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 δ15N 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, NO3⁻ leaching to stream, and soil NO3⁻ concentration were low and NO3⁻ 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 NO3⁻ leaching to stream, and negatively with pH values in soil and stream water. Foliar δ15N was between −6.6‰ and 0.7‰, and was negatively correlated with soil NO3⁻ concentration and NO3⁻ leaching to stream across the entire transect, demonstrating that an increased N supply does not necessarily increase forest δ15N values. We proposed several potential mechanism that could contribute to the δ15N pattern, including (1) increased plant uptake of 15N-depleted soil NO3⁻, (2) foliage uptake of 15N-depleted NH₄⁺, (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

Received 6 April 2010; revised version received 16 June 2010 and accepted 19 June 2010

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 (NO3⁻), 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 NO₃⁻ loss starts, but tree growth is still high. In stage 3, tree growth declines and nitrification and NO₃⁻ loss continue to increase as the fraction of mineralized NH₄⁺ 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 δ¹⁵N for ¹⁵N 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 ¹⁵N when chemical bonds are broken, such that the product generally has lower ¹⁵N/¹⁴N ratio than the remaining substrate (Nadelhoffer & Fry, 1994; Robinson, 2001). During nitrification, the NO₃⁻ produced is depleted in ¹⁵N (lower ¹⁵N/¹⁴N) relative to the ammonium (NH₄⁺) substrate (Mariotti et al., 1981). When ¹⁵N-enriched NH₄⁺ is retained in the soil and ¹⁵N-depleted NO₃ 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 ¹⁵N (Pardo et al., 2006, 2007a, b). Thus, as an ecosystem moves toward N saturation (i.e., nitrification and consequent increase in NO₃ loss), it is predicted that soils and plants would become enriched in δ¹⁵N (Nadelhoffer & Fry, 1994; Pardo et al., 2006, 2007a, b).

Foliar δ¹⁵N has been used to assess the regional patterns of N saturation in Europe and North America. Näsholm et al. (1997) observed elevated foliar δ¹⁵N in spruce stands in southern Sweden with elevated NO₃ leaching. Pardo et al. (2001) suggested that even a small disruption of N cycle could cause a detectable increase in δ¹⁵N in organic soil horizons. Increases in foliar δ¹⁵N 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 δ¹⁵N 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 δ¹⁵N (Jussy et al., 2002; Pardo et al., 2002; Templer et al., 2007) and foliar δ¹⁵N (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 δ¹⁵N (Stewart et al., 2002; Falkengren-Gerup et al., 2004; Bragazza et al., 2005; Kranabetter & MacKenzie, 2010; Takebayashi et al., 2010) and that δ¹⁵N can be used only as an indicator of the N saturation stage, when δ¹⁵N 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 δ¹⁵N 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 δ¹⁵N 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 δ¹⁵N 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

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**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; Kjonaas, 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 is sufficient to collect N input equivalent to a field solution, which was equivalent to the deposition rates with the IER columns preloaded with simulated throughfall to July 2009 (Table S2). The preliminary laboratory test kept out debris (Fig. S1). The columns were retrieved and 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. NH4+ concentration in the resin extract was determined by the indophenol blue method followed by colorimetry, and NO3− concentration was determined after cadmium reduction to NO2−, followed by the sulfanilamide–NAD reaction (Liu et al., 1996).

**N concentration and 15N natural abundance (δ15N)**

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, sunlit, 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 (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 15N 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 (δ15N = 1.7‰), glycine (δ15N = 10.0‰), and histidine (δ15N = 8.0‰) were used as the internal standards. The 15N natural abundance of the sample relative to the standard (atmospheric N2) was expressed as the following:

\[
\delta^{15}N = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \text{ (‰)}
\]

where \( R \) represents the isotope ratio (15N/14N), and \( R_{\text{standard}} \) is the 15N/14N for atmospheric N2. The analytical precision for δ15N 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 (NH4+ and NO3−) 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. NH4+ concentration in the extract was determined by the indophenol blue method followed by colorimetry, and NO3− concentration was determined after cadmium reduction to NO2−, followed by the sulfanilamide–NAD reaction (Liu et al., 1996). Soil pH(H2O) 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, $\delta^{15}$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$^{-1}$)

