N isotopes and N cycle in the TieShanPing subtropical forest ecosystem, Southwestern China

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Received: 17 November 2007 / Accepted: 22 May 2008 / Published online: 10 July 2008
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Abstract Nitrogen is essential for forest growth and forest stand development. It is commonly a limited factor for forest productivity. We examined $\delta^{15}\text{N}$ values in soils and plants by studying the sources of N used by vegetation and cycles of N in a 43-year-old plantation of the TieShanPing forest ecosystem in southwestern China, dominated by massone pine (*Pinus massoniana*). The N concentration of plant materials ranges from 1.1% to 2.2%. The nitrogen concentration of *P. massoniana* was 1.3% while soils showed the concentration of 0.04–0.15%. Regarding natural abundance of ^{15}N , large significant variation (-6.0‰ to -3.8‰) in $\delta^{15}\text{N}$ values was observed among shrub and tree leaves. $\delta^{15}\text{N}$ values were also significantly varied from -4.7‰ to -3.8‰ among the pioneer species in the plantation. Soil $\delta^{15}\text{N}$ values ($3.1\text{--}6.3\text{‰}$) were significantly enriched compared to those values in plant samples. Despite the negative $\delta^{15}\text{N}$ values of the vegetation cover, the high $\delta^{15}\text{N}$ values in the topsoil indicate that return of N to soils by litter-fall is minimal

on TieShanPing and the present forests do not change very much the soil ^{15}N signals at the surface layer. The positive $\delta^{15}\text{N}$ values may also indicate large N losses from the soil system via leaching, volatilization and plant uptake.

Keywords Massone pine plantation · N cycling · ^{15}N natural abundances · Soil-plant system

Introduction

Nitrogen is an essential element for forest growth, and usually acts as a limiting element for the productivity of forest ecosystem. In natural conditions, N is generally accumulated in a forest ecosystem, which means that the balance of the N input to the system minus the output is positive until an equilibrium is attained (Chen et al. 2000).

Nitrogen inputs to a system can occur by atmospheric deposition (Aranibar et al. 2004) or biogenic N_2 fixation (reduction of atmospheric). Both stemflow and throughfall contribute to the N input through precipitation, throughfall representing the major contribution. The process of N uptake by vegetation and returns of N to the soil system via periodical litterfall is one of the basic features of a forest ecosystem. More than 90% of the soil N reserves are in the soil organic pool, which is a potential source of soil available N through mineralization (Xu et al. 1995). Litterfall

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is the major contribution of soil organic matter in a forest ecosystem. Inorganic N is the most mobile pool in a forest ecosystem, but plays a decisive role in the forest production and the stability of the system.

Stable isotope of nitrogen is a powerful tool for evaluating N cycling because of their ability to integrate over time and space (Nadelhoffer and Fry 1994). Natural abundance of ^{15}N has been used to help evaluate N losses and patterns of N mineralization (Johanisson and Högberg 1994; Austin and Vitousek 1998; Emmett et al. 1998; Högberg 1993; Garten 1993; Kramer et al. 2003; Pardo et al. 2007), to compare plant species patterns of N uptake (Nadelhoffer et al. 1996), and to determine effect of land-use history on N cycles (Piccolo et al. 1994; Eshetu and Högberg 2000; Eshetu 2004). The abundance of stable isotopes in plant tissues reflects the isotopic composition of the N sources available to them (Nadelhoffer et al. 1996; Högberg 1997). Factors that may cause variation in the abundance of ^{15}N in plant tissues include N source (mineralization or rainfall), rooting depth (deep or shallow), the form of N absorbed (ammonium, nitrate or N from organic litter), mycorrhizal uptake, N fixation (Nadelhoffer et al. 1996; Högberg 1997; Tatenio et al. 2003) and land use history (Piccolo et al. 1994; Eshetu and Högberg 2000; Eshetu 2004). Disturbed sites have elevated soil ^{15}N signals, while less disturbed and natural forest ecosystems have depleted in ^{15}N abundance in the surface soil layer relative to the lower horizons (Eshetu and Högberg 2000). Hence, the change in soil ^{15}N is considered as an indicator of rehabilitation of soil degradation afforded by afforestation, as shown in (Eshetu 2004).

