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Dynamics of soil inorganic nitrogen and their responses to nitrogen additions in three subtropical forests, south China

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Abstract: Three forests with different historical land-use, forest age, and species assemblages in subtropical China were selected to evaluate current soil N status and investigate the responses of soil inorganic N dynamics to monthly ammonium nitrate additions. Results showed that the mature monsoon evergreen broadleaved forest that has been protected for more than 400 years exhibited an advanced soil N status than the pine (*Pinus massoniana*) and pine-broadleaf mixed forests, both originated from the 1930's clear-cut and pine plantation. Mature forests had greater extractable inorganic N pool, lower N retention capacity, higher inorganic N leaching, and higher soil C/N ratios. Mineral soil extractable NH_4^+-N and NO_3^--N concentrations were significantly increased by experimental N additions on several sampling dates, but repeated ANOVA showed that the effect was not significant over the whole year except NH_4^+-N in the mature forest. In contrast, inorganic N (both NH_4^+-N and NO_3^--N) in soil 20-cm below the surface was significantly elevated by the N additions. From 42% to 74% of N added was retained by the upper 20 cm soils in the pine and mixed forests, while 0%—70% was retained in the mature forest. Our results suggest that land-use history, forest age and species composition were likely to be some of the important factors that determine differing forest N retention responses to elevated N deposition in the study region.

Keywords: N deposition; N saturation; extractable inorganic N; soil solution inorganic N; subtropical China

Introduction

Some heavily populated areas in tropical and subtropical regions are facing acid rain events and high nitrogen (N) deposition, due to the intensification of fossil fuel use and agricultural practices; and the rate of N deposition in these regions are expected to increase by as much as several folds by 2050 (Matson *et al.*, 1999, 2002; Galloway *et al.*, 2003). Yet the impact of N deposition on natural tropical and subtropical ecosystems is not well understood (Matson *et al.*, 1999, 2002; Galloway *et al.*, 2003; Fang *et al.*, 2004). In China the use and emission of reactive N increased from 14 Tg N/a in 1961 to 68 Tg N/a in 2000 and is expected to reach 105 Tg N/a in 2030 (Zheng *et al.*, 2002). Currently this leads to the deposition of 30—70 kg N/(hm^2·a) in some forests of SE China (Fang *et al.*, 2004). However, relatively little work has been done to evaluate their biological and ecological effects on forest ecosystems (Chen *et al.*, 2004).

Forest ecosystems differ in their responses to increased N deposition (Gundersen *et al.*, 1998; Aber *et al.*, 1998; Rueth *et al.*, 2003; Hall and Mastson, 2003), but it is not completely understood what factors control the rate and magnitude of the responses. Land-use history, soil N pool size, species composition, stand age, and growing season length have been shown to be some of the important factors, because they influence the balance between N availability and demand (Aber *et al.*, 1998; Gundersen *et al.*, 1998; Fenn *et al.*, 1998; Rueth *et al.*, 2003). A nitrogen addition experiment has been established in Dinghushan Biosphere Reserve (DHSBR) located in south China subtropical moist life zone (Holdridge, 1967) since July 2003. This experiment contains three forests: pine, pine-broadleaf mixed (mixed), and monsoon evergreen broadleaf forest (mature), with various historical land-use, forest age, and species assemblages (Wang *et al.*, 1982; Brown *et al.*, 1995; Mo *et al.*, 1995, 2003; Fang *et al.*, 2003). The mature forest is a regional climax forest type and has been protected for more than 400 years by monks in the nearby temples. Both the pine and mixed forests originated from the 1930s clear-cut and pine plantation. N deposition to these forests was very high, with reported wet deposition at 35—38 kgN/(hm^2·a) in the 1990s (Huang *et al.*, 1994, 2000; Zhou and Yan, 2001). About 80% of N deposited was leached from the mature forest (Huang *et al.*, 1994, 2000). A critical characteristic of N-saturated ecosystems is increased leaching of various forms of N. Therefore, the mature forest can be considered as N-saturated system. There was no available data on N output from pine and mixed forests up to date. The aim of this experiment was to determine the current forest N-status and the impact of chronic elevated N deposition on ecosystem processes and N retention in the three forests.

