rural transect in southern China, with an emphasis on examining whether foliar $\delta^{15}N$ can be used as an indicator of N saturation. Bulk deposition ranged from 16.2 to 38.2 kg N ha^{-1} yr^{-1}, while the throughfall covered a larger range of 11.7–65.1 kg N ha^{-1} yr^{-1}. Foliar N concentration, NO_3^- leaching to stream, and soil NO_3^- concentration were low and NO_3^- production was negligible in some rural forests, indicating that primary production in these forests may be limited by N supply. But all these N variables were enhanced in suburban and urban forests. Across the study transect, throughfall N input was correlated positively with soil nitrification and NO_3^- leaching to stream, and negatively with pH values in soil and stream water. Foliar $\delta^{15}N$ was between −6.6‰ and 0.7‰, and was negatively correlated with soil NO_3^- concentration and NO_3^- leaching to stream across the entire transect, demonstrating that an increased N supply does not necessarily increase forest $\delta^{15}N$ values. We proposed several potential mechanism that could contribute to the $\delta^{15}N$ pattern, including (1) increased plant uptake of ^{15}N-depleted soil NO_3^-, (2) foliage uptake of ^{15}N-depleted NH_4^+, (3) increased utilization of soil inorganic N relative to dissolved organic N, and (4) increased fractionation during plant N uptake under higher soil N availability.

Keywords: nitrogen deposition, nitrogen isotope, nitrogen saturation, soil nitrogen availability, tropical and subtropical forests

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Introduction

Anthropogenic emissions of reactive forms of nitrogen (N) to the atmosphere due to fossil fuel combustion and modern agriculture have increased N deposition over much of the industrialized world (Gruber & Galloway, 2008; Reay *et al*., 2008). Increased N deposition can result in N saturation when the biotic demand for N is exceeded, leading to increased rates of N cycling and losses of nitrate (NO_3^-), soil and surface water acidification, plant nutrient imbalances, even forest decline (Aber *et al*., 1998, 2003; Fenn *et al*., 1998; Gundersen *et al*., 1998b). Concerns over the adverse effects of N deposition have initiated many studies in temperate and boreal forests since the late 1980s (Aber *et al*., 1998; Gundersen *et al*., 1998b; Nave *et al*., 2009). Galloway and his colleagues (Galloway, 2000; Galloway *et al*., 2004) have drawn attention to the potential deleterious consequences of the sharply increasing fertilizer inputs of reactive N to agro-ecosystems in Asia. N deposition in precipitation >10 kg N ha^{-1} yr^{-1}, a threshold above which N leaching often increases in temperate and boreal forests (e.g., MacDonald *et al*., 2002), has frequently been reported in southern China associated with rapid economic growth (e.g., Chen & Mulder, 2007; Lu & Tian, 2007; Fang *et al*., 2008). This elevated atmospheric N deposition, which may have persisted for decades in some parts of the region (Fang *et al*., 2008), is expected to change the C and N cycles in forest ecosystems. Concerns over the impact of increased N deposition are still at the initial stage. Little is known about the current forest N status, the fate of deposited N, and the change in ecosystem N cycling following decadal-long elevated deposition (Chen & Mulder, 2007; Fang *et al*., 2008, 2009a; Mo *et al*., 2008; Lu *et al*., 2010).

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The process of N saturation has been qualitatively classified into several progressive stages (Aber *et al*., 1998; Gundersen *et al*., 1998b, 2006). The conceptual model of N saturation developed by Aber *et al*. (1998) suggests that in most forest ecosystems, primary production is limited by N supply and they are efficient in retaining atmospheric N input or experimentally added N (stage 0 and stage 1). In stage 2, nitrification is induced and NO_3^- loss starts, but tree growth is still high. In stage 3, tree growth declines and nitrification and NO_3^- loss continue to increase as the fraction of mineralized NH_4^+ that is nitrified increases. Nevertheless, some complex questions remain, such as the time course to N saturation for a given forest ecosystem (Pardo *et al*., 2006). It is thus valuable to develop useful indicators to determine whether a forest has reached N saturation and to predict when a forest is nearing a particular N saturation stage. Such indicators would facilitate both forest management and enhance understanding of N cycling in forest ecosystems (Gundersen *et al*., 1998a; Pardo *et al*., 2006, 2007a, b; Templer *et al*., 2007).

Most evaluations of N cycling and the N status of ecosystems rely on long-term monitoring of stream water chemistry to determine the input–output budgets of N for the ecosystem (Moldan *et al*., 2006; Pardo *et al*., 2007a). This approach provides much useful information about sites, including the seasonal and annual variability of N fluxes. However, the intensity of measurement and the long-term nature of this approach have limited its use (Pardo *et al*., 2007a). Forest floor %N and C/N have long been suggested as good indicators of N saturation for boreal and temperate forests (McNulty *et al*., 1991, 2005; Gundersen *et al*., 1998a; Aber *et al*., 2003; Nave *et al*., 2009). But, those indicators may not be appropriate for tropical and subtropical forests, as there is sometimes no forest floor layer present due to fast litter turnover and the frequent and high precipitation therein.

The stable N isotope ratio (expressed as $\delta^{15}N$ for ^{15}N natural abundance), in contrast, can be used as a tool for the regional assessment of N saturation because it provides an integrated measure of current and past N cycling of a site with a single sampling (Robinson, 2001; Pardo *et al*., 2007a, b). Isotopic fractionation occurs during enzymatic and other biological processes, discriminating against the heavier ^{15}N when chemical bonds are broken, such that the product generally has lower $^{15}N/^{14}N$ ratio than the remaining substrate (Nadelhoffer & Fry, 1994; Robinson, 2001). During nitrification, the NO_3^- produced is depleted in ^{15}N (lower $^{15}N/^{14}N$) relative to the ammonium (NH_4^+) substrate (Mariotti *et al*., 1981). When ^{15}N-enriched NH_4^+ is retained in the soil and ^{15}N-depleted NO_3^- is leached from the ecosystem or is lost by denitrification, the net effect of nitrification is to enrich the soil and subsequently the plant in ^{15}N (Pardo *et al*., 2006, 2007a, b). Thus, as an ecosystem moves toward N saturation (i.e., nitrification and consequent increase in NO_3^- loss), it is predicted that soils and plants would become enriched in $\delta^{15}N$ (Nadelhoffer & Fry, 1994; Pardo *et al*., 2006, 2007a, b).

Foliar $\delta^{15}N$ has been used to assess the regional patterns of N saturation in Europe and North America. Näsholm *et al*. (1997) observed elevated foliar $\delta^{15}N$ in spruce stands in southern Sweden with elevated NO_3^- leaching. Pardo *et al*. (2001) suggested that even a small disruption of N cycle could cause a detectable increase in $\delta^{15}N$ in organic soil horizons. Increases in foliar $\delta^{15}N$ were noted also after N saturation was induced via N addition to a Scots pine forest in northern Sweden (Högberg & Johannoisson, 1993). Emmett *et al*. (1998) observed that $\delta^{15}N$ of plant and soil increased along an N deposition gradient across NITREX study sites in Europe. Net N mineralization and/or nitrification have been positively correlated to soil $\delta^{15}N$ (Jussy *et al*., 2002; Pardo *et al*., 2002; Templer *et al*., 2007) and foliar $\delta^{15}N$ (Garten & van Miegroet, 1994; Pardo *et al*., 2006; Craine *et al*., 2009b). However, some studies have shown that N-saturated forests may not be necessarily characterized by elevated $\delta^{15}N$ (Stewart *et al*., 2002; Falkengren-Grerup *et al*., 2004; Bragazza *et al*., 2005; Kranabetter & MacKenzie, 2010; Takebayashi *et al*., 2010) and that $\delta^{15}N$ can be used only as an indicator of the N saturation stage, when $\delta^{15}N$ of the deposited N and the isotope fractionation factors are taken into consideration (Koopmans *et al*., 1997). In addition, previous studies mainly concentrated on boreal and temperate forests in Europe and North America, where N limitation is widespread and the forests receive low to moderate N deposition. The applicability of $\delta^{15}N$ measurement remains to be tested in other regions, particularly tropical and subtropical forests and forests where there exists a large variation of N deposition.