<table>
<thead>
<tr>
<th>Location</th>
<th>Site</th>
<th>NH$_4^+$</th>
<th>NO$_3^-$</th>
<th>DIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>SFS</td>
<td>18.8 (1.9)b</td>
<td>10.9 (1.0)bc</td>
<td>29.8 (2.5)b</td>
</tr>
<tr>
<td></td>
<td>MFS</td>
<td>13.0 (0.3)c</td>
<td>8.9 (1.3)c</td>
<td>21.9 (1.5)c</td>
</tr>
<tr>
<td>Suburban</td>
<td>LKS</td>
<td>22.5 (1.0)a</td>
<td>15.7 (1.0)a</td>
<td>38.2 (0.8)a</td>
</tr>
<tr>
<td></td>
<td>DHS</td>
<td>20.8 (0.8)ab</td>
<td>13.3 (0.6)ab</td>
<td>34.1 (1.3)ab</td>
</tr>
<tr>
<td>Rural</td>
<td>HSD</td>
<td>10.0 (0.4)c</td>
<td>8.1 (0.4)c</td>
<td>18.1 (0.5)c</td>
</tr>
<tr>
<td></td>
<td>LSC</td>
<td>9.6 (0.3)c</td>
<td>7.2 (0.3)d</td>
<td>16.7 (0.4)c</td>
</tr>
<tr>
<td></td>
<td>YST</td>
<td>9.7 (0.6)c</td>
<td>6.5 (1.0)d</td>
<td>16.2 (1.5)c</td>
</tr>
</tbody>
</table>

SE in parentheses. Values within columns sharing the same letter are not significantly different (one-way ANOVA with Tukey’s HSD; P < 0.05).

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). Spatial patterns of N concentration of Oi layer was also significantly lower in the rural forests than in urban and suburban broadleaved forests (Table S5). N concentration of the Oi layer across all forests was 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). The mean %N of Oi layer across all forests was 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). The mean %N of 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 transect 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 $\delta^{15}$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 $\delta^{15}$N was close to those of the Oa + e layer, particularly in the pine forests. The mean $\delta^{15}$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 $\delta^{15}$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...
\( \delta^{15}N \) was \(-4.1\%o, -3.6\%o, \) and \(-2.1\%o \) at urban, suburban, and rural sites, respectively; in the pine forests, it was \(-6.4\%o, -3.9\%o, \) and \(0.7\%o \) (Table S5). The difference among sites in the broadleaved forests was significant \((P = 0.012, \text{Table S5})\).

Soil \( \delta^{15}N \) values were positive \((0.4–2.8\%o)\) at all sites except at the urban SFS site (Fig. 3a). The \( \delta^{15}N \) values were lower in the urban forests soils than in the rural forest soils \((P = 0.002 \text{ and } <0.001 \text{ in the broadleaved and pine forests, respectively, \text{Table S5})}\). Foliar \( \delta^{15}N \) was correlated with \( \delta^{15}N \) of the forest floor layers \((\text{Oi and Oe} + a) \) in both forest types \((P = 0.002–0.07) \text{ or across all forests } (P = 0.001 \text{ and } 0.006), \) but not with soil \( \delta^{15}N \) (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 \( \text{NO}_3^- \) 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, \( \text{NO}_3^- \) 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\(^{-1}\) mon\(^{-1}\) 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 \( \text{NH}_4^+ \) 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 \( \text{NO}_3^- \) 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 urban. U, S, and R denote urban, suburban, and rural sites, respectively.
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 $\delta^{15}$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

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Fig. 3 $\delta^{15}$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.
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). 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 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

### Table 2: The pH values and NO\(_3^-\) concentrations in stream water from selected forests along the study transect

<table>
<thead>
<tr>
<th>Location</th>
<th>Forest</th>
<th>Sample number</th>
<th>pH</th>
<th>NO(_3^-) (mg N L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broadleaved forest</td>
<td>Urban</td>
<td>SFS</td>
<td>4</td>
<td>6.3 (0.2)c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MFS-1</td>
<td>5</td>
<td>6.3 (0.4)c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MFS-2</td>
<td>4</td>
<td>6.6 (0.2)bc</td>
</tr>
<tr>
<td></td>
<td>Suburban</td>
<td>LKS</td>
<td>8</td>
<td>4.5 (0.1)d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DHS</td>
<td>5</td>
<td>4.1 (0.1)d</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>HSD-1</td>
<td>10</td>
<td>7.2 (0.1)ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSD-2</td>
<td>5</td>
<td>7.3 (0.1)a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LSC</td>
<td>10</td>
<td>6.9 (0.1)ab</td>
</tr>
<tr>
<td>Pine forest</td>
<td>Suburban</td>
<td>LKS</td>
<td>3</td>
<td>4.5 (0.0)b</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>YST</td>
<td>5</td>
<td>7.1 (0.2)a</td>
</tr>
</tbody>
</table>