In this paper, the dynamics of soil extractable inorganic N and solution inorganic N was reported in

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order to compare soil initial N status among the three forests and monitor their responses to simulated N deposition. We hypothesized that: (1) the mature forest had higher soil N status than both pine and mixed forests due to their difference in historical land-use, forest age, and species assemblages; (2) soil inorganic N in the mature forest would be more pronounced towards N addition than in both pine and mixed forests, and consequently the mature forest would retain a lower proportion of the N added than both pine and mixed forests.

1 Methods

1.1 Study site

Dinghushan Biosphere Reserve lies in the middle of Guangdong Province (112°10′E and 23°10′N) in southeast China. The reserve is located in a subtropical moist forest life zone. The mean annual rainfall of 1927 mm has a distinct seasonal pattern, with 75% falling from March to August and only 6% from December to February (Huang and Fan, 1982). Mean annual relative humidity is 80%. Mean annual temperature is 21.0℃, with average January and July temperature of 12.6℃ and 28.0℃, respectively (Huang and Fan, 1982). During the study period, precipitation and temperature largely followed this long-term seasonal pattern (Fig.1). The reserve occupies an area of approximately 1200 hm^2. The mature forest, at about 200—350 m above sea level (asl), occupies 20% of the reserve, the mixed forest, at about 200 m asl, occupies 50%, and the pine forest, from 50 to 200 m asl, occupies 20% (Mo *et al.*, 2003).

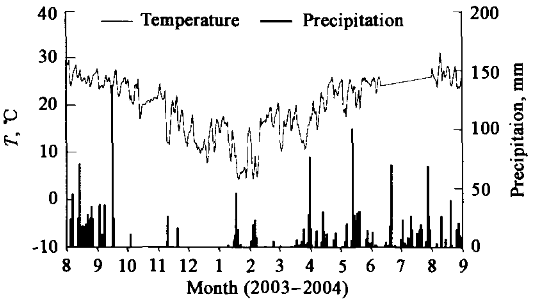

Fig.1 Daily precipitation and temperature of DHSBR during the study period from August 2003 to August 2004 Data from Dinghushan Forest Research Station, South China Botanic Garden, the Chinese Academy of Sciences; temperature from June 11, 2004 to August 1, 2004 was unavailable due to equipment problems

The mature forest is a regional climax forest type and has been protected for more than 400 years. Both pine and mixed forests originated from the 1930s clear-cut and pine plantation. However, the pine forest has been under continuous human disturbances (generally the harvesting of understory and litter) so that the tree layer remained predominated by *Pinus massoniana* (Mo *et al.*, 1995, 2003; Brown *et al.*, 1995). On the other hand, colonization from natural dispersal of regional broadleaf species has changed plant composition in the mixed forest (Wang *et al.*, 1982; Mo *et al.*, 2003). The survey conducted in June 2003 (before the start of N addition experiment) showed that the major species in the mature forest were *Castanopsis chinensis*, *Machilus chinensis*, *Schima superba*, *Cryptocarya chinensis*, *Syzygium rehderianum* in the canopy and sub-canopy layers, which represented up to 80% of total basal area. The major species in the canopy layer of the mixed forest were *Pinus massoniana*, *Schima superba*, and *Castanopsis chinensis*. Pine forest was dominated by *Pinus massoniana*. The mature forest had almost twice basal area as the pine and mixed forests (Table 1). Stem density, tree height and diameter at the breast height in the three forests are given in Table 1. Standing floor litter varied markedly among the forests, with 8.9 ±3.0, 23.7 ±4.8 and 20.0 ±0.4 mg/hm^2 (mean±SE, n=3) in mature, pine and mixed forests, respectively.