In this study, we quantified atmospheric N deposition and revealed current plant and soil N status in 14 forests along a 150 km urban to rural transect in southern China. Our objectives were twofold, (1) to examine the spatial patterns of atmospheric N deposition and current ecosystem N status along the transect; (2) to relate N deposition to N status and soil N availability, with an emphasis on testing whether foliar $\delta^{15}N$ can be used as an indicator of N saturation in the study region. To this end, N deposition fluxes in precipitation and throughfall, N concentration and $\delta^{15}N$ in leaves, forest floor and mineral soils, soil inorganic N concentration, potential rates of mineralization and nitrification, and stream water chemistry were measured from 2008 through 2009. We initially hypothesized that (1) N

deposition, soil N cycling rates and plant N concentration would be elevated in urban and suburban forests compared with those in rural forests; (2) foliar $\delta^{15}N$ would be elevated with the increased N deposition and soil N availability.

Methods and materials

Study sites

Seven sites were selected along a 150 km urban-to-rural transect in the Pearl River Delta, extending from Guangzhou metropolitan area (Shunfengshan and Maofengshan, abbreviated to SFS and MFS, respectively), through suburban Dinghushan (DHS) and Lankeshan (LKS) in Zhaoqing and ending in rural Heishiding (HSD), Yanshuitian (YST) and Lengshuichao (LSC) (all three in the Heishiding Natural Reserve in Zhaoqing, Fig. 1). Longitude ranges from E111°50.6′ to E113°27.6′ and latitude ranges from N22°48.4′ to N23°29.4′ (Table S1). One to three forests were selected at each site. In total, there were 14 forests along this transect, of which eight were broadleaved forests and six were pine forests (Table S1). There are no visible symptoms of forest decline in all selected forests. Except for the DHS broadleaved forest that is more than 400 years old (old-growth), all other forests are naturally regenerated, unmanaged secondary growth after being cut, with neither forest fires nor prior fertilization recorded. The ages of these forests range from 30 to 70 years (Table S1). We did not find exceptionally high or lower values for plant N stable isotope composition or soil N availability in the old-growth forest. Thus the results for that forest were included in this paper. The major species are listed in Table S1. The soil is lateritic red earth (Ultisols in the USDA soil taxonomy or Acrisols in the FAO soil classification) formed from sandstone at suburban sites, whereas it is formed from granite at urban and rural sites. The climate is warm and humid in the region. Annual precipitation ranges from 1380 to 1927 mm, and mean

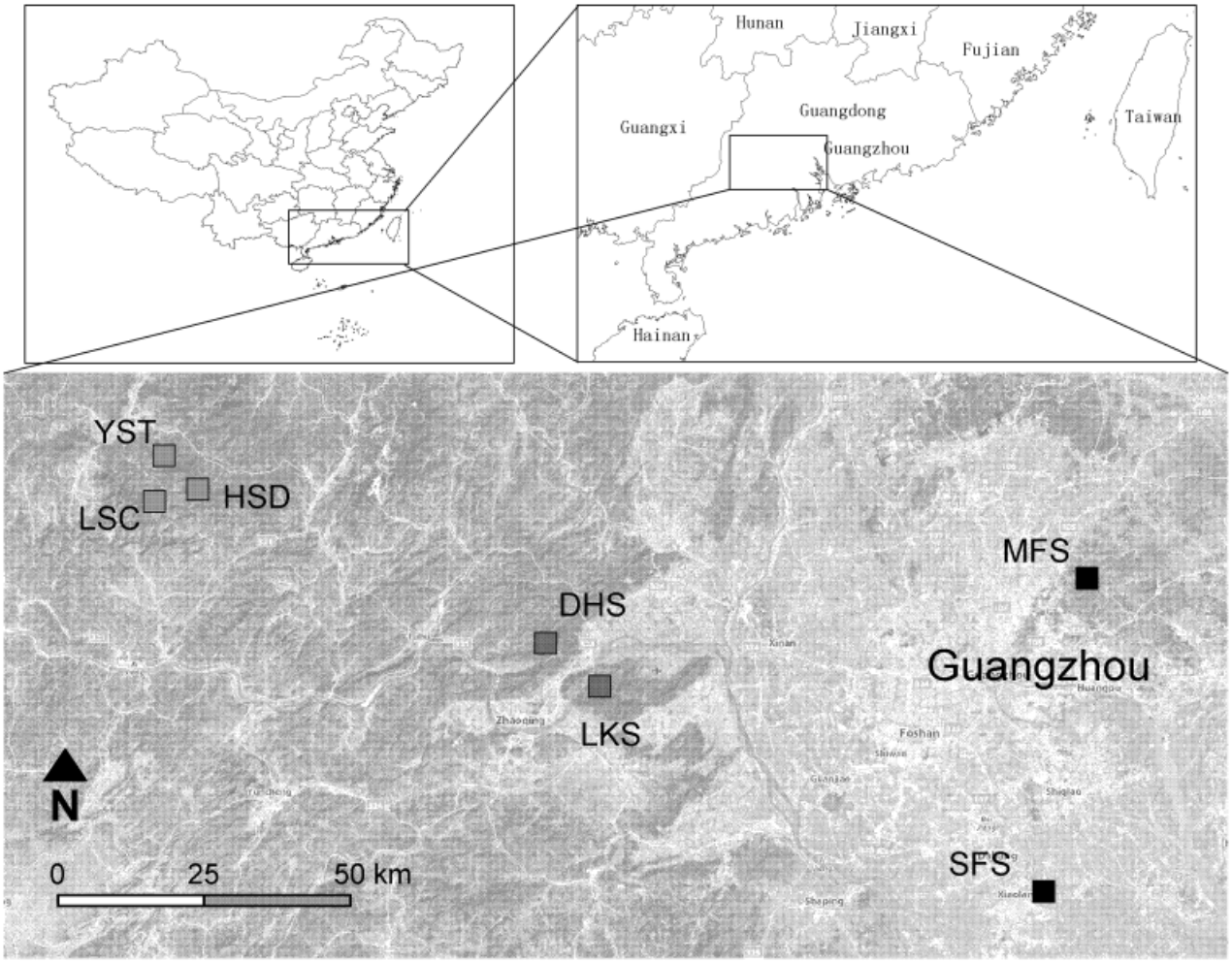

Fig. 1 A 150 km long urban-to-rural transect in the Pearl River Delta, southern China, running from highly urbanized Guangzhou city to rural Heishiding Natural Reserve in Zhaoqing. A total of seven sites (filled squares) were selected along the transect. One to three forests were selected at each site. The detailed information for each site is listed in Table S1.

annual air temperature ranges from 19.5 to 21.9 °C for the study sites.

Atmospheric N deposition

We chose to use ion-exchange resin (IER) columns to monitor N deposition in precipitation (bulk deposition) at seven sites and in throughfall at all 14 forests along the study transect. It is generally not feasible to monitor atmospheric concentrations and deposition of a suite of important atmospheric pollutants over an extensive number of sites. The ion IER technique is advantageous over the conventional collection methods (i.e., water is frequently collected and N concentration is directly measured for water samples. N fluxes are calculated based on N concentrations and water fluxes), which are labor intensive and analytically expensive to implement at broad scales (Van Dam *et al*., 1991; Kjønaas, 1999; Köchy & Wilson, 2001; Fenn & Poth, 2004; Simkin *et al*., 2004).