Sino-Norwegian cooperated Integrated Monitoring Program on Acidification of Chinese Terrestrial Systems (IMPACTS) project selected and established five monitoring sites in Southern and Southwestern China; long-term monitoring on deposition, soil, soil water, stream water as well as vegetation has been conducted. In this study, TieShanPing (TSP) of IMPACTS sites was selected for detailed N research. This paper provides data on nitrogen cycling in a 40-year old plantation based on the nitrogen concentration and the nitrogen stable isotopic composition of plant and

soils under the massone pine plantation, which represents a young growth plantation forests in Southwestern China.

Materials and methods

Site description

TSP (29°38' N, 106°41' E) is a 16.3 ha catchment in a protected forest area, about 25 km northeast of the center of Chongqing city in the Sichuan basin. The altitude ranged from 450 to 500 m. The climate at TSP is sub-tropical and humid with Mean annual temperature of about 18.2°C, and the total annual rainfall of about 1230 mm. TSP is a coniferous-broad-leaved mixed forest ecosystem, dominated by massone pine (*Pinus massoniana*); Chinese fir (*Cunninghamia lanceolata*) is also common. The forest in the area has been planted since 1962, because large parts of the forests in China were logged between 1958 and 1962 during the Great Leap Forward (Guo et al. 2007). Prior to this, the forest area had several plots used for rice and vegetable production. The forest stand stocking varies between 700 and 1556 trees per hectare, with an average of 1095 trees per hectare. The average height and mean diameter at breast height of dominant Masson pines (Kraft class 1–3) in January 2002 were 15.5 m and 193 mm, respectively. The corresponding values of all trees (Kraft class 1–5 and all species) were 12.3 m and 153 mm, respectively. The forest has a relatively dense under-vegetation of shrubs and fern. The soil type is yellow soil in the Chinese classification system (*Haplic Acrisol* in the FAO system). The N fluxes of TSP are shown in Table 1 (IMPACTS, 2004).

Table 1 The fluxes on $\text{NH}_4^+ - \text{N}$, $\text{NO}_3^- - \text{N}$, total N through the Massone pine ecosystem

	$\text{NH}_4^+ - \text{N}$	$\text{NO}_3^- - \text{N}$	total N
Bulk N precipitation (Kg N ha ⁻¹ year ⁻¹)	17.98	8.69	26.67
Through fall N (Kg N ha ⁻¹ year ⁻¹)	26.67	11.06	43.46
Stream water N (Kg N ha ⁻¹ year ⁻¹)	0.05	6.14	6.79

Table 2 Nitrogen concentration (%) and isotopic composition ($\delta^{15}\text{N}$ in ‰) of vegetations collected in a Massone pine dominated plantation

Samples	N (%)	$\delta^{15}\text{N}$ (‰)	C (%)	$\delta^{13}\text{C}$ (‰)
<i>P. massoniana</i>	1.3 ± 0.1	−4.7 ± 0.2	46.6	−29.2
<i>C. lanceolata</i>	1.1 ± 0.2	−3.9 ± 0.1	43.1	−28.1
<i>S. sumuntia</i>	1.9 ± 0.2	−3.8 ± 0.3	52.1	−31.1
Shrub	2.0	−6.0	42.8	−26.4
Grass	2.2	−4.8	47.2	−30.2

Soil and plant sampling and analysis

To determine N and C isotopic composition of the existing vegetation and SOM, soil and plant samples were collected from TieShanPing forest in September 2005. The grass samples from the four sites within a circle were mixed into one composite sample regardless of species. With regard to tree and shrub foliage samples, leaves were collected from lower, middle and upper canopy layers of all age at four sites within a circular plot. For tree foliar, each the dominant species sample were mixed together to form a simple sample. For shrub foliage, the leave samples were mixed together to represent a composite sample for the plot regardless of age, canopy layer and species.

