Key nitrogen cycling processes in pine plantations along a short urban–rural gradient in Nanchang, China

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ABSTRACT
We used pine (Pinus elliottii Engelm.) forests located along a short urban–rural gradient in Nanchang, China to study nitrogen (N) cycling responses to urbanization. Annual average rates of nitrification and net N-mineralization in soils (0–15 cm depth) measured from February 2007 to January 2009 increased from rural (8 and 37 kg ha−1 year−1) to suburban (69 and 79 kg ha−1 year−1) and urban sites (114 and 116 kg ha−1 year−1) (P < 0.05). Soil nitrate and mineral N pools exhibited the same spatial patterns in response to urban location. In comparison to rural sites, urban and suburban sites experienced soil microbial biomass N that increased by 98% and 38%, urease activity that increased by 40% and 26%, and uricase activity that decreased by 35% and 25%, respectively. Soil microbial biomass C:N and free amino acids varied little along the urban–rural gradient. Foliar N concentrations and N resorption efficiencies were higher in urban (12.3 and 4.8 g kg−1) and suburban (12.3 and 6.2 g kg−1) than in rural (9.9 and 3.6 g kg−1) sites, while N resorption efficiencies (from 58% to 72%) were not statistically different. These results indicate that forests in suburban and especially in urban areas are moving rapidly towards a state of “N saturation” and increased potential N loss most likely attributable to higher N deposition to these sites.

1. Introduction

Urbanization is accelerating worldwide, with about 50% of the human population currently living in urban areas, and this figure is projected to increase to 60% by 2025 (Pickett et al., 2001). As a result of rapid economic growth for three decades since the initiation of economic reform in 1978, China has experienced a dramatic and unprecedented increase in urbanization. The Chinese urban population rose from 18% in 1978 to 45% in 2008 and is projected to be 65% by 2030 (Ni, 2008). Urbanization directly transforms landscapes and affects biodiversity, ecosystem productivity, watershed discharge characteristics, and biogeochemical cycles (Pickett et al., 2001). Urbanization also indirectly influences ecosystems across various scales by altering abiotic environmental conditions, including atmospheric chemistry, climate, and soil properties (Lovett et al., 2000; Pataki et al., 2006) and biotic components, such as introduced exotic species (Heckmann et al., 2008). Yet, with a few exceptions (see Pickett et al., 2001) urban areas have been largely ignored in general ecological studies and are among the least understood of all ecosystems (Grimm et al., 2008); and this is especially true for urban areas in tropical, less-developed regions of the world.

In many terrestrial ecosystems, nitrogen (N) availability exerts strong controls on primary production (Reich et al., 1997), and affects soil processes such as litter decomposition (Carreiro et al., 2000), C storage (Shaver et al., 1998) and trace gas production (Davidson et al., 1993). Increasingly, anthropogenic N fixation and deposition are affecting the global and local N budgets (Vitousek et al., 1997). In urban and suburban areas, forest N cycling may be altered because they receive much greater wet and dry atmospheric N deposition than rural areas (Lovett et al., 2000; Lohse et al., 2008) and prolonged elevated N deposition may eventually cause N saturation, as the nutrient balance of the plant–soil system is disturbed and the capacity of the ecosystem to retain added N is exceeded (Aber et al., 1998). Nitrogen deposition levels are increasing in China and other rapidly developing countries due to increases in industrial activity, fertilizer production, domestic animal waste, the number of motor vehicles (Klimont et al., 2001).

Forest N cycling responses to urbanization also could be influenced by a variety of other distinctive features of urban environments. For example, cities are usually warmer as a result of the “heat island” effect (Oke, 1995), which could influence microbial and plant processes (Shen et al., 2008). Some urban...
pollutants such as O₃ and heavy metals can alter plant and soil microbial activity (Pouyat and McDonnell, 1991). Some local enrichment of atmospheric CO₂ in the urban atmosphere is common (Fataki et al., 2003) which could influence plant production and C/N dynamics. And in many less-developed countries, removal of detritus for human use is common (e.g., for fuel and animal bedding). How these and other influences on forest N cycling might interact with enhanced deposition remains uncertain.