The IER columns were made according to the method developed by Fenn & Poth (2004). A funnel was connected to each IER column (a 16 mm × 330 mm polyvinylchloride [PVC] tube) with a septum and a fitting (Fig. S1). A white PVC pipe (44 mm i.d.) was placed outside the IER column to protect it from direct solar radiation (Fig. S1). The resin used for the IER collector is a mixture of strong base styrene anion exchange resin (201 × 7[717], similar to Amberlite IRA-400) and strong acid styrene cation exchange resin (001 × 7[732], similar to Amberlite IR-120; Guangzhou, China). Sixty-four milliliters of mixed resin (half cation and half anion), with a total ion exchange capacity of about 86 $mmol_c$, was added to each PVC column and rinsed with distilled water. This volume of resin is sufficient to collect N input equivalent to a field deposition flux of 1040 kg N ha^{-1}, assuming equal amounts of NO_3^- and NH_4^+. Polyester floss was inserted at the bottom (as a support platform) and top (as a filter) of the resin columns. The bottom end of the IER column was closed using a PVC cap with *x* cuts allowing for drainage (Fenn & Poth, 2004).

At each site, five IER columns were installed to collect precipitation in an open area. Meanwhile, two IER columns with the both ends sealed were installed to determine the background N (e.g., potential NH_4^+ release from amine group, Fenn & Poth, 2004) in the ion resin. Background N in the resin, although minimal (0.01–0.02 kg NH_4^+–N ha^{-1} and 0.12–1.06 kg NO_3^-–N ha^{-1}), was subtracted from the deposition data to determine the actual throughfall/bulk N deposition. Five IER columns were installed in all forests except at DHS where 10 IER columns were set in each of the three forests. A fine mesh screen was placed in the funnel opening to keep out debris (Fig. S1). The columns were retrieved and new ones were installed about every 4 months from April 2008 to July 2009 (Table S2). The preliminary laboratory test with the IER columns preloaded with simulated throughfall solution, which was equivalent to the deposition rates of 7–71 kg N ha^{-1} yr^{-1} (half in NH_4^+ and half in NO_3^-), showed that the resin had high removal efficiency of 94–100% for NH_4^+ and 90–99% for NO_3^-, and the recovery efficiency of 97% for NH_4^+ and 90% for NO_3^- after two KCl extractions.

At the end of each field sampling, the resin columns were unscrewed from the funnel assembly, sealed at both ends, and returned to the laboratory. The resin from each column was well mixed and 6.4 mL of the resin was extracted with 50 mL 2 M KCl solution. NH_4^+ concentration in the resin extract was determined by the indophenol blue method followed by colorimetry, and NO_3^- concentration was determined after cadmium reduction to NO_2^-, followed by the sulfanilamide–NAD reaction (Liu *et al*., 1996).

N concentration and ^{15}N natural abundance ($\delta^{15}N$)

Sampling of leaves and soils was performed in September and October 2009, around the end of the rainy season (Table S3). In each forest, four plots (10 m × 10 m each) were set up for sample collection. In each plot, sun-lit, mature, and healthy leaves or needles of each dominant species were collected from 1 to 3 trees. Three points were randomly selected in each plot for forest floor and soil sampling. Oi (newly fallen litter) and Oe + a (partially decomposed litter) layers were separately collected using a 10 cm × 10 cm sampling frame at each sampling location. After forest floor collection, 0–10 cm mineral soil was sampled using a soil core (2.5 cm diameter). The materials from the same plot were mixed. In the rural YST pine forest, leaves and floor materials were not collected due to the height of the canopy and the dense understory.

In the laboratory, forest floor materials were dried at 60 °C to constant weight and weighed. Mineral soil from each plot was passed through a 2 mm mesh sieve to remove fine roots and coarse fragments. Subsamples of oven-dried foliage (leaves and needles), floor materials, and mineral soils were ball milled and analyzed for ^{15}N natural abundance and total N and C concentrations by EA-IRMS (EA1112 coupled with Delta-XP, ThermoFisher Scientific K.K., Yokohama, Japan). Calibrated DL-alanine ($\delta^{15}N = 1.7‰$), glycine ($\delta^{15}N = 10.0‰$), and histidine ($\delta^{15}N = 8.0‰$) were used as the internal standards. The ^{15}N natural abundance of the sample relative to the standard (atmospheric N_2) was expressed as the following:

$$\delta^{15}N = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000\,(‰),$$

where *R* represents the isotope ratio ($^{15}N/^{14}N$), and R_{standard} is the $^{15}N/^{14}N$ for atmospheric N_2. The analytical precision for $\delta^{15}N$ was, in general, better than 0.2‰.

Potential N mineralization and nitrification

The sieved fresh mineral soil was used to determine the extractable inorganic N (NH_4^+ and NO_3^-) pool, and the potential rates of net N mineralization and nitrification, using methods similar to those described in Fang *et al*. (2009b). Briefly, soil was extracted with 2 M KCl solution. NH_4^+ concentration in the extract was determined by the indophenol blue method followed by colorimetry, and NO_3^- concentration was determined after cadmium reduction to NO_2^-, followed by the sulfanilamide–NAD reaction (Liu *et al*., 1996). Soil pH(H_2O) was measured using a glass electrode (Liu *et al*., 1996). For the measurement of net N mineralization and nitrification rates, sieved soil was adjusted to soil water

content of 60% of the water holding capacity and then incubated for 30 days at 20 °C. At the end of incubation, extractable inorganic N concentration of the incubated soil was determined using the same method as that mentioned above. Net N mineralization rate was calculated as the difference between the initial and incubated inorganic N concentrations, and nitrification was calculated as the difference between the initial and incubated NO_3^- concentrations. Percent nitrification was defined as the percentage of net nitrification rate of the net N mineralization rate.

Stream water

Stream water from all broadleaved forests and two pine forests was collected with prerinsed polypropylene syringes, at the time when IER collectors were set out or exchanged, or soil and leaves were sampled (Table S2). Sample was filtered in the field through 0.45 μm filters into 50 mL plastic bottles. No stream water was available for the other four pine forests because there were no apparent streams draining the study forests. Stream water pH was determined in the field with a digital pH meter when water was collected. Concentrations of NH_4^+ and NO_3^- were determined for all water samples by ion-chromatography (Dionex DX-120, Osaka, Japan).

Statistics

One-way analysis of variance was performed for the broadleaved forests and pine forests, respectively, in order to examine the differences in each investigated variable among sites (urban, suburban, and rural) and among forests. Correlation analysis with two-tail significance test was used to examine the relationships between variables across the study transect. To compare the differences in %C, %N, δ^{15}N, and C/N ratio among foliage, forest floor layer, and mineral soil, paired *t*-tests were used across the transect. All analyses were conducted using the PASW STATISTICS 18.0 for Windows. Statistically significant differences were set at P values <0.05 unless otherwise stated.

Results

N deposition

Bulk deposition varied from 16.2 to 38.2 kg N ha^{-1} yr^{-1} along the study transect, with 55% to 63% in the form of NH_4^+ (Table 1). N deposition was the highest in the suburban sites, significantly higher than the urban sites, and the lowest in rural sites (Table 1 and Table S3). Throughfall N input varied greatly from 11.7 to 65.1 kg N ha^{-1} yr^{-1}, of which 36–64% was in the form of NH_4^+ (Fig. 2). Throughfall N input was also highest in the suburban sites and lowest in the rural sites (Table S3).

Table 1 Precipitation N deposition at seven sites along the study transect in southern China (kg N ha^{-1} yr^{-1})

Location	Site	NH_4^+	NO_3^-	DIN
Urban	SFS	18.8 (1.9)b	10.9 (1.0)bc	29.8 (2.5)b
	MFS	13.0 (0.3)c	8.9 (1.3)cd	21.9 (1.5)c
Suburban	LKS	22.5 (1.0)a	15.7 (1.0)a	38.2 (0.8)a
	DHS	20.8 (0.8)ab	13.3 (0.6)ab	34.1 (1.3)ab
Rural	HSD	10.0 (0.4)c	8.1 (0.4)cd	18.1 (0.5)c
	LSC	9.6 (0.3)c	7.2 (0.3)d	16.7 (0.4)c
	YST	9.7 (0.6)c	6.5 (1.0)d	16.2 (1.5)c

SE in parentheses. Values within columns sharing the same letter are not significantly different (one-way ANOVA with Tukey's HSD; $P<0.05$).
ANOVA, analysis of variance; DHS, Dinghushan; HSD, Heishiding; LKS, Lankeshan; LSC, Lengshuichao; MFS, Maofengshan; SFS, Shunfengshan; YST, Yanshuitian.