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, Lengshuchao; MFS, Maofengshan; SFS, Shunfengshan; YST, Yanshuitian.
(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

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 $\delta^{15}$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 through-
fall (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). Our
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 δ¹⁵N 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 δ¹⁵N was negatively correlated with soil NO₃⁻ concentration (Fig. 6d) and stream NO₃⁻ concentration (R = 0.65, P = 0.06, data not
shown). In the broadleaved forests, foliar δ¹⁵N was also
negatively correlated with soil percent nitrification (Fig.
6h). There was a trend for foliar δ¹⁵N to decrease with
increasing atmospheric N deposition and soil net nitrifi-
cation although the trends were not statistically sig-
ificant (Fig. 6b and f). This negative correlation be-
 tween foliar δ¹⁵N 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 δ¹⁵N 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 gra-
dient pattern of δ¹⁵N was not species related. To further
separate possible plant species effect from urban–rural
effect on δ¹⁵N, we sampled foliage material of Syzygium
rehdarianum. Foliar δ¹⁵N 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 broad-
leaved forest, five different species had been collected
and δ¹⁵N 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 δ¹⁵N 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 NOx and N₂O (Zheng
et al., 2002). In contrast, both fertilizer N use and NOx
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 δ¹⁵N 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 δ¹⁵N 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 δ¹⁵N and atmospheric
deposition can be much more complicated than the
simple assertion that increased N losses in N-saturated
sites would have higher δ¹⁵N remaining. Instead, the
change in foliar δ¹⁵N could reflect either a shift in N
sources (plant usage of NH₄⁺ vs. NO₃⁻, 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 sev-
eral potential mechanisms here. First of all, plant uptake
of N in the urban and suburban forests may be dominated
by NO₃⁻. Foliar δ¹⁵N was negatively correlated to soil
NOS⁻ concentration (Fig. 6d). Compared with soil NH₄⁺
and organic matter N, soil NO₃⁻ is largely depleted of
heavy ¹⁵N (Garten, 1993; Koba et al., 1998; Takebayashi
et al., 2010). Our data at the suburban DHS site showed
that the δ¹⁵N values of soil NO₃⁻ 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 NH₄⁺ and bulk soil in the broadleaved
forest (Koba et al., 2010). Takebayashi et al. (2010) reported
that the δ¹⁵N values of NO₃⁻ 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 NH₄⁺
from the atmosphere may alter foliar N stable composi-
tion in our study. The whole-forest canopy N fertiliza-
tion 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
¹⁵NH₄⁺NO₃ by seedling leaves suggested a preference
for NH₄⁺, and foliage uptake rate as high as 9 kg N ha⁻¹ yr⁻¹ has been reported (Gaige et al., 2007 and references therein). The δ¹⁵N value of NH₄⁺ in this
study was analyzed only for rainwater collected in 2008
at the DHS site; the results showed that rain NH₄⁺ 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. Takebayashi & 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. Takebayashi & M. Yoh, unpublished results; Y. T. Fang, K. Koba, X. M. Wang, D. Z. Wen, J. Li, Y. Takebayashi, 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 broad-leaved 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; Portl 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 mycorrhizae 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.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 40703030 and 30725006), Provincial Natural Science Foundation of Guangdong (Nos. 8351065005000001 and 7006915), Pearl River Delta Forest Ecosystem Research Station, CAF, Grant-in-Aid for Scientific Research of Japan Society for Promotion of Science (JSPS) (Nos. 21310008 and 19310039), Grants-in-Aid for Creative Scientific Research (Nos. 187801172 and 20780113) and the Program to Create an Independent Research Environment for Young Researchers from the Ministry of Education, Culture, Sports, Science and Technology, Japan. Yunting Fang was also supported by the JSPS with a Postdoctoral Fellowship for Foreign Researchers and a Grant-in-Aid for JSPS Fellows (No. 20-08421). We thank X. M. Fang, J. Z. Su, J. Guo, T. Zhang, Y. W. Kuang, the staff in Lankeshan Natural Reserve, and others for their assistance in sample collection and chemical analysis.

References


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 $^{15}$N, C and N concentrations, and C/N ratios for each sampled species in 13 forests along the transect.

Table S5. $^{15}$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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