We quantified N cycling parameters in replicated pine (Pinus elliottii Engelm.) forest plantations along a short urban–rural gradient in Nanchang, China, where recent growth in population and motor vehicle usage is typical of many urban areas in less-developed countries. We sought to demonstrate the responses of key N transformations to the combined effects of urbanization, with the overall hypothesis that these responses would conform to the overriding influence of high urban N deposition. Hence, our specific hypotheses concerning N cycling shifts along the rural to urban gradient (Lovett et al., 2000), especially nitrification rates, in pine forest ecosystems would increase along the rural to urban gradient (Lovett et al., 2000), and increased soil N transformation rates in urban forests would be a trend to N saturation (Aber et al., 1998); (2) the combined effects of N deposition together with other key environmental changes along the urban–rural gradient (e.g., temperature, O₃, etc.) would result in shifts in seasonal patterns of N cycling parameters such as N-mineralization and nitification (Zhu and Carreiro, 2004a); (3) soil microbial biomass N content would decrease along the urban–rural gradient (Zhu and Carreiro, 2004b); however, soil microbial biomass C:N would be lower in suburban and urban than rural forests reflecting奢侈品 consumption at higher soil N availability; (4) soil microbial extracellular enzyme activity would increase along the urban–rural gradient in accordance with previous reports (Saiya-Cork et al., 2002; DeForest et al., 2004); therefore, soil monomers (e.g., amino acids) released from depolymerization of N-containing polymers by microbial extracellular enzymes would be lower in urban than in rural sites; and finally (5) foliar N concentration and N resorption proficiency would be higher in suburban and urban than in rural forests, while foliar C:N and N resorption efficiency would show the opposite trends (Chapin, 1980; Kobe et al., 2005).

2. Materials and methods

2.1. Study area

This study was conducted in the mid-subtropical zone, in Nanchang City (115°27′–116°35′E, 28°09′–29°11′N). The total metropolitan area is 7402 km² with a population of 4.8 million. Nanchang is the capital of Jiangxi Province, China, and a typical rapidly expanding city. Population increased rapidly from 2.4 million in 1970 to 4 million in 1996, and the number of motor vehicles increased almost 8-fold between 1995 and 2006. Currently there are about 200,000 cars and trucks registered in the city (Statistical Yearbook of Nanchang in 2007; http://www.chinayearbook.com/%CD%B3%BC%C6%C4%EA%BC%FR2007/book_904.htm). The subtropical monsoon climate in Nanchang is wet and mild, with abundant precipitation. Mean annual precipitation is about 1700 mm, with mean annual relative humidity about 77%. Mean annual temperature is 17.5 °C. The frost free period averages 291 d year⁻¹. Vegetation cover is relatively high (38.2%) with a green space area of 7.5 m² per capita in Nanchang City, and rapidly expanding urban forest area resulting from plantation establishment (Nanchang Forestry Bureau, 2009, unpublished data).

2.2. Plot selection

Nine pine plantations were selected for the study located along a 30 km by 5 km belt transect extending from central urban, through suburban, and rural areas (Fig. 1). Three plantations were selected within each stratum along the gradient in 2007. All plots were on well-drained sites with no evidence of disturbance in the past decade. The ages of the pine plantations ranged from 16 to 20 years old, stand density from 600 to 800 trees ha⁻¹, mean diameter at breast height (DBH) from 10 to 16 cm and canopy height from 12 to 16 m. All sites are located on low hills at elevation range of 30–80 m in the hilly red soil region and underlain by Ultisols (or local name, red soil), which is a typical soil type in the mid-tropical region of China. Ultisols derived from Quaternary red clay cover over 10% of 1.14 million km² of total land area in southeast China (Zhao et al., 1988). The soil of the area was formed from arenaceous shale and is approximately 1 m in depth. In the urban plantations gathering of surface detritus by humans has mostly eliminated the litter layer, while litter accumulation of 4.1–6.3 t ha⁻¹ was observed in the suburban and rural sites.

2.3. Soil properties

Soil sampling was conducted at random locations within four 10 m x 10 m subplots in each plantation. In each subplot after removing the litter layer, five soil cores (2.5 cm in diameter) were randomly collected from 0 to 15 cm depth in August 2007 for pH, total soil organic carbon (C), total N, total phosphorus (P) analysis. Soil pH was measured in a 1:2.5 mixture of soil and deionized water using a glass electrode. Soil organic C was determined by dichromate oxidation and titration with ferrous ammonium sulfate. Total N was determined by the microKjeldahl method and samples were analyzed for total P by a phosphomolybdic acid blue color method (Liu et al., 1996). In addition, soil samples were collected within each subplot using a 5.0-cm-sampling cylinder for determining soil bulk density at each of three depths: 0–5, 5–10, and 10–15 cm in August 2007. The soil bulk density (g cm⁻³) was determined based on the dry soil weight per unit volume of the soil core at each depth.