N concentration and ^{15}N natural abundance (δ^{15}N)

Mean foliar %N ranged from 1.5% to 2.2% in the broadleaved forests, and from 0.9% to 1.6% in the pine forests (Fig. 3b, Table S4). In both broadleaved and pine forests, foliar %N showed decreasing trends along the study transect from urban to rural areas (Fig. 3b), with leaf %N in rural broadleaved forests significantly lower than in urban and suburban broadleaved forests (Table S5). N concentration of the Oi layer was also significantly lower in the rural forests than in urban and suburban forests (Fig. 3b and Table S5). Spatial patterns of N concentration in the Oa + e layer and in the mineral soil along the transect were not clear (Fig. 3b, Table S5). Foliar %N was correlated with %N of the Oi layer across all forests ($P = 0.02$, $n = 13$), but not with %N of the Oa + e layer or %N of the mineral soil (Fig. 3).

The C/N ratios were from 25 to 57 for the foliage, 27 to 61 for the Oi layer, and 25 to 50 for the Oa + e layer (Fig. 3c). The mean C/N ratios of foliage and floor materials generally increased along the study gradient from urban to rural areas for both forest types (Fig. 3c, Table S6). Mineral soils had narrow C/N ratios (9–15; Fig. 3c, Table S6). Similar to N concentrations, the C/N ratios of mineral soils showed no clear pattern along the transect (Fig. 3c).

The mean δ^{15}N of foliage and forest floor layer were all negative (−7.6‰ to −1.8‰) except for the rural HSD pine forest (0.7‰; Fig. 3a, Table S4). Foliar δ^{15}N was close to those of the Oa + e layer, particularly in the pine forests. The mean δ^{15}N of both foliage and forest floor layer was significantly lower (by 0.5–8.0‰) than those of the corresponding mineral soil ($P<0.05$). The δ^{15}N of both foliage and forest floor layer increased along the gradient from urban to rural areas, regardless of forest type (Fig. 3a, Table S5). In the broadleaved forests, foliar

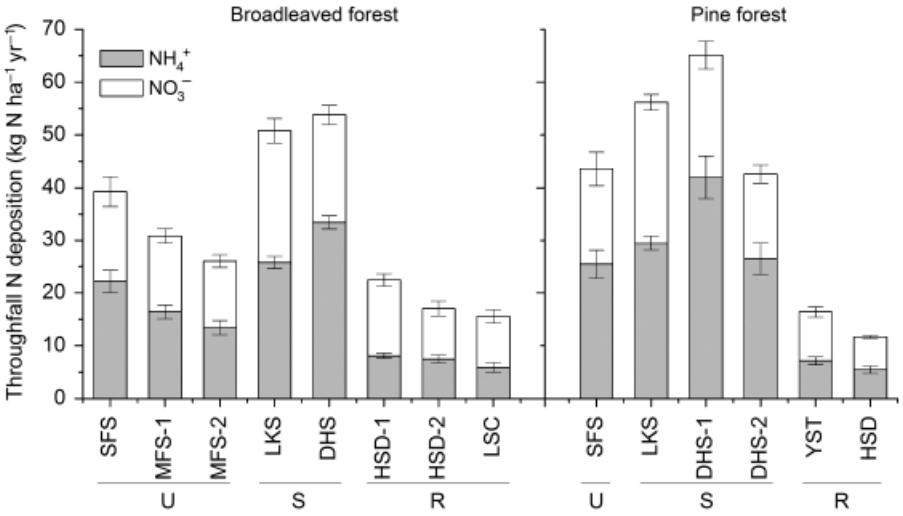

Fig. 2 Nitrogen deposition in throughfall (mean ± 1 SE) in 14 forests along the study transect ($n = 5$, except at DHS where $n = 10$ in each forest). U, S, and R denote urban, suburban, and rural sites, respectively.

δ^{15}N was −4.1‰, −3.6‰, and −2.1‰ at urban, suburban, and rural sites, respectively; in the pine forests, it was −6.4‰, −3.9‰, and 0.7‰ (Table S5). The difference among sites in the broadleaved forests was significant ($P = 0.012$, Table S5).

Soil δ^{15}N values were positive (0.4–2.8‰) at all sites except at the urban SFS site (Fig. 3a). The δ^{15}N values were lower in the urban forests soils than in the rural forest soils ($P = 0.002$ and <0.001 in the broadleaved and pine forests, respectively, Table S5). Foliar δ^{15}N was correlated with δ^{15}N of the forest floor layers (Oi and Oe + a) in both forest types ($P = 0.002$–0.07) or across all forests ($P = 0.001$ and 0.006), but not with soil δ^{15}N (Fig. 3a).

Soil net N mineralization and nitrification

Soil extractable inorganic N varied greatly from 7.6 to 14.1 mg N kg^{-1}, and the difference among sites was not so clear in the broadleaved forests while it decreased from urban to rural sites in the pine forests (Table S7). Soil NO_3^- concentration was significantly ($P < 0.001$) lower in the rural forests than in the urban and suburban forests for both forest types, Fig. 4a, Table S7). Nitrate accounted for 40–79% of the total inorganic N in the broadleaved forest soils at the urban and suburban sites, compared with 5–19% at the rural sites. In the pine forest, NO_3^- accounted for 20–42% at the urban and suburban sites whereas 5–14% at the rural sites.

Soil potential N production rates showed even wider ranges than inorganic N concentration, being from 1.3 to 24.6 mg N kg^{-1} mon^{-1} for net N mineralization and from 0.2 to 21.2 mg N kg^{-1} mo^{-1} for net nitrification, respectively (Fig. 4b). In the broadleaved forests, net N mineralization was 20.9, 20.4, and 11.3 mg N kg^{-1} mon^{-1} at the urban, suburban, and rural sites, respectively, and net nitrification was 20.5, 19.0, and 3.9 mg N kg^{-1} mon^{-1} (Table S7). In the pine forests, the highest rates of N mineralization and nitrification were observed at the suburban sites (Fig. 4b), where the N deposition was the highest and the forests were relatively older (Table S1). Percent nitrification, defined as the fraction of mineralized NH_4^+ that nitrified, was also higher at urban and suburban sites (95–102% in the broadleaved forests and 38–67% in the pine forests, respectively) than at rural sites (35% and 28%, Table S7). Soil NO_3^- concentration was significantly and positively correlated with net nitrification rate across the broadleaved forests ($R = 0.87$, $P = 0.005$, $n = 8$) or across all forests ($R = 0.82$, $P < 0.001$, $n = 14$).

All soils were highly acidic (Fig. 4c), with pH values significantly ($P < 0.001$) lower in both urban and suburban forests (4.0 and 3.9, respectively) than in the rural forests (4.5). The lowest pH value of 3.6 was found in the suburban DHS broadleaved forest (Fig. 4c), a forest that is >400 years old and is N-saturated due to long-term N deposition and N accumulation (Fang *et al.*, 2008).