Soil samples were taken in the vicinity of the lysimeters. Four soil profiles were dug at four different plots. From each soil profile, the litter layer (L) and the fermentation + humus layer (F + H) were sampled separately. For three soil profiles, the underlying mineral soil layer (0–90 cm) was sampled from each of four successive soil depth intervals (0–5, 5–10, 10–20, 20–40 cm). Other one profile was sampled according to 0–5, 5–10, 10–

20, 20–40, 40–60, 60–90 cm of mineral soil layer. All plant and soil samples were air dried at room temperature (approximately 22°C) for 7–10 days. Mineral soils were then sieved through a 2 mm sieve to remove roots, gravel and stones. All samples were put into plastic bags and transported to Beijing where they were kept at 4°C until analyzed. All samples were dried at 70°C for 24 h, ground in a ball mill to a fine powder and loaded into tin capsules for analysis of %N and $\delta^{15}\text{N}$, using an elemental analyzer (FlashEA 1112N, USA) connected to an Isotope Ratio Mass Spectrometer (Finnigan MAT DeltaplusXP, Germany). Soil samples with a pH >6.5 were acidified with 0.1 M HCl to assure carbonate removal, rinsed with H₂O, then oven dried at 70°C. These samples were weighed into silver capsules and analyzed for $\delta^{13}\text{C}$ values and C content. From these analyses, we obtained both isotope ratio ($\delta^{15}\text{N}$; $\delta^{13}\text{C}$) and elemental content (%N; %C) for carbon and nitrogen.

Data are expressed in “delta” notation ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) as:

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000 \left[\frac{0}{\infty} \right] \quad (1)$$

Fig. 1 Distribution of ^{15}N natural abundance, nitrogen content, ^{13}C natural abundance and carbon content in soil profiles at the TSP site

Table 3 Analysis of variance for the significance of grasses vs. tree leaves on ^{15}N , %N, ^{13}C and %C; and for the significance of soil depth on ^{15}N , %N, ^{13}C and %C in the TSP site

Source	df	¹⁵ N		%N		¹³ C		%C	
		F-test	P-value	F-test	P-value	F-test	P-value	F-test	P-value
Significance of grasses vs. tree leaves									
Grass vs. tree leaves	1	0.125	0.736	2.006	0.206	0.041	0.847	0.057	0.819
Intercept	1	132.845	< 0.001	72.199	< 0.001	1073.003	< 0.001	457.457	< 0.001
Error	6								
Significance of soil depth									
Depth	5	83.114	< 0.001	22.066	< 0.001	72.848	< 0.001	158.964	< 0.001
Intercept	1	5705.862	< 0.001	213.460	< 0.001	19244.405	< 0.001	1600.173	< 0.001
Error	12								

where R_{sample} and R_{standard} are the $^{15}\text{N}/^{14}\text{N}$ OR $^{13}\text{C}/^{12}\text{C}$ ratios of the samples and the standard, respectively. The international standards for carbon and nitrogen isotope ratio values are $\delta^{13}\text{C}$ of V-PDB defined as 0.011237 (Coplen 1995, 1996) and $\delta^{15}\text{N}$ of atmospheric N_2 defined as 0.00365 (Mariotti 1983), Respectively. Precision of the measurement was $\pm 0.2\text{‰}$ for n and C.

Statistical analysis

Univariate analysis of variance (ANOVA; Statistical Package for Social Sciences version 11.5) was used to test differences in $\delta^{15}\text{N}$ values and %N between grasses and tree leaves. For the mineral

soils, similar statistical approach was used to test effects of soil depths as fixed factors on the $\delta^{15}\text{N}$ values and total soil N content. The plot has been used as replicates in the ANOVA. Significant differences refer to $P \leq 0.05$ unless otherwise stated.