2.4. Soil N-mineralization rate and extractable N concentration

Soil N-mineralization including ammonification, nitrification and net N-mineralization rates were estimated monthly using the closed-top tube in situ incubation method (TUBE method) (Chen et al., 2006) from February 2007 to January 2009. Near the center of each subplot, the forest floor was carefully removed and two covered polyvinyl chloride (PVC) tubes with a diameter of 4.0 cm were sunk into the soil to a depth of 15 cm. Twenty-three field incubation cycles were run for 1-month periods except for the 2-month period from January to March 2008. At the beginning of each cycle, pre-incubation soil was sampled near each tube to estimate the initial concentration of NH₄⁺-N, NO₃⁻-N and mineral N (NH₄⁺-N plus NO₃⁻-N). A subsample of 25 g from pre- or post-incubation soil samples was mixed with 100 ml 2 mol L⁻¹ KCl, shaken for 0.5 h, and left to stand overnight at 4 °C. The NH₄⁺-N and NO₃⁻-N in the supernatant were separately measured by spectrophotometry following filtration using the indophenol blue method and the cadmium reduction method (Liu et al., 1996), respectively. In addition, 10 g of soil from each of the pre- or post-incubation soil samples was dried in an oven at 105 °C to a constant weight to determine soil moisture content.

We used ion exchange resin bags (IERB method) to evaluate relative N-mineralization rates (Binkley and Matson, 1983). Ten grams of resin beads (Amberlite resin, which is a 1:1.5 mixture...
resin of IR-120 and IRA-400) were sealed in each 4 cm × 4 cm nylon bag (1 mm × 1 mm grid). Four resin bags were buried at 4 and 11 cm depths, respectively, to estimate the relative ammonification and nitrification rates in 0–15 cm soil layer in the center of each plot. The method was repeated four times (spring, summer, fall and winter) with a 45-d incubation period for each season. Upon collection, the plant roots and soil adhering to the bags were removed, and the resin bags were transferred and stored at 4 °C. We used 2 mol L⁻¹ KCl solutions to extract the NH₄⁺-N and NO₃⁻-N, and the colorimetric methods to measure NH₄⁺-N and NO₃⁻-N concentrations (Liu et al., 1996), as above.

2.5. Soil microbial biomass C and N

Five soil cores (2.5 cm in diameter) were collected randomly from each subplot at 0–15 cm layer March (spring), June (summer), September (autumn) and December (winter) 2008. The fresh soils from each subplot were composited and stored at 4 °C and used to measure soil microbial biomass C and N (MBC and MBN).

The soil MBC and MBN were measured by the fumigation–extraction procedure (Brookes et al., 1985). The field moist soils were sieved (2 mm), adjusted to 40% of water holding capacity, conditioned for 7 d at room temperature and then stored at 4 °C for analysis. A subsample of 25 g from incubated soil was saturated with purified liquid CHCl₃ for 24 h as a fumigation treatment; simultaneously, another 25 g subsample was drawn from the incubated soil as an unfumigated control. Both pretreated soil samples were separately extracted with 100 ml 0.5 mol L⁻¹ K₂SO₄ solution, soil extracts were measured for organic C by dichromate oxidation and total N using the microKjeldahl digestion procedure (Liu et al., 1996). MBC and MBN were estimated from the ratio of difference in C and N concentration between the fumigated and unfumigated samples using a correction factor of 0.38 and 0.54, respectively (Brookes et al., 1985).

2.6. Soil free amino acids and extracellular enzyme activities

Subsamples from the MBC and MBN analysis were used to measure soil free amino acids (FAA), sucrase, urease and acid phosphatase activities in each season during 2008. Soil FAA, which represents amino acids, proteins and peptides (Joergensen and Brookes, 1990), were extracted in 0.5 mol L⁻¹ K₂SO₄ solution, and measured colorimetrically at 570 nm following addition of ninhydrin solution (Putnam and Schmidt, 1959). The activities of sucrase, urease and acid phosphatase were measured by the Hoffmann–Seegerer, Nesslerization and disodium phenyl phosphate (C₆H₅Na₂O₄P) colorimetric method (Guan and Shen, 1984), respectively.