Stream water

Mean pH values in stream water were between 4.1 and 7.3, with significantly low values in the suburban forests and highest values in the rural forests (Table 2). Ammonium concentrations were very low (from 0 to 0.40 mg N L^{-1}, averaging 0.05 mg N L^{-1}), thus are not shown here. Nitrate concentrations were between 0.3 and 4.3 mg N L^{-1}, and were significantly ($P < 0.001$) higher in the suburban and urban forests than in the

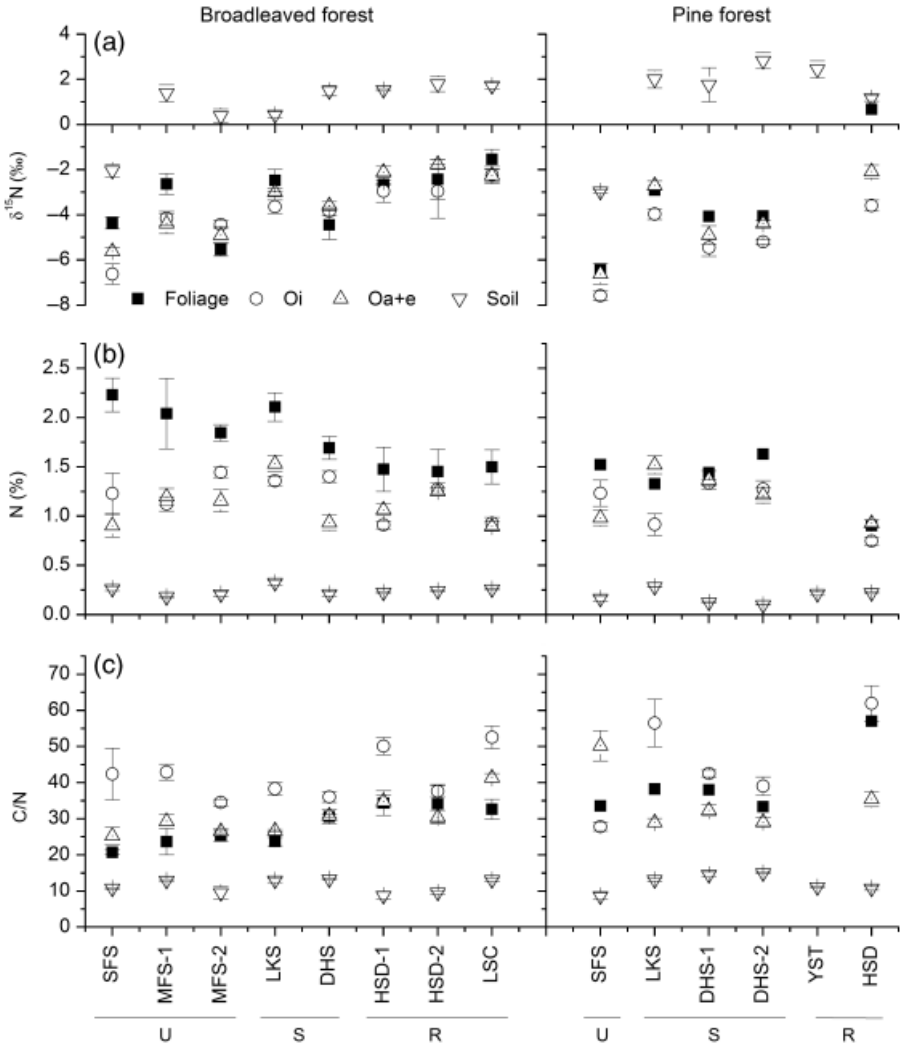

Fig. 3 δ^{15}N (a), %N (b), and C/N ratio (c) of foliage, forest floor (Oi and Oa + e layer), and 0–10 cm mineral soil from 14 forests along the study transect. U, S, and R denote urban, suburban, and rural sites. Shown are mean ± 1 SE. Leaves and forest floor materials were not collected from the YST pine forest.

rural forests, for both forest types. The pH values and NO_3^- concentration was negatively correlated ($R = -0.30$, $P < 0.001$, $n = 51$ for the broadleaved forests; $R = -0.85$, $P = 0.001$, $n = 8$ for the pine forests) along the study transect (data not shown). In the broadleaved forests, the pH value in stream water significantly decreased and NO_3^- concentration increased with increasing N deposition (Fig. 5e and f). Across all forests, stream water NO_3^- concentration was negatively related to foliar δ^{15}N ($P = 0.06$, data not shown).

Discussion

Atmospheric N deposition

N fluxes in precipitation (bulk deposition, mostly wet deposition plus particulate deposition, Lovett *et al*., 2000) are frequently monitored because this is the least complex and most reproducible component to measure. However, in areas of significant fog or dry deposition, wet deposition is often the smallest component of total deposition inputs, particularly in forest ecosystems with high leaf area index values, where plant canopies serve as efficient scavengers of air pollutants in the wet and dry forms (Fenn & Poth, 2004; Fenn *et al*., 2008). At the suburban DHS site, bulk deposition measured using IER was 34.1 kg N ha^{-1} yr^{-1} in this study (Table 1), which is comparable with the previous measurements of 34.2 kg N ha^{-1} yr^{-1} in 2004 and 31.6 kg N ha^{-1} yr^{-1} in 2005 using conventional open collectors (Fang *et al*., 2008). The bulk deposition was slightly higher at the suburban LKS (38.2 kg N ha^{-1} yr^{-1}, Table 1), a site near DHS. The N input in throughfall was substantially higher (on average by 18 kg N ha^{-1} yr^{-1}) than that in

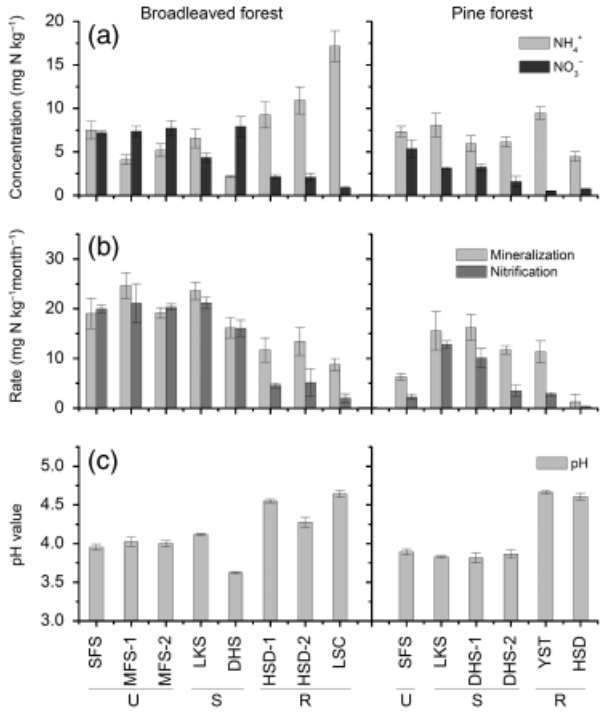

Fig. 4 Extractable inorganic N (NH_4^+ and NO_3^-) concentrations (a), potential rates of net N mineralization and nitrification (b), and pH values (c) in the 0–10 cm mineral soil from 14 forests along the transect. U, S, and R denote urban, suburban, and rural sites, respectively. Shown are mean ± 1 SE.

bulk deposition at these two sites (Table 1 and Fig. 2), pointing to the great contribution of dry particles or gases attached to the leaf surface.

At DHS, the throughfall N input estimated in this study was higher than the previous estimates using the open collectors and biweekly collection of water samples (Fang *et al*., 2008). Lower estimates in the previous study were likely caused by some dry deposition in the dry season (from October 2004 to February 2005) that was not included, because the low amounts of rain made it difficult to obtain uncontaminated throughfall samples (Fang *et al*., 2008). This was demonstrated by unusually high concentration in a few winter throughfall samples (Fang *et al*., 2008). In contrast, the IER collectors retained whatever ions were transported by gravitational flow from the funnel collector to the resin column, even in low volumes (low precipitation events). In some areas with prolonged dry period such as our study region, the long dry periods result in atmospheric pollutants accumulation on plant canopies. In the San Bernardino Mountains in southern California with a summer-dry climate, precipitation N deposition was 12.3 kg N ha^{-1} yr^{-1} at a high N deposition site and 3.3 kg N ha^{-1} yr^{-1} at a relatively low one, respectively, whereas their respective throughfall N deposition was

Table 2 The pH values and NO_3^- concentrations in stream water from selected forests along the study transect

Location	Forest	Sample number	pH	NO_3^- (mg N L^{-1})
Broadleaved forest				
Urban	SFS	4	6.3 (0.2)c	2.0 (0.8)bc
	MFS-1	5	6.3 (0.4)c	1.6 (0.2)b
	MFS-2	4	6.6 (0.2)bc	1.5 (0.4)bc
Suburban	LKS	8	4.5 (0.1)d	0.8 (0.1)bc
	DHS	5	4.1 (0.1)d	4.3 (0.8)a
Rural	HSD-1	10	7.2 (0.1)ab	0.8 (0.1)bc
	HSD-2	5	7.3 (0.1)a	0.6 (0.1)bc
	LSC	10	6.9 (0.1)ab	0.3 (0.1)c
Pine forest				
Suburban	LKS	3	4.5 (0.0)b	1.2 (0.1)a
Rural	YST	5	7.1 (0.2)a	0.5 (0.0)b

SE in parentheses. Values within columns from the same forest type sharing the same letter are not significantly different (one-way ANOVA with Tukey's HSD; $P < 0.05$).
ANOVA, analysis of variance; DHS, Dinghushan; HSD, Heishiding; LKS, Lankeshan; LSC, Lengshuichao; MFS, Maofengshan; SFS, Shunfengshan; YST, Yanshuitian.