Results

Table 2 presented the Nitrogen concentration of the plant materials, and the values ranged from 1.3% to 2.2%. Nitrogen content amounted to 2.0% in shrub and to 2.2% in grasses. The N concentration in foliages collected from the dominant species ranged between 1.1% and 1.9%, while the

Fig. 2 Correlations between: **a** $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, **b** $\delta^{15}\text{N}$ values and %N, and **c** %N and %C of forest soils on TieShanPing. The respective correlation was described by: **a** a linear equation ($r^2 = 0.45$, $P < 0.001$), **b** an exponential decay, three parameters ($r^2 = 0.46$, $P < 0.001$), and **c** a simple exponent, three parameters ($r^2 = 0.94$, $P < 0.001$)

values in soils samples varied from 0.04% to 1.6% (Fig. 1). The $\delta^{15}\text{N}$ values ranged between -6.0‰ and -3.8‰ for plant materials (Table 2).

In the mineral soils, a range of $\delta^{15}\text{N}$ values from 3.1‰ to 6.3‰ was observed (Fig. 1). For the observed soil profiles, the $\delta^{15}\text{N}$ values increased significantly with soil depth, and the values observed were 3.1‰ in the 0–5 cm and $>5.9\text{‰}$ in the >20 cm soil depth (Fig. 1, Table 3). In contrast, the soil profiles showed continuous decreases in total N content from 0.15% to 0.04% with soil depth (Fig. 1). The differences between the grasses and tree leave in $\delta^{15}\text{N}$ values and %N were not significant (Table 3). Foliar $\delta^{13}\text{C}$ in vegetations ranged from -26.3‰ to -31.1‰ . The soil $\delta^{13}\text{C}$ values increased with depth, and the values ranged from -24.3‰ at 0–5 cm to -17.5‰ at 20–40 cm soil depth. Carbon content in topsoil was 3.4–5.2% and in tree leaves was 42.8–52.1% (Fig. 1, Table 2). Overall, $\delta^{15}\text{N}$ values of SOM, even at the surface mineral soils (0–5 cm), were higher by 3.1‰ than the $\delta^{15}\text{N}$ values of plants (Table 3). Generally, Soils were ^{15}N -enriched relative to plants, and ^{15}N -enriched at depth relative to the surface. However, there were no significant differences in concentration of nitrogen between plants and soils.

A significant correlation between ^{15}N and ^{13}C ($r^2 = 0.45$; $P < 0.001$), ^{15}N and %N ($r^2 = 0.46$; $P < 0.001$) as well as between %N and %C ($r^2 = 0.94$; $P < 0.001$) was observed in forest soils on TieShanPing (Fig. 2a–c).

Discussion

In this study the TSP site was examined, a representative of Southwestern China plantation. N deposition inputs of $>20 \text{ kg ha}^{-1} \text{ year}^{-1}$ in bulk precipitation, increasing in throughfall to $>40 \text{ kg ha}^{-1} \text{ year}^{-1}$, and with leaching losses of N matching up 25% of the total N input (Table 1). These values were significantly greater than those at other three IMPACTS sites (IMPACTS 2004). On TSP, the observed $\delta^{15}\text{N}$ value in vegetation ranged from -6.0‰ to -3.8‰ and in soil ranged from 3.1‰ to 6.3‰. Martinelli et al. (1999) pointed out that the average $\delta^{15}\text{N}$ value for tropical foliage was $3.7 \pm 3.5\text{‰}$ ($n = 73$), which is

significantly greater ($P < 0.01$) than the temperate forest value of $-2.8 \pm 2.0\text{‰}$ ($n = 90$). Compared our data, the results clearly showed that the ^{15}N values of tree leaves seem fairly low even for temperate forests. In tropical forest, $\delta^{15}\text{N}$ values in soils between $+2\text{‰}$ and $+23\text{‰}$ were reported (Piccolo et al. 1996; Martinelli et al. 1999; Vervaet et al. 2002). In the literature, $\delta^{15}\text{N}$ values in temperate forest soil profiles range from -8‰ in the forest floor to $+8\text{‰}$ in the mineral soil (Högberg et al. 1996; Martinelli et al. 1999; Vervaet et al. 2002). The $\delta^{15}\text{N}$ values of subtropical soils measured in the TSP site was significantly lower than those reported from tropical forests. However, they did not significantly differ from those of temperate forests. Natural ^{15}N abundance levels (3.1–6.3‰) in mineral soils were different significantly from plant samples. Soils were ^{15}N -enriched relative to plants, and ^{15}N -enriched at depth relative to the surface. The causes for ^{15}N enrichment and for decrease of %N with increasing soil depth are well discussed (Nadelhoffer and Fry 1994; Högberg 1997).