2.7. Foliar nutrients, resorption efficiency and proficiency

Three representative trees were selected on the basis of the average DBH and height in each plot. For each sample tree, we collected the leaves from one first order branch from each of four cardinal directions and mixed them together to obtain a composite sample for each plot in each month from September 2007 to August 2008. We also collected fresh litterfall from December 2007 to March 2008 under each sample tree, and mixed them together as a composite sample for each plot. The leaf samples were washed for 1 min with demineralized water to remove dust, oven-dried at 70 °C for 48 h, ground in a mill and screened with a 0.25 mm sieve. Foliar organic C was determined by dichromate oxidation and titration with ferrous ammonium sulfate. Total N was determined by the microKjeldahl method (Liu et al., 1996). N resorption
efficiency (NRE) was calculated as a percentage of the maximum pool of nutrients in the leaf before senescence (Aerts, 1996).

\[
NRE = \frac{[N] \text{ of fresh leaf} - [N] \text{ of senescent leaf}}{[N] \text{ of fresh leaf}}
\]

where \([N] \text{ of fresh leaf}\) is the N concentration corresponding to the month with maximum among 12 months; \([N] \text{ of senescent leaf}\) is the N concentration of the litterfall from December to March. Nutrient resorption proficiency (NRP) was calculated as the minimum concentration of N remaining in senesced leaves (litterfall) every year (Killingbeck, 1996). Although changes in leaf mass during senescence varied from 5% to 10%, the effects of these changes on resorption were not considered in this study.

2.8. Statistical analysis

For each sample, data on soil properties including soil bulk density, pH, organic C, total N, total P, C:N, N:P were analyzed using one-way analysis of variance (ANOVA) with position along the gradient as the fixed main effect. Tukey's multiple comparisons method was used to identify significant differences along the gradient after significant ANOVA tests.

Soil mineral N concentrations, N-mineralization, and nitrification (TUBE method) measured from February 2007 to January 2009 were analyzed by two-way, repeated measures ANOVA with month and position along gradient as fixed effects. Distributions for soil NO\textsubscript{3}−-N concentration and nitrification rate did not conform to the normal distribution, and data were log-transformed to achieve normality.

Data of the four seasonal measurements from each plot of soil relative N-mineralization and nitrification rates (IERB method), MBC and MBN, FAA and extracellular enzyme activities were used to examine the effects of position along the gradient, season and their interactions over the course of a year. Two-way repeated measures ANOVA was used to examine these effects. The Tukey's multiple comparisons method was used to identify significant differences in annual average values among different positions after significant ANOVA tests.

Two-way ANOVA was used to examine the effects of position along the gradient, month, and their interactions on foliar nutrient concentrations. The Tukey's multiple comparisons method was used to identify significant differences in foliar N concentration and pine tree NRE and NRP along the urban–rural gradient after significant one-way ANOVA tests. SPSS software (SPSS Inc., 2001, Version 10.0) was used to perform all these analyses. For all data, all differences reported in the text were tested and considered significant at \(\alpha = 0.05\).

3. Results

3.1. Soil properties

No differences in soil organic C, total N, total P and their ratios were detected across the urban–rural gradient, but soil bulk density was lower in rural than in suburban and urban sites, and soil pH was lower in rural than suburban than urban sites (Table 1).

3.2. Soil mineral N concentrations and N-mineralization rates

The effects of position and month on soil NH\textsubscript{4}+-N, NO\textsubscript{3}−-N and total mineral N were significant, while the interaction effects were not significant (Table 2). Overall average concentrations of NO\textsubscript{3}−-N and total mineral N gradually increased along the urban–rural gradient, whereas NH\textsubscript{4}+-N was not significantly different between rural and suburban sites. The monthly variations in soil NO\textsubscript{3}−-N and total mineral N over 2 years were generally similar with lower concentrations in the warm season (April to August) than in cool season, whereas NH\textsubscript{4}+-N showed minimal seasonal variation (Fig. 2).

The effects of position, month and their interaction on soil nitrification and net N-mineralization rates (TUBE method) were significant (Table 3A). The annual nitrification rate increased markedly along the urban–rural gradient (rural = 5 mg kg\textsuperscript{−1} year\textsuperscript{−1}; suburban = 41 mg kg\textsuperscript{−1} year\textsuperscript{−1}; urban = 64 mg kg\textsuperscript{−1} year\textsuperscript{−1}) and the same spatial pattern was observed for net N-mineralization (rural = 25 mg kg\textsuperscript{−1} year\textsuperscript{−1}; suburban = 47 mg kg\textsuperscript{−1} year\textsuperscript{−1}; urban = 66 mg kg\textsuperscript{−1} year\textsuperscript{−1}) (Fig. 3). Seasonal variations in N-mineralization and nitrification (Fig. 3) were less consistent than for mineral N concentrations; most notable were the relatively smaller monthly variations in the rural than the suburban and urban sites.

Patterns of relative N-mineralization and nitrification (IERB method) (Table 3B) across sites were generally consistent with those using TUBE method. Seasonal variations in N-mineralization and nitrification measured with the IERB method were lower in the rural than the urban/suburban sites, matching the results of the TUBE method. However, the timing of peak rates of mineralization and nitrification differed between methods, with spring peaks in the IERB (Fig. 4) compared with summer peaks with the TUBE method (Fig. 3).