143.9 and 15.0 kg N ha^{-1} yr^{-1}, respectively (Fenn & Poth, 2004). Similar differences between precipitation and throughfall N deposition are also obtained using IER collectors (Fenn & Poth, 2004). At two urban sites in this study, we also observed that the throughfall input was higher (by 4–14 kg N ha^{-1} yr^{-1}) than the input in precipitation (Table 1 and Fig. 2). N input in both bulk deposition and throughfall was higher at the suburban sites than at the urban sites (Table S3), probably because the suburban sites are located in a downwind area of the Pearl River Delta, one of the rapid economically developing centers of China (Fig. 1).

Impact of N deposition on ecosystem N cycling

In this study, N concentration in forest floor organic matter ranged from 0.73% to 1.53% (Fig. 3b), of which most was lower than the critical value for the onset of nitrification at 1.4% in the spruce-fir forests of New England (McNulty *et al*., 1991). Forest floor material had C/N ratio of 25–62 (Fig. 3c), >25 above which nitrification and NO_3^- leaching is often elevated in temperate and boreal forests in Europe (Gundersen *et al*., 1998a, 2006; MacDonald *et al*., 2002; Kristensen *et al*., 2004) and the northeastern United States (Goodale & Aber, 2001). Nevertheless, in urban and suburban broadleaved forests, net nitrification was high and was 95–100% of mineralization (percent nitrification, Table S7). In the broadleaved forests soil nitrification was positively correlated with throughfall N input (Fig. 5a), leaf %N

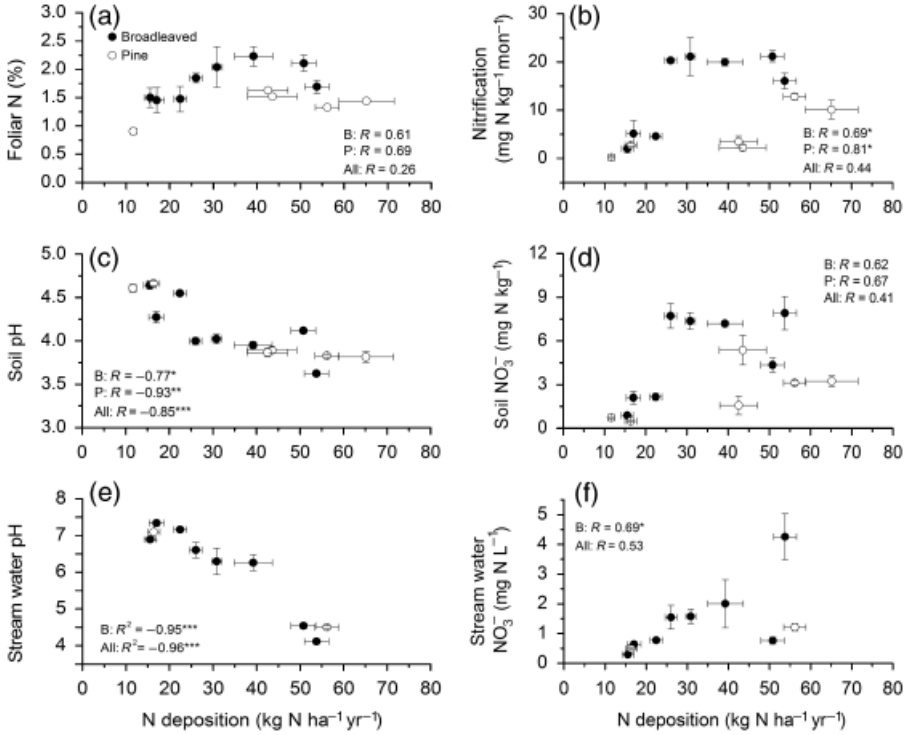

Fig. 5 Correlations of N deposition (in throughfall) with foliar %N (a), net nitrification (b), pH in soil (c) and stream water (d), NO_3^- concentration in soil (e) and stream water (f) across the study transect. Correlation coefficients (R) are shown across the entire gradient and for each forest type. B and P indicate broadleaved forests and pine forests, respectively. *, **, ***Significance at the level of 0.05, 0.01, and 0.001, respectively.

(Fig. 6e), and negatively with the C/N ratios of leaf and Oa + e materials (data not shown), while in the pine forests nitrification was only correlated with Oa + e N concentration (data not shown). However, soil nitrification was not correlated to either %N or C/N ratio of the mineral soils

In the rural forests, the surface mineral soil had relatively low NO_3^- concentration which accounted for 5–19% of total inorganic N (Fig. 4b). Soil NO_3^- production rate (net nitrification) was also low, especially in the pine forests. Combined with low %N in the foliage and forest floor organic matter, these results indicate primary production in the rural forests may still be limited by N supply and that these forests are in stage 1 according to the concept of N saturation of Aber *et al*. (1998), although N deposition was elevated there (throughfall N input was 12–22 kg N ha^{-1} yr^{-1}, which is greater than 10 kg N ha^{-1} yr^{-1}, a threshold above which N leaching often increases in temperate and boreal forests (e.g., MacDonald *et al*., 2002)). Soil N availability was higher at suburban and urban sites than the rural sites. Soil NO_3^- pool, proportion of NO_3^- relative to total inorganic N, potential rates of net N mineralization and nitrification all generally increased moving from rural to urban sites (Fig. 4, Table S7).

The pattern of N saturation along the urban–rural transect was supported by many other indicators, including %N and C/N in leaves and floor materials (Fig. 3), pH values and NO_3^- concentration in stream water draining from the forested catchments (Table 2). These results point out strongly the enhanced N status and fast N cycling in the urban and suburban forests. Several factors may have potentially contributed to the variation in soil N availability along this urban to rural transect. First of all, atmospheric N deposition was measured to be 26–65 kg N ha^{-1} yr^{-1} at the urban and suburban sites, which was significantly higher than the 12–22 kg N ha^{-1} yr^{-1} at the rural sites (Fig. 2). Increased N input can induce nitrification activity and NO_3^- leaching, as demonstrated in many N-addition experiments and along the natural N deposition gradients (e.g., Aber *et al*., 1998, 2003; Gundersen *et al*., 1998a). In the present study soil net nitrification was significantly ($P < 0.05$) related to throughfall N deposition for both forest types (Fig. 5b). Secondly, the atmospheric input of other nutrients, although not measured in the present study, such as phosphorus and cations that can stimulate soil microbial N turnover, is perhaps also higher in urban and suburban sites than in rural sites. In addition, temperature differences may exist across the entire