Table 1 Indices of the tree layers in pine, mixed and mature forests of DHSBR

Species	Stem density, tree/hm^2	Mean height, m	Mean DHB, cm	Basal area, m^2/hm^2	Relative basal area, %
Pine forest					
Pinus massoniana	456	6.9	17.5	13.3	95.1
Other plants	311	4.3	4.4	0.7	4.9
Total	767			14.0	100
Mixed forest					
Pinus massoniana	133	10.2	22.0	5.6	41
Schima superba	1567	5.2	6.4	7.4	53.6
Other plants	233	4.2	5.1	0.8	5.4
Total	1933			13.8	100
Mature forest					
Castanopsis chinensis	83	12.7	23.5	9.6	37
Machilus chinensis	208	7.1	8.6	4.1	15.8
Schima superba	183	7.7	10.3	3.8	14.5
Cryptocarya chinensis	113	11.5	20.6	2.3	8.9
Syzygium rehderianum	129	11.1	29.4	1.6	6.2
Other plants	1013	5.4	5.8	4.6	17.6
Total	1729			26.0	100

The topography is highly heterogeneous, with slopes ranging from 15° to 35°. The soil is lateritic red earth formed from sandstone (He *et al.*, 1982), but the soil depths vary with forests. In mature and mixed forests the soil depth ranges from 30 to 55 cm, and in pine forest the depth is generally less than 40 cm (Mo *et al.*, 2003). Soil bulk density from the 0—10 cm mineral soil was 0.98±0.06, 1.16±0.05 and 1.22±

0.01 g/cm^3 in the mature, pine and mixed forests, respectively.

1.2 Experimental design

Four N treatments, control, low-N, medium-N and high-N (three replicated plots per treatment) were established within the mature forest, and three N treatments (control, low-N, and medium-N) within both pine and mixed forests. Total applications were 0, 50, 100, 150 kgN/(hm^2·a) for the control, low-N, medium-N and high-N treatments, respectively. The high-N treatment was employed in the mature forest in order to test hypotheses related to the concept of N saturation (Aber *et al.*, 1998; Fenn *et al.*, 1998). The objective was to accelerate ecosystem responses so they might become evident within a more practical experimental period (within 10 years). Each plot measures 10 × 20 m, with about 10 m buffer strips around each of the plot. Monthly fertilizer additions of dissolved NH_4NO_3 began July 2003 as equal applications over the whole year. For each N application, fertilizer was weighed, mixed with 20 L of water, and applied to the plots using a backpack sprayer below the canopy. Two passes were made across each plot to ensure an even distribution of fertilizer. The control plots received the same amount of water without additional N.

1.3 Soil

Mineral soils of the upper 10 cm were sampled approximately 25 d after the latest N applications. In each plot, 12—15 soil cores (2.5 cm in diameter) were collected randomly, and combined to one composite sample in the field. In the laboratory, soils were sieved (2 mm) and mixed thoroughly by hand. One 10 g sub-sample from each composite sample was shaken for 1 h in 50 ml of 1 mol/L KCl, and filtered through pre-leached Whatman No.1 filters; the filtrate was frozen immediately for later analysis. Another 10 g sub-sample was dried at 105℃ to constant weight (at least 24 h) to determine gravimetric moisture content. Extractable NH_4^+-N was determined by the indophenol blue method followed by colorimetry. NO_3^--N was determined after cadmium reduction to NO_2^--N, followed by sulfanilamide-NAD reaction (Liu *et al.*, 1996).

Mineral soils collected in December 2004 were analyzed for soil pH, concentration of total C, N and P. Soil pH was measured in deionized water suspension using glass electrode, after shaken for 1 h at a ratio of 25 ml water to 10 g mineral soil. The soil samples were dried in air condition and ground. Total C was measured by dichromate oxidation before titration with Fe^{2+} solution (Liu *et al.*, 1996). Total N concentration was determined with semimicro-Kjeldahl digestion followed by the detection of ammonium, and total P concentration was analyzed colorimetrically after digestion (Liu *et al.*, 1996).