The most important nutrient cycling mechanism in forest ecosystems is litterfall and litter decomposition. Litterfall plays a particular role in the N cycle of a forest ecosystem, by which large amount of N assimilated by vegetation returns to the soil for recycling and subsequent uptake by plants. According to the data of no publication, average N input through litterfall was $109 \text{ kg ha}^{-1} \text{ year}^{-1}$ in the TSP site during the period of 2001–2003. Symbiotic N fixer is not significant at TSP. Accordingly, atmospheric N input is the dominant N input. When precipitation passes through the forest canopy, the N content in throughfall was increased in TSP (Table 1). This is may be due to the washing of dry deposited N from leaves. N in throughfall is also one of the nutrient sources for a forest ecosystem. Throughfall can influence soil physical and chemical properties by adding available nutrients or allelopathic chemicals (Chen et al. 2000; Likens and Bormann 1995; Lian and Zhang 1998). Although a portion of the N in soil and precipitation, especially NO_3^- , was lost through runoff when precipitation occurs (Table 1), general N from precipitation is accumulated in the TSP site. Nutrient flux to the forest floor in kilograms per hectare per year for

litterfall and aqueous sources (throughfall) was 135.67 for N. Although the return of N to soil through throughfall was smaller than through litterfall, throughfall were sufficient to benefit all species growing in the ecosystem directly.

Forest floor and underlying mineral soil contain large reserves of nutrient, but only a small portion of it participates in the cycling processes (Chen et al. 2000). Availability of the N in a forest ecosystem is determined by the rates of decomposition and mineralization. The N content in the upper 15 cm of the mineral soils at TSP was 1460 kg N ha⁻¹ (Chen and Mulder 2007). Soil at TSP lacks a continuous and well-developed forest floor with decomposing litter. Nitrogen in the O/A horizon (0 to 3 cm depth) at TSP contributes 25–55% to the total N pool of the upper 15 cm of the soil. Inorganic N is the most mobile pool in a forest ecosystem, but plays a decisive role in the N cycle of forest ecosystems. At TSP, the internal N cycle is characterized by relatively high net mineralization and nitrification rates. Net N mineralization rates, including the litter layer, at TSP may be around 72 kg N ha⁻¹ year⁻¹. Net nitrification rates are substantial at TSP, with average values for the upper 15 cm of the mineral soil of 35 kg N ha⁻¹ year⁻¹. Along the profile, the nitrification rate increases with the depth (Chen and Mulder 2007).

The $\delta^{15}\text{N}$ of leaves can reflect the nutrient pools that the vegetation is exploiting, nitrogen fixation, soil pools, re-translocation within the plant, among others (Ometto et al. 2006). $\delta^{15}\text{N}$ varied widely (-6.0‰ to -3.8‰) between different species. The average $\delta^{15}\text{N}$ value for forestfloor was $-6.1 \pm 0.3\text{‰}$. Symbiotic N fixer is not significant at TSP. According, N fixation cycle was probably not a major determinant of foliar $\delta^{15}\text{N}$. Variations in ^{15}N natural abundances suggested that most N uptake by trees was from the forest floor and that dissolution occurred in soils and canopies by precipitation. In addition, gaseous, aerosol and liquid anthropogenic nitrogen deposition may have influenced local ^{15}N of the plants in TSP.