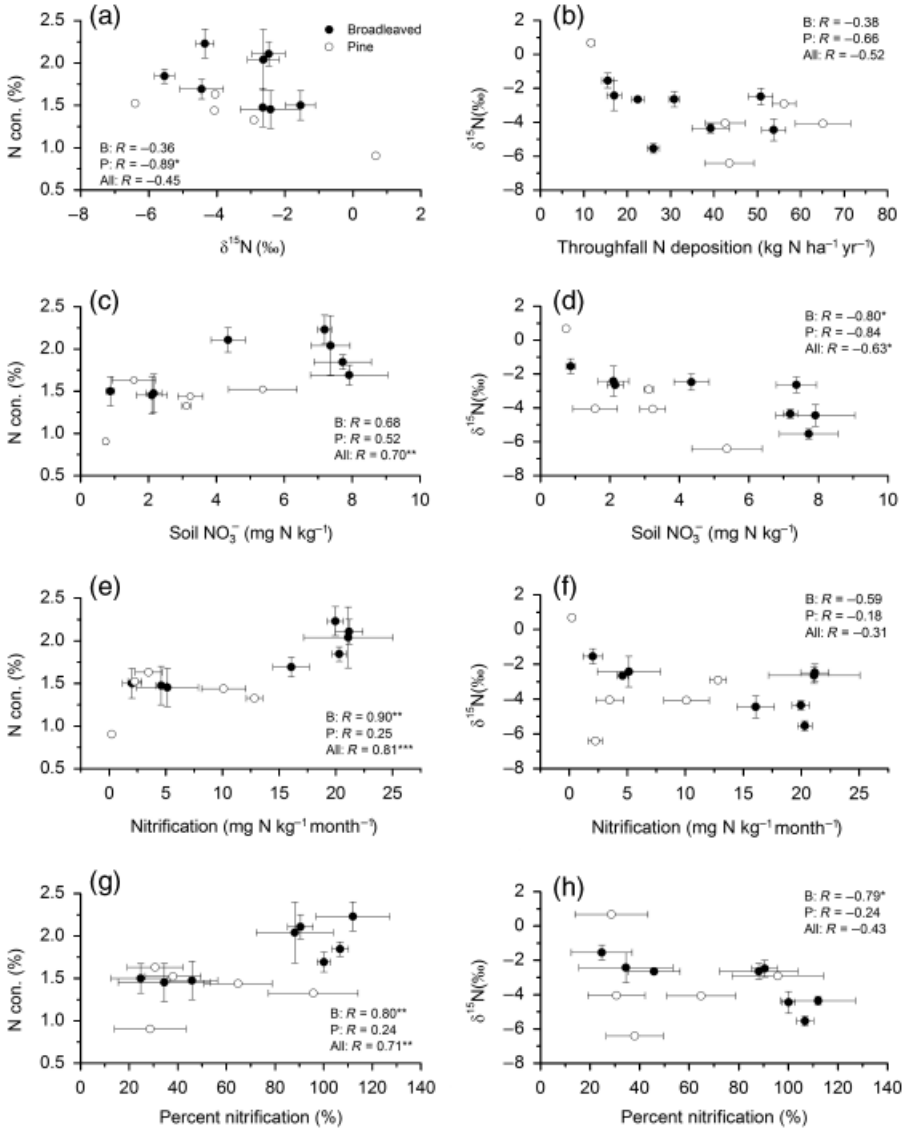

Fig. 6 Correlations of foliar %N with δ^{15}N (a), of foliar δ^{15}N with N deposition in throughfall (b), of foliar %N and δ^{15}N with soil NO_3^- concentration (c, d), net nitrification rate (e, f), and percent nitrification (g, h) across the study transect. Correlation coefficients (*R*) are shown across the entire gradient and for each forest type. B and P indicate broadleaved forests and pine forests, respectively. *, **, ***Significance at the level of 0.05, 0.01, and 0.001, respectively.

transect due to the 'urban heat island effect' and the relatively higher elevation of rural forests than urban and suburban ones (Table S1). Finally, litter quality has been widely considered as one of the critical factors regulating soil N cycling (Zhu & Carreiro, 2004). We found that nitrification rate was correlated positively with N concentrations in both foliage (Fig. 6e) and the Oi layer ($P = 0.06$), and negatively with C/N ratios of foliage ($P = 0.005$) and the Oa + e layer ($P = 0.01$) across the entire transect. However, both foliar N status and soil N availability were likely to be a result of increased atmospheric deposition of N and other nutrients in the urban end of the transect (Figs 5 and 6).

Ecosystem ^{15}N natural abundance

Our current understanding of global patterns of climate control of ecosystem ^{15}N natural abundance is primarily based on studies conducted in North America, Europe, South America, Australia, and Africa (e.g., Martinelli *et al*., 1999; Amundson *et al*., 2003; Craine *et al*., 2009b). There is an apparent data gap over a large area in eastern Asia, particularly subtropical China. In the present study, foliar δ^{15}N varied from −6.6‰ to 0.7‰ along the study transect. This range is comparable to that of −5.0‰ to 2.7‰ that has been observed across three forested catchments in southwestern China

receiving different amount of N depositions in throughfall (6.5–43.5 kg N ha^{-1} yr^{-1}, Jiang *et al*., 2009) and that of −3.1‰ to 0.9‰ in three Masson's pine forests in southern China (Kuang *et al*., 2010). Similar results were also observed in the tropical forests of Tanzania (−3.1‰ to −1.1‰) and Hawaii, USA (−5‰ to 2‰), and in other temperate forests (see Amundson *et al*., 2003). Out results are well within the worldwide range of −10.1‰ to 21.7‰ (Martinelli *et al*., 1999) for both tropical and temperate forests. The average $\delta^{15}N$ values of our study, −3.8‰ for foliage and 0.9‰ for the 0–10 cm mineral soil across the entire transect, are both much lower than the averages of tropical forests (3.7‰ and 9.0‰ for foliage and soil, respectively) but close to those of temperate forests (−2.8‰ and 2.0‰) compiled by Martinelli *et al*. (1999).

In our study, we found that foliar $\delta^{15}N$ was negatively correlated with soil NO_3^- concentration (Fig. 6d) and stream NO_3^- concentration ($R = 0.65$, $P = 0.06$, data not shown). In the broadleaved forests, foliar $\delta^{15}N$ was also negatively correlated with soil percent nitrification (Fig. 6h). There was a trend for foliar $\delta^{15}N$ to decrease with increasing atmospheric N deposition and soil net nitrification although the trends were not statistically significant (Fig. 6b and f). This negative correlation between foliar $\delta^{15}N$ and N availability is in sharp contrast to the positive correlation observed in many previous studies (Garten, 1993; Emmett *et al*., 1998; Pardo *et al*., 2006; Craine *et al*., 2009b). However, our results are consistent with a number of individual studies where foliar $\delta^{15}N$ values did decrease or not increase after N supply had been increased (Koopmans *et al*., 1997; Stewart *et al*., 2002; Falkengren-Grerup *et al*., 2004; Bragazza *et al*., 2005; Pardo *et al*., 2007a, b; Templer *et al*., 2007; Craine *et al*., 2009a; Kranabetter & MacKenzie, 2010; Kuang *et al*., 2010; Takebayashi *et al*., 2010).

No leguminous (N-fixing) species was found in our selected forests. In pine forests, all sites had the same dominant species *Pinus massoniana*, suggesting the gradient pattern of $\delta^{15}N$ was not species related. To further separate possible plant species effect from urban–rural effect on $\delta^{15}N$, we sampled foliage material of *Syzygium rehderianum*. Foliar $\delta^{15}N$ of that species was −6.4‰ at the suburban DHS site that received highest N input while it was −1.5‰ at the rural LSC site that received lowest N input (Fig. 2, Table S4). In the DHS broadleaved forest, five different species had been collected and $\delta^{15}N$ showed a narrow variation of −6.6‰ to −3.8‰ (Table S4). Narrow ranges of species variation were also observed in other broadleaved forests (Table S4). Thus, we consider that the difference in species composition cannot explain the spatial pattern of foliar $\delta^{15}N$ observed along this study gradient.