1.4 Soil solution

Soil solution from 20 cm below the surface was collected from all plots except in one of the medium-N plots in the mature forest due to its shallow soil condition. Two replicate zero tension plastic tray lysimeters (755.4 cm^2/tray) per plot were installed in April/May 2003, 3—4 months before the first sampling. Soil solution was sampled twice a month (one prior to the N addition and another 15 d after the N addition). Solution from the two lysimeters was combined within each plot on the date of collection. Concentrations of NH_4^+-N and NO_3^--N were determined for each sample date after filtration using the same method described above. These concentrations were multiplied by the water volume for the same period, and then summed to determine N fluxes in kg/hm^2 for each plot.

1.5 Statistical analysis

For soil extractable NH_4^+-N and NO_3^--N and concentrations of NH_4^+-N and NO_3^--N in soil solution, with samples collected and analyzed continuously throughout the study period, we performed a repeated multivariate analysis to examine temporal fluctuations of N concentrations and an univariate analysis to examine the overall N treatment effects. One-way ANOVA was also performed for each sampling date. One-way ANOVA with Tukeys HSD was used to identify N treatment effect on soil properties (soil pH, concentrations of total C, N and P and C/N ratios) for each forest. One-way ANOVA with Tukeys HSD was also used to identify N treatment effect on DIN (dissolved inorganic N) flux of soil solution at 20 cm depth for each forest. Residual plots were examined to check equal variance assumptions for ANOVA. All analyses were conducted using SPSS 10.0 for Windows. Statistically significant differences were set with $P<0.05$ unless otherwise stated.

2 Results

2.1 Soil general characteristics

The mature forest had the highest concentrations of total C, N and P in the 0—10 cm mineral soils, but had the lowest soil pH and C/N ratios. Seventeen months of N fertilization had not yet significantly altered the examined properties in any of three forests, although soil pH decreased with increasing N treatment (Table 2).

2.2 Soil extractable NH_4^+-N and NO_3^--N

Soil extractable inorganic N (NH_4^+-N, NO_3^--N, from the upper 10 cm soil) in the control plots varied depending on forest type and sampling date (Fig.2). In the pine forest, concentrations of extractable inorganic N varied from 4.2 to 9.9 mg/kg, with a mean value of 5.9 mg/kg. Comparable N concentrations were also observed in the mixed forest (2.2 to 15.8 mg/kg, with a mean value of 6.4 mg/kg). Much higher concen-

Table 2 Soil general characteristics (mean±*SE*, *n*=3) in the pine, mixed and mature forests of DHSBR

Forest	N treatment	pH	Total C, g/kg	Total N, g/kg	C/N ratio	Total P, mg/kg
Pine	Control	4.04±0.04	28.3±0.31	1.1±0.1	25.1±1.1	43±3
	Low-N	3.87±0.07	27.7±0.42	1.2±0.1	24.5±6.2	43±1
	Medium-N	3.93±0.03	25.9±0.24	1.1±0.0	22.7±2.8	42±4
Mixed	Control	3.95±0.01	26.4±2.6	1.0±0.1	27.8±0.7	44±4
	Low-N	3.96±0.04	28.7±3.8	1.0±0.1	28.9±5.0	41±4
	Medium-N	3.87±0.04	28.0±2.3	0.9±0.1	31.7±4.3	39±3
Mature	Control	3.83±0.02	45.5±2.3	1.9±0.1	22.1±1.3	59±3
	Low-N	3.81±0.07	38.3±5.2	1.8±0.1	20.9±1.7	55±3
	Medium-N	3.78±0.06	38.6±1.9	1.8±0.1	21.4±0.2	57±5
	High-N	3.72±0.01	43.0±0.6	1.9±0.1	22.7±1.1	63±2

Note: Soils were collected on December 2004, 17 months after the experimental N additions

trations of extractable inorganic N, however, were found in the mature forest (4.6 to 27.9 mg/kg, with an average value of 14.9 mg/kg). This difference between forests was mainly contributed by the difference in extractable NO_3^--N (Fig. 2). Correspondingly, the mature forest had a much higher NO_3^--N proportion (on average 75.8%) than both the pine and mixed forests (43.6% and 43.2%, respectively).