3.3. Microbial biomass, free amino acids and extracellular enzymes

The effects of position, season and their interactions on soil MBC and MBN were significant, but there were no significant effects of position and the interaction with season on soil microbial biomass C:N (Table 4). In general, soil MBC and MBN were lower in rural (189 and 23 mg kg\textsuperscript{−1}, respectively) than in urban sites (497 and 45 mg kg\textsuperscript{−1}), while suburban sites were intermediate (395 and 31 mg kg\textsuperscript{−1}) (Fig. 5). Seasonal trends in MBC and MBN were not significant.

Table 1

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>Rural</th>
<th>Suburb</th>
<th>Urban</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density (g cm\textsuperscript{−1})</td>
<td>1.00(0.02)a</td>
<td>1.13(0.01)b</td>
<td>1.19(0.06)b</td>
<td>P &lt;0.032</td>
</tr>
<tr>
<td>pH</td>
<td>4.48(0.09)a</td>
<td>4.47(0.21)a</td>
<td>5.63(0.17)b</td>
<td>P &lt;0.004</td>
</tr>
<tr>
<td>Organic C (g kg\textsuperscript{−1})</td>
<td>9.3(2.39)a</td>
<td>14.6(4.14)a</td>
<td>10.3(2.10)a</td>
<td>P =0.468</td>
</tr>
<tr>
<td>Total N (g kg\textsuperscript{−1})</td>
<td>0.75(0.25)a</td>
<td>1.16(0.29)a</td>
<td>0.70(0.10)a</td>
<td>P =0.349</td>
</tr>
<tr>
<td>Total P (g kg\textsuperscript{−1})</td>
<td>0.14(0.10)a</td>
<td>0.35(0.03)a</td>
<td>0.49(0.15)a</td>
<td>P =0.097</td>
</tr>
<tr>
<td>Soil C:N</td>
<td>14.0(2.4)a</td>
<td>12.6(1.4)a</td>
<td>14.7(1.7)a</td>
<td>P =0.753</td>
</tr>
<tr>
<td>Soil N:P</td>
<td>5.2(1.6)a</td>
<td>3.2(0.6)a</td>
<td>1.6(0.2)a</td>
<td>P =0.103</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>NH\textsubscript{4}+-N</th>
<th>NO\textsubscript{3}−-N</th>
<th>Mineral N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>2</td>
<td>3.53a</td>
<td>39.71a</td>
<td>41.28a</td>
</tr>
<tr>
<td>Month</td>
<td>22</td>
<td>3.02**</td>
<td>2.33**</td>
<td>2.92**</td>
</tr>
<tr>
<td>Position × month</td>
<td>44</td>
<td>1.08 NS</td>
<td>1.03 NS</td>
<td>1.13 NS</td>
</tr>
</tbody>
</table>

Note: NS, not significant.

\( P < 0.05. \)
\( ** P < 0.01. \)
\( *** P < 0.001. \)
consistent across the gradient. Concentrations of free amino acids and extracellular enzyme activities did not differ significantly or consistently either among sites along the gradient or between seasons in the pine plantations (Table 5).

3.4. Foliar N concentration and resorption

The effects of position, month and their interactions on foliar N concentration and C:N ratio were highly significant (Table 6).

Foliar N concentrations were significantly lower in rural (9.9 g kg\(^{-1}\)) than in suburban (12.3 g kg\(^{-1}\)) and urban (12.3 g kg\(^{-1}\)) sites, with a corresponding pattern in C:N ratios (rural = 46.5; suburban = 37.3; urban = 37.4). The patterns of seasonal variation in foliar N concentration differed across the urban–rural gradient, with generally smaller variation in the rural than the suburban and urban sites (Fig. 6). Although NRE (from 58% to 72%) did not differ significantly along the urban–rural gradient, NRP was significantly greater in the rural than the urban and suburban sites.

4. Discussion

Urban areas in most less-developed regions are experiencing population growth, industrialization and rapid increase in motor vehicle transportation. The implications of urban growth on terrestrial ecosystem dynamics have received limited study. We hypothesized that the overriding effects on N cycling in urban forests would be a trend towards N saturation (Aber et al., 1998) with attendant responses of key N cycling parameters like soil inorganic N concentrations, N-mineralization and nitrification, microbial biomass and foliar N concentrations and resorption. Based upon replicate pine plantations along a short urban–rural gradient in Nanchang, China, we can conclude that forest N cycling...
has responded locally to very recent increases in urbanization and industrialization. In particular, we observed a dramatic increase in soil NO$_3^-$–N, N-mineralization and nitrification, microbial biomass C and N, and foliar N along this relatively short urban–rural gradient. These responses coincide with expectations based on the theory of N saturation and suggest that the N cycle in subtropical, urban forest is sensitive to the short-term, localized increased in N deposition most likely from fossil fuel combustion.