Although we have documented extremely high N deposition in southern China (this study, Fang *et al*., 2008, 2009a), the history of N pollution in China is likely to be short. Economic development starts to rise in the early 1980s in China, associated with the heavy use of N fertilizer and increased emission of NO*x* and N_2O (Zheng *et al*., 2002). In contrast, both fertilizer N use and NO*x* emission increased sharply in the 1960s and 1970s and peaked at 1980s in the United States (Howarth *et al*., 2002) and Europe (van Egmond *et al*., 2002). One may suspect that relatively shorter history of increased N deposition compared with that in Europe and the United State might be one of the reasons for decreased $\delta^{15}N$ pattern along the N availability. However, the preliminary data on the tree rings of three pine trees in the suburban DHS site showed a significant decline in $\delta^{15}N$ of wood materials over the past 60 years, from 2.1‰ in the 1940s to −1.5‰ in the 2000s (Y. W. Kuang, personal communication).

The relationship between foliar $\delta^{15}N$ and atmospheric deposition can be much more complicated than the simple assertion that increased N losses in N-saturated sites would have higher $\delta^{15}N$ remaining. Instead, the change in foliar $\delta^{15}N$ could reflect either a shift in N sources (plant usage of NH_4^+ vs. NO_3^-, DIN vs. DON dominance in soil, and foliage uptake of deposited N vs. root uptake of soil N), or a change in isotopic discrimination during N uptake and assimilation under different N supply conditions, or both. We propose several potential mechanisms here. First of all, plant uptake of N in the urban and suburban forests may be dominated by NO_3^-. Foliar $\delta^{15}N$ was negatively correlated to soil NO_3^- concentration (Fig. 6d). Compared with soil NH_4^+ and organic matter N, soil NO_3^- is largely depleted of heavy ^{15}N (Garten, 1993; Koba *et al*., 1998; Takebayashi *et al*., 2010). Our data at the suburban DHS site showed that the $\delta^{15}N$ values of soil NO_3^- in the surface 10 cm soil were −3.2‰ to 1.0‰ and were, on average, 3.8‰ to 2.7‰ lower than those of NH_4^+ and bulk soil in the broadleaved forest (Koba *et al*., 2010). Takebayashi *et al*. (2010) reported that the $\delta^{15}N$ values of NO_3^- in the surface soils from seven forest stands in central Japan were even more negative, ranging from −2.0‰ to −10.7‰.

Second, canopy uptake of ammonia and/or NH_4^+ from the atmosphere may alter foliar N stable composition in our study. The whole-forest canopy N fertilization of a mature spruce-hemlock forest suggested that the forest canopy retained more than 70% of the applied N (Gaige *et al*., 2007). In the greenhouse, assimilation of $^{15}NH_4^{15}NO_3$ by seedling leaves suggested a preference for NH_4^+, and foliage uptake rate as high as 9 kg N ha^{-1} y^{-1} has been reported (Gaige *et al*., 2007 and references therein). The $\delta^{15}N$ value of NH_4^+ in this study was analyzed only for rainwater collected in 2008 at the DHS site; the results showed that rain NH_4^+ was

greatly ^{15}N-depleted, with $\delta^{15}N$ values ranged from −16.6‰ to −1.3‰ (K. Koba, Y. T. Fang, J. M. Mo, W. Zhang, X. K. Lu, L. Liu, T. Zhang, Y. Takebayshi & M. Yoh, unpublished results), probably due to the large discrimination against ^{15}N relative to ^{14}N when ammonia gas is produced in the process of volatilization. The $\delta^{15}N$ value of NO_3^- was analyzed for the rainwater collected in the City of Guangzhou, suburban DHS and rural HSD sites. Our data showed positive $\delta^{15}N$ values for almost all samples and no significant difference among sites (K. Koba, Y. T. Fang, J. M. Mo, W. Zhang, X. K. Lu, L. Liu, T. Zhang, Y. Takebayshi & M. Yoh, unpublished results; Y. T. Fang, K. Koba, X. M. Wang, D. Z. Wen, J. Li, Y. Takebayshi, X. Y. Liu & M. Yoh, unpublished results), suggesting that foliar direct NO_3^- uptake may not be important in the forests we studied.

Third, dissolved organic N (DON) in soils may be a predominant N form for roots in some extremely N-limited forests and becomes less important as an ecosystem approaches N saturation (Schimel & Bennett, 2004; Jones *et al*., 2005; LeDuc & Rothstein, 2010; Takebayashi *et al*., 2010). Measurements of the upper 10 cm soils in the DHS broadleaved forest showed that $\delta^{15}N$ of DON was 3.6–10.2‰ higher than those of inorganic N and 1.7–5.2‰ higher than those of bulk soil N (Koba *et al*., 2010). Soil DON was also shown to be enriched in ^{15}N by other researchers (Houlton *et al*., 2007; Pörtl *et al*., 2007; Takebayashi *et al*., 2010). Therefore, foliar $\delta^{15}N$ likely decreases when plant requisition of inorganic N increases relative to DON.

Finally, the fractionation during plant N uptake may be larger when soil available N is abundant than when N is limited (Evans, 2001). Tropical broadleaved trees often have their roots infected with arbuscular mycorrhizal fungi and the isotope effect during plant N uptake is considered to be minimal (Högberg, 1997; Evans, 2001; Hobbie & Colpaert, 2003; Houlton *et al*., 2007). And the reliance of N uptake on mycorrhizaes may decrease with increased N supply (Treseder, 2004). However, foliar $\delta^{15}N$, on average 9.0‰ lower than $\delta^{15}N$ of NH_4^+ and 4.7‰ lower than $\delta^{15}N$ of NO_3^- in the surface 10 cm soils in the suburban DHS broadleaved forest suggests an apparent fractionation during N incorporation to plant biomass even in the most N-saturated forest (Koba *et al*., 2010). The smaller spatial variation of $\delta^{15}N$ in bulk soil (ranging from −3.0‰ to 2.8‰) than that in leaves (ranging from −6.4‰ to 0.7‰; Fig. 3a) indicates that a difference in the magnitude of fractionation during N uptake and assimilation may exist among sites with different N supply, which in turn could contribute to the lower foliar $\delta^{15}N$ in urban and suburban forests.

Conclusions

Our study is among the first to assess regional N saturation using foliar ^{15}N abundance in tropical and subtropical forests. Our results showed that N deposition increased soil N availability and N turnover in urban and suburban forests, compared with those in rural forests. However, decreased foliage $\delta^{15}N$ with increasing N availability demonstrates that an increased N supply does not necessarily increase forest $\delta^{15}N$ values. We propose that the decreased foliar $\delta^{15}N$ values could be due largely to the increased uptake of ^{15}N-depleted soil NO_3^-, although foliar uptake of atmospherically deposited NH_4^+ and increased utilization of DIN relative to DON, as well as increased fractionation during N uptake and assimilation might additionally contribute to the decreased $\delta^{15}N$ values in the urban and suburban sites. Our results highlight that foliar $\delta^{15}N$ alone may not be a good indicator to assess regional pattern of N saturation, but can provide insights into N status and the mechanisms of N cycling in combination with other measures, such as soil N availability, foliar N concentration, and stream N export. Elevated N deposition in southern China has altered N biogeochemistry in forests, yet due to complicated N transformation pathways, the detailed mechanisms leading to altered $\delta^{15}N$ pattern remain to be tested in future studies.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Schematic drawing of throughfall/precipitation ion exchange resin collectors used in this study.
Table S1. Site characteristics along an urban-rural transect in southern China.
Table S2. Sampling date of ion exchange resin columns, soil and leaf, and stream water during the study period.
Table S3. Bulk deposition or throughfall N input along the urban-rural transect ($kg\,N\,ha^{-1}\,yr^{-1}$).
Table S4. Foliar $\delta^{15}N$, C and N concentrations, and C/N ratios for each sampled species in 13 forests along the transect.
Table S5. $\delta^{15}N$ values and %N for foliage, forest floor, and mineral soil from 14 forests along the transect.
Table S6. C/N ratios for foliage, forest floor, and mineral soil from 14 forests along the transect.
Table S7. Selected characteristics of the 0–10 cm mineral soil from 14 forests along the transect.

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