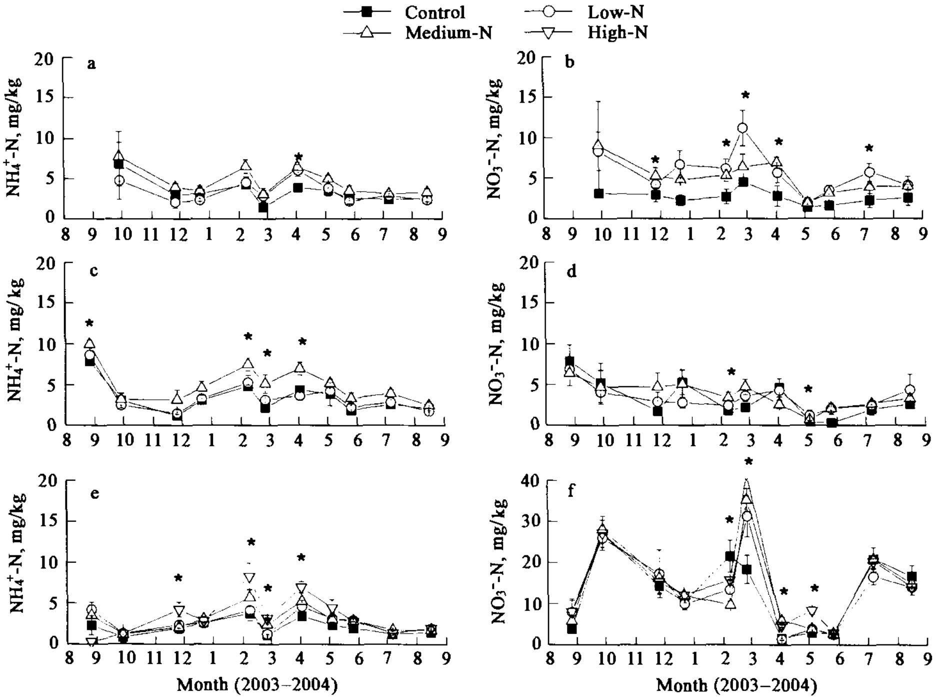

Fig.2 Soil extractable NH_4^+-N and NO_3^--N concentrations in the upper 10 cm mineral soils of the pine (a, b), mixed (c, d) and mature forests (e, f) of DHSBR

Samples in the pine forest were not taken in time for August 2003 owing to high rainfall event; asterisks (*) indicate significant difference between treatments on the same sampling date (p<0.05)

Soil extractable inorganic N concentrations responded to N addition in all three forests, but differed between forest types and between components of inorganic N (Fig.2). In the pine forest, concentrations of extractable NH_4^+-N and NO_3^--N were significantly increased by experimental N addition on several sample dates (Figs.2a, b). Repeated ANOVA, however, showed no statistical significant difference between treatments over the entire study period (p = 0.089 for NH_4^+-N, and p=0.078 for NO_3^--N), due to concentration fluctuations both between sampling dates and between replicate plots (Figs.2a, b). There were trends, however, indicating N additions had increased both NH_4^+-N and NO_3^--N concentrations in the mineral soil. Average concentrations for the 10 samplings in the control, low-N and medium-N plots in the pine forest were 3.34, 3.37, and 4.58 mg/kg for NH_4^+-N, and 2.60, 5.73 and 4.92 mg/kg for NO_3^--N, respectively. In mixed forest, concentrations of soil extractable NH_4^+-N and NO_3^--N were also significantly increased by the experimental N addition on several sample dates (Figs.2c, d), but the differences were not statistically significant over the entire study period (p=0.162 for NH_4^+-N, and p=0.737 for NO_3^--N, respectively). Average NH_4^+-N concentration was 3.38, 3.61, and 5.10 mg/kg and average NO_3^--N concentration was 3.03, 3.33 and 3.64 mg/kg in the control, low-N, and medium-N treatments, respectively. Similar to those in pine and mixed forests, concentrations of both NH_4^+-N and NO_3^--N in mature forest were significantly increased by N addition on several sample dates (Figs.2e, f). Repeated ANOVA showed no significant difference between N treatments for NO_3^--N (p=0.644), but significant for NH_4^+-N (p=0.009). Concentrations of NO_3^--N in mature forest, however, were 4—5 times higher than in pine and mixed forests, with averages of 12.73, 12.68, 13.18 and 15.10 mg/kg in the control, low-N, medium-N, and high-N plots, respectively. Average NH_4^+-N concentrations were 2.10, 2.76, 3.08 and 3.71 mg/kg, respectively.