Other environmental changes associated with urbanization also could have contributed to the N cycle responses we observed. In particular, higher air and soil temperatures associated with the heat-island effect of urbanization could stress the trees or affect microbial activity in ways that could contribute to these responses in N cycling. Similarly, other pollutants (O$_3$, heavy metals), soil compaction and litter raking could affect N cycling, and increased atmospheric CO$_2$ could stimulate primary productivity and alter plant and soil C:N balances. Although more controlled experiments are warranted to more rigorously test the effects of these coincident changes in urban environments, the consistency of the N cycling responses with a simple N saturation phenomenon suggests the possibility that the urban–urban gradient was dominated by effects of N deposition. To our knowledge, N cycling variations along such a locally short (30 km) and short-term (motor vehicle use increased 8-fold from 1995 to 2006) urbanization/industrialization gradient have not been reported previously, and this appears to be the first such analysis for an urban area of a humid-tropical, less-developed country.

The most comprehensive study of N cycling and other ecosystem processes along urban–rural gradients comes from New York City (NYC) (McDonnell et al., 1997; Carreiro et al., 1999; Groffman et al., 2006). Lovett et al. (2000) quantified N deposition fluxes in oak (Quercus rubra L.) forests along that gradient and showed that concentrations and fluxes of NH$_4^+$-N and NO$_3^-$–N decreased significantly with distance from the city. Patterns of concentration and flux with increasing distance from NYC were distinctly nonlinear, with the steepest decline occurring between the urban and suburban sites (just over 30 km distance).

Lovett et al. (2000) found that dry N deposition exceeded wet N deposition in their urban sites in NYC, but the opposite was likely true at the rural sites; N dry deposition approximately equaled canopy N uptake in the suburban and rural sites, but dry deposition greatly exceeded canopy uptake at the urban sites. Unfortunately, we were unable to quantify N deposition at our sites due to repeated vandalism; however, data from Changsha,
China (a nearby capital city of Hunan province) indicated high atmospheric N deposition in the area (78 kg N ha$^{-1}$ year$^{-1}$; Chen et al., 2004). Moreover, the patterns of soil N cycling that we observed suggest that the gradient in deposition was much shorter for Nanchang than NYC, probably reflecting both the difference in metropolitan area and the duration and intensity of the urban N source. Lovett et al. (2000) suggested that co-deposition of NO$_3^-$-N and basic cations (for example, Ca$^{2+}$) contributed significantly to the urban deposition patterns; however, in some urban locations NH$_4^+$-N was the predominant N form even where NO$_3^-$-N levels in soils were elevated (Lohse et al., 2008; Koerner and Klopatek, 2009) and it seems possible that this process contributed to the higher soil pH observed in the urban sites in Nanchang (Table 1). The co-deposition process could also help to explain the apparently short gradient in N availability at Nanchang (i.e. strong decrease within 20 km of city center).

Table 4
F-values from the ANOVA for the effects of position and season on soil microbial biomass C, N and their ratio in 2008 under pine plantations along a rural-suburban-urban gradient in Nanchang city and vicinity, China.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>Microbial biomass C</th>
<th>Microbial biomass N</th>
<th>Microbial biomass C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>2</td>
<td>6.38$^*$</td>
<td>18.36***</td>
<td>1.00 NS</td>
</tr>
<tr>
<td>Season</td>
<td>3</td>
<td>4.48$^*$</td>
<td>6.92**</td>
<td>3.96**</td>
</tr>
<tr>
<td>Position x season</td>
<td>6</td>
<td>3.52$^*$</td>
<td>3.43$^*$</td>
<td>2.10 NS</td>
</tr>
</tbody>
</table>

Note: NS, not significant.

$^*$ $P < 0.05$.

$^*$ $P < 0.01$.

$^*$ $P < 0.001$. 

Fig. 4. Seasonal variations in soil relative ammonification, nitrification and net N-mineralization rates under pine plantations along a rural–suburban–urban gradient in Nanchang City in 2007. Bars represent the mean (SE), of three samples (data pooled for four seasons with four subsamples per site, three sites per area). Different letters in the same season denote significant differences among three areas (three sites per area) at $\alpha = 0.05$.