The restoration of the N cycles in the soil–plant system is required for the sustainability of forests. To evaluate accurately the efficacy of restoration techniques, it is necessary to use natural ^{15}N abun-

dance (Nadelhoffer and Fry 1994; Liu et al. 2006). A very thin litter layers was observed in TSP. The positive $\delta^{15}\text{N}$ values (3.1‰) in 0–5 cm, despite the negative $\delta^{15}\text{N}$ (-6.0‰ to -3.8‰) of the vegetation cover, indicated that the current forest cover did not change very much the $\delta^{15}\text{N}$ values of these soils, providing lines of evidence that redeposition of ^{15}N depleted N to soils by litter-fall was minimal on TSP during a period of 43-year forest growth. A range of $\delta^{13}\text{C}$ values from -24.3 to -17.48 (20–40 cm) was observed in the mineral soil (Fig. 1), these data indicated contributions from both C₃ and C₄ plants. Thus, there was strong evidence of a substantial land-use change at TSP. Thus, the higher $\delta^{15}\text{N}$ values in 0–5 cm might be related to the effects of long-term forest disturbances. There was a significant correlation between the two stable isotopes, ^{15}N and ^{13}C (Fig. 2a). The latter is linked to accumulation of SOM from forest litter. The top soils at the TSP site was up to 3‰ lighter than the lower soil horizons indicating a trend of ^{15}N depleted SOM buildup through litter-fall in the TSP site.

Atmospheric N input is the dominant N input. However, all-year $\delta^{15}\text{N}$ value has not been measured for ammonium deposition and nitrate deposition in TSP. Long-term measurements of precipitation nitrogen suggest that both ammonium and nitrate inputs to forests are relatively depleted in ^{15}N with values for these inputs ranging from roughly -10‰ to 0‰ (Yoneyama et al. 1986; Garten 1991; Martinelli et al. 1999; Nadelhoffer and Fry 1994). At the TSP site, positive soil $\delta^{15}\text{N}$ values were observed, the low of deposition relative to the soil N pool (Chen and Mulder 2007), the net effect of N deposition would probably be a negligible decrease in soil $\delta^{15}\text{N}$. Högberg et al. (1996) and Vervaet et al. (2002) pointed out that forest ecosystems, which in general maintain a much tighter cycling of N (N limitation), have been found to have comparatively low $\delta^{15}\text{N}$ in the superficial soil layer and a rapid increase in $\delta^{15}\text{N}$ with increasing soil depth. The higher $\delta^{15}\text{N}$ value in the surface layers indicates N surpluses (open N cycle), which in humid conditions correlate with loss of N (nitrate) from the system. The TSP site shows high N deposition and considerable NO_3^- loss (Table 1), the internal N cycle is characterized by relatively high net mineralization and nitrifica-

tion rates, so the N cycle should be open. Thus the positive $\delta^{15}\text{N}$ values in the surface layers may also indicate large N losses from the soil system via leaching, volatilization and plant uptake.

Conclusions

Nutrient flux to the forest floor in kilograms per hectare per year for aqueous sources (throughfall) was 26.67 for N in TSP. Although the return of N to soil through throughfall was smaller than through litterfall, throughfall was sufficient to benefit all species growing in the ecosystem directly. In TSP, the ^{15}N values of tree leaves seem fairly low even for temperate forests. The $\delta^{15}\text{N}$ values of subtropical soils measured in the TSP site was significantly lower than those reported from tropical forests. However, they did not significantly differ from those of temperate forests. There was a large range of $\delta^{15}\text{N}$ among tree leaves. Interpretation of the $\delta^{15}\text{N}$ pattern is that plants assimilate the products of mineralization and nitrification of soil humus. High $\delta^{15}\text{N}$ values in the topsoil indicate that return of N to soils by litterfall is minimal on TSP, and may also indicate large N losses from the soil system via leaching, volatilization and plant uptake on TSP.

Acknowledgements This study was supported by National Basic Program of China (No. 2005CB422206) and Sino-Norwegian IMPACTS project. We thank all partners of the Sino-Norwegian IMPACTS project for their contributions to this study.

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