2.3 Inorganic N in soil solution

In the control plots, while NH_4^+-N concentration in soil solutions was mostly low in all three forests, the pine forest had a significantly (p=0.011) higher concentrations (on average 0.42 mg/L) than the mature forest(0.11 mg/L), with mixed forest had intermediate values (0.30 mg/L). NO_3^--N concentrations were much higher than NH_4^+-N in all three forests. In control plots, the mature forest had a significantly (p=0.007) higher NO_3^--N concentration (on average 9.10 mg/L) than both the pine (3.22 mg/L) and mixed forests (3.00 mg/L). N additions increased NH_4^+-N concentrations in soil solutions in all three forests. A significant (p=0.020) and a marginal (p= 0.068) effect of N addition were observed in mature and pine forest, respectively. However, NH_4^+-N concentration remained very low in all N-amended plots. Unlike NH_4^+-N, repeated ANOVA showed NO_3^--N concentration was significantly (p=0.006—0.041) elevated by N additions in all three forests. Average NO_3^--N concentrations over the study period were 3.22, 8.65 and 9.65 mg/L in the control, low-N, and medium-N plots in pine forest, and were 3.00, 7.10 and 12.72 mg/L in the mixed forest. In mature forest, average NO_3^--N concentrations were 9.10, 15.02, 13.76, and 14.78 mg/L in the control, low-N, medium-N, and high-N plots respectively (Fig.3).