Fig. 5. Seasonal variations in soil microbial biomass C, N and their ratio under pine plantations along a rural–suburban–urban gradient in Nanchang City in 2007. Bars represent the mean (SE), of three samples (data pooled for four seasons with four subsamples per site, three sites per area). Different letters in the same season denote significant differences among three areas (three sites per area) at $\alpha = 0.05$. 

center), as local deposition of atmospheric NOx emissions would be favored over long-distance transport. Additionally, Bettez (2009) documented the pattern of increasing soil N availability adjacent to heavily used roadways that was attributed to localized deposition of vehicular N emissions.

As noted above, some other environmental differences along the urban–rural gradient could have contributed to the N cycling responses. Surface soil pH was much higher in the urban than the suburban and rural sites (Table 1). Based on the sensitivity of autotrophic nitrification to low soil pH (Robertson and Vitousek, 1981; Rudebeck and Persson, 1998), this difference probably contributed to the contrasting nitrification and soil NO3−N pools between rural and urban sites (Figs. 2 and 3); however, pH was similar in the rural and suburban sites, yet NO3−N and nitrification were much higher in the latter. Soil bulk density was significantly higher in the urban and suburban than in the rural sites, presumably as a result of compaction by human related activity; the consequent reduction in porosity should reduce aeration and soil moisture content, but the consequences for N cycling are uncertain. In the urban sites, litter raking has mostly eliminated the surface organic horizon from the pine forest soil; hence, the increase in N availability occurred in spite of this chronic disruption of N recycling. Whether this disturbance could contribute to differences between the urban and suburban sites deserves further experimental study. Finally, increased air temperatures could result in plant water stress, with possible consequences for root uptake, while increased soil temperatures would be expected to accelerate microbial activity, with possible consequence for C and N processing (Parao-Zuckerman and Coleman, 2005).

The seasonal variations in soil N dynamics observed in the present study provide some useful insights into N cycling in subtropical forest under varying conditions of N availability. Net

<table>
<thead>
<tr>
<th>Soil variables</th>
<th>Rural</th>
<th>Suburb</th>
<th>Urban</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free amino acids (g kg−1)</td>
<td>0.30 (0.05)a</td>
<td>0.41 (0.05)a</td>
<td>0.37 (0.02)a</td>
<td>P = 0.205</td>
</tr>
<tr>
<td>Sucrase (mg g−1 soil d−1)</td>
<td>1.21 (0.05)a</td>
<td>1.53 (0.11)a</td>
<td>1.70 (0.09)a</td>
<td>P = 0.085</td>
</tr>
<tr>
<td>Urease (mg g−1 soil d−1)</td>
<td>0.20 (0.02)a</td>
<td>0.15 (0.11)a</td>
<td>0.13 (0.01)a</td>
<td>P = 0.060</td>
</tr>
<tr>
<td>Acid phosphatase (mg g−1 soil d−1)</td>
<td>1.07 (0.08)b</td>
<td>0.62 (0.06)a</td>
<td>1.08 (0.07)b</td>
<td>P = 0.020</td>
</tr>
</tbody>
</table>

Values are the mean (SE) for three samples, and each sample is the average of four subsamples during four seasons within a plot. Different letters within the same row denote significant differences between the three areas at α = 0.05.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>F values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C concentration</td>
</tr>
<tr>
<td>Position</td>
<td>2</td>
<td>0.24 NS</td>
</tr>
<tr>
<td>Month</td>
<td>11</td>
<td>2.11 *</td>
</tr>
<tr>
<td>Position × month</td>
<td>22</td>
<td>1.33 NS</td>
</tr>
</tbody>
</table>

Note: NS, not significant.
* P < 0.05.
*** P < 0.001.

Fig. 6. The monthly variation in foliar N concentration under pine plantations along a rural–suburban–urban gradient in Nanchang City during 2007 and 2008. Bars represent the mean (SE), of three samples. Different letters in the same season denote significant differences among three areas (three sites per area) at α = 0.05.
N-mineralization was somewhat higher during the warm season than the cool season in the N rich urban and suburban sites, a pattern that coincided with the seasonal pattern of net nitrification (Figs. 2 and 3). This result suggests that, at least at high N levels, the supply of NH$_4^+$ -N limits nitrification (Zhu and Carreiro, 2004a). In contrast, soil NO$_3^-$ -N concentrations exhibited the reverse pattern with lowest levels in the warm season probably reflecting seasonal variation of root uptake. This pattern was evident even in the rural sites where soil NO$_3^-$ -N levels were always very low (Fig. 2) and further reinforces the likely seasonality of root N demand in this subtropical, humid region.