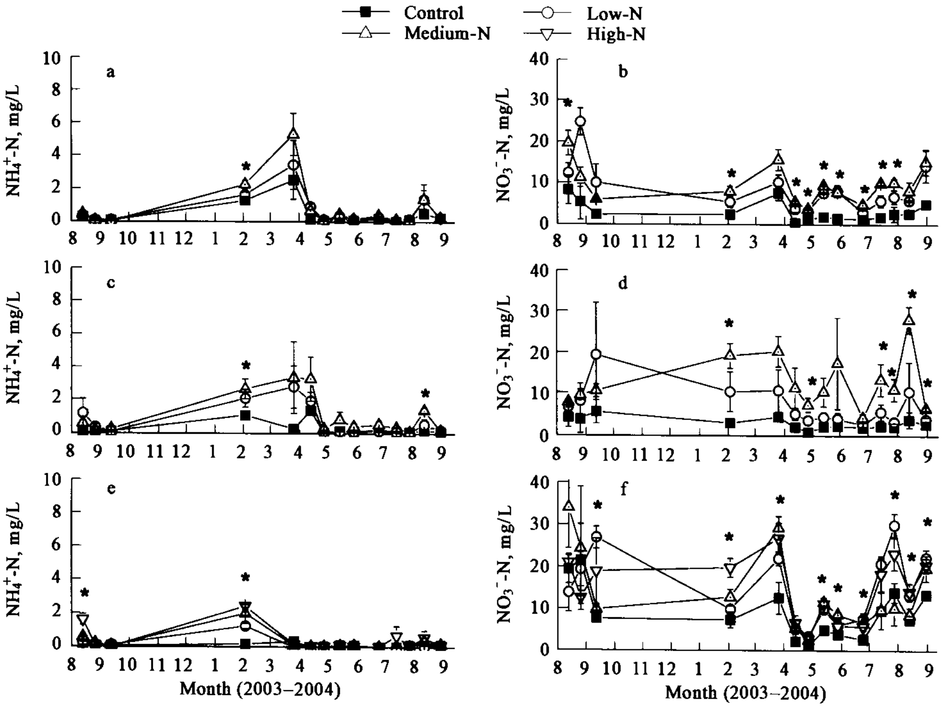

Fig.3 Concentrations of NH_4^+-N and NO_3^--N in soil solutions at 20 cm depth in the pine (a, b), mixed (c, d) and mature (e, f) forests of DHSBR Asterisks (*) indicate significant difference between treatments on the same sampling date (p < 0.05)

There were great within treatment variations in DIN flux of soil solution over the period from August 2003 to July 2004 in all the three forests (Fig. 4). For example, in control plots, the flux varied among replicate plots from 9.3 to 27.4, 8.2 to 18.9, and 32.1 to 64.9 kg N/(hm^2·a) in pine, mixed and mature forests, respectively, with respective averages of 16.5, 14.2 and 50.9 kg N/(hm^2·a). NH_4^+-N was less important in N flux in all three forests than NO_3^--N, and mostly represented less than 10% of the total N fluxes. N flux had a clear seasonal pattern of distribution. Among total N flux, 29%—50% was distributed in autumn (Aug. to Oct., 2003), 6%—12% in winter (Dec. 2003 to Jan. 2004), 9%—30% in spring (Feb. to Apr. 2004), and 28%—45% in summer (May—Jul. 2004). A similar precipitation distribution pattern occurred at the same period. Rainfall in these seasons represented 35%, 7%, 19% and 39% of the total annual precipitation of 1776 mm (Fig. 1), respectively, indicating that DIN leaching was partially controlled by precipitation.

Experimental N addition had a significant effect on N fluxes in both the pine (p=0.004) and mixed forests (p=0.006, Fig.4). The increases in N flux due to N additions in low-N and medium-N plots accounted for 50% and 42% of the N added in pine forests, and accounted for 22% and 34% in mixed forests. In mature forest, there was a marginal (p= 0.058) effect of N treatment on N flux, due to great variations within the replicate plots. The increases in N flux accounted for 100%, 27% and 43% of N added, respectively, in low-N, medium-N and high-N plots.

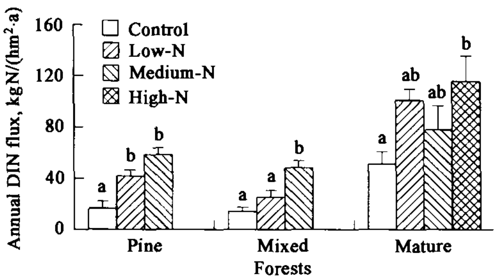

Fig.4 DIN flux of soil solution at 20 cm depth over the first treatment year in the pine, mixed and mature forests of DHSBR

Different letters indicated significant treatment effects of N addition in each type of forest (p<0.10)