The N-mineralization fluxes we observed using in situ incubations (37 kg ha$^{-1}$ year$^{-1}$ for 0–15 cm soil) were somewhat higher than for a warm temperate Pinus taeda plantation in NC, USA (25 kg ha$^{-1}$ year$^{-1}$ for 0–15 cm soil; Vitousek and Matson, 1985). Seasonal variation was less pronounced in our sites than the NC pine plantations, presumably because of less temperature variation at the lower latitude. Previous studies of urban effects on forest soil N transformations have provided mixed results. As summarized recently by Groffman et al. (2006), the effects of urban vs. rural environment on N-mineralization can be complicated by inherent site fertility differences, variation in earthworm abundance and the heat-island effect on soil temperature. The straightforward increase in N-mineralization and nitrification that we observed may be explained in part by limited effects of these and other confounding factors on the short urban–rural gradient in this warm climate.

An additional insight into these interactions is provided by a comparison of seasonal patterns of N transformations measured by the TUBE and IERB methods. Although these methods exhibited similar patterns of response along the urban–rural gradient (Figs. 3 and 4), the IERB method indicated a spring peak in potential N-mineralization and nitrification at the high N sites, compared with the summer peak in the TUBE method. The former method eliminates root N uptake whereas the IERB method accommodates a degree of “competition” for mineralized N between resins and uptake sinks (especially roots) (Binkley and Hart, 1989). Thus, it is likely that high root demand for inorganic N in the warm season reduces nitrification. The lack of such a pattern in the rural sites suggests the overriding effect of low N availability on seasonal N dynamics in relatively unpolluted soils.

Soil microbial biomass generally reflected the trends in N cycling across the urban–rural gradient with higher MBC and MBN in the urban than rural sites. One striking seasonal contrast was observed between the low N and high N sites: the microbial biomass C:N ratio was much higher in the cool season in the high N urban sites than the low N rural sites, but this difference was not evident in the warm season as microbial C:N significantly declined in the high N urban sites. These results suggest that N availability influences seasonal shifts in dominant microbial groups (Hall et al., 2009), or luxury uptake of N during seasons of excessive N availability (Zhu and Carreiro, 2004b). Several previous studies have indicated that excessive N deposition could reduce the activity of extracellular enzymes (Saija-Cork et al., 2002; DeForest et al., 2004). Therefore, we were surprised that soil sucrose and urease activities, and the products of extracellular enzyme (FAA) effect did not decrease significantly along the urban–rural gradient (Table 5). In fact, the trend in sucrose ($P = 0.0085$) was the reverse, suggesting that C-acquiring enzyme activity might increase with urbanization, possibly to counteract the imbalance in C and N supply (Stursova et al., 2006). Acid phosphatase activity was significantly lower in the suburban than the rural and urban sites (Table 5); hence, shifts in the P enzyme activity did not conform to the simple hypothesis that increased N availability would result in greater P limitation (Robinson et al., 2004). Perhaps litter raking influenced the difference in phospahase activity between urban and suburban sites. Total P was somewhat lower, and soil N:P higher in the rural than the urban/ suburban sites, but these trends were not statistically significant (Table 1).

Forest N concentrations were about 24% higher in the suburban and urban sites than the rural sites, illustrating the high sensitivity of foliar nutrition to changing N availability. The canopies could take up some deposited N (Lovett et al., 2000), part of which could be directly absorbed by tree needles (Vose and Swank, 1990). The “keystone process” (Killingbeck, 1996) of nutrient resorption (retranslocation) was expected to decrease along the urban–rural gradient, coinciding with increased N availability and foliar N (Kobe et al., 2005). In fact, NRP did conform to this pattern, as reflected in the lower N concentration in pine needle litter in the rural than the suburban and urban sites (Fig. 5). In contrast, NRE did not differ significantly among sites. This result conforms to the suggestion of Killingbeck (1996) and the observation of Norris and Reich (2009) that NRP is a more sensitive index of nutrient availability than NRE.

In conclusion, forest N cycling has been altered by recent urbanization in Nanchang, China. As urban forests have been exposed to elevated temperature, N deposition, O$_3$ and other pollutants, they may be ahead of the global change “response curve” for forests. Therefore, not only might forests along urbanization gradients provide us with natural experiments for studying current responses to global change factors, but their legacy of response to past urbanization may also constitute space–time substitution experiments for predicting likely regional forest responses to continued environmental change. The specific implications of N saturation in urban ecosystems for urban forest health and downstream ecosystems also deserve continuing attention.

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References