3 Discussion

3.1 Characteristics of soil initial N status

Our data showed clearly that there was a distinct difference in soil initial N status between studied forests as we hypothesized, with higher advanced stage of N saturation in the mature forest than both the pine and mixed forests. In the control plots, there was no significant difference in extractable NH_4^+-N concentration in the 0—10 cm mineral soils among three forests (Fig.2). However, NO_3^--N concentration in the mature forest was substantially higher than those in pine and mixed forests at most of the sampling dates (Fig.2). Mean extractable NO_3^--N concentrations over the study period were 2.6, 3.0 and 12.7 mgN/kg in the pine, mixed and mature forests, respectively (Fig.2). At the same time, the mature forest had higher NO_3^--N concentration and DIN leaching in soil solution (at 20 cm depth) than both pine and mixed forests (Fig.3). Average NO_3^--N concentrations in mature forest (control plots) across 14 samplings during the study period was about three times higher than those in pine and mixed forests. Correspondingly, the N flux of soil solution at this depth in control mature forest was nearly three and four times higher than those in the control pine and mixed forests, respectively (Fig.4). DIN input in throughfall to the pine, mixed and mature forests was measured at 36.1, 37.6 and 53.5 kgN/(hm^2·a), respectively (our unpublished data), that combined with data on N flux of soil solution suggested that about 54% and 62% of N input was retained by the upper 20 cm soils in the pine and mixed forest, but only 5% was retained in the mature forest. According to the N-saturation model (Aber *et al.*, 1998), the undisturbed mature forest should be near the middle of stage 2 where N losses are sufficient to balance N inputs in deposition, while the pine and mixed forest should stand to the beginning of stage 2 since a considerable extractable NO_3^--N pool was observed in these two forest soils.

This difference in soil N status between N-saturated mature forest and relatively N-limited pine and mixed forests was consistent with mineral soil C/N ratios. Soil C/N ratios was lower in the mature forest (on average 22) than pine and mixed forests (average 25 and 28, respectively, Table 2). The soil C/N ratios had long been used as an important indicative of N status. Data from five European coniferous forest sites showed that nitrification potential and to some extent the actual nitrification rates are negatively related to the C/N ratio of the forest floor with a range of 25—27 as critical ratios (Gundersen *et al.*, 1998). A study of Yoh (2001) showed that mineral soil C/N ratio strongly regulated the NO_3^--N production in central Japanese forests, and that the net NO_3^--N production practically initiated below a C/N ratio of 20 and became progressively dominant along with a decreasing C/N ratio.

The difference in N status may result from the difference in species composition which often leads to the different N content in plant leaves and foliage litter and subsequently soil N processes (Gundersen *et al.*, 1998; Lovett *et al.*, 2002). Nitrogen concentrations of plant leaf in mature forest ranged from 0.95% to 2.54%, with a mean of 1.82%, and were relatively higher in comparison with those reported in other Chinese forests (including tropical, subtropical and temperate forests) (Mo *et al.*, 2000). For example, N concentration of leaves averaged 1.54% and 1.58% in tropical primary and secondary forests from Hainan Island, China (Zeng *et al.*, 1997). However, the trees in pine forest may be still N limited with an average concentration of 1.4% in the needle and 0.55% in the litter (Mo *et al.*, 1995, 1996). Litter C/N ratio was much lower in the mature forest than in the pine forest (36 and 83, respectively; Mo *et al.*, 2003). Pine needles were found immobilizing N during the first 200—250 d of decomposing in the pine forest, indicating that pine needles contained insufficient nitrogen to support microbial use of carbon (Mo *et al.*, 1996). In a separate experiment, N addition significantly increased early decomposition rate of pine needle (Mo *et al.*, 2006). In contrast, there was a negative effect of N addition on litter decay in mature forest (Mo *et al.*, 2006), suggesting that N added combined with atmospheric input had likely exceeded both plant and microbial N demand in the mature forest ecosystem.

3.2 Responses of soil extractable inorganic N and solution inorganic N to N addition

Many of the observed responses of terrestrial systems to N deposition can be understood within the context of “nitrogen saturation”, a concept model that has been developed largely based on the temperate forests responses to chronic N input in North America and Europe (Matson *et al.*, 1999, 2002). The N saturation model (Aber *et al.*, 1998) predicts that N addition would increase soil mineral N availability. This is supported by the results found in most Europe and North America forests (Gundersen *et al.*, 1998; Rueth *et al.*, 2003; Micks *et al.*, 2004). However, in our study, despite periodical higher NH_4^+-N and NO_3^--N concentrations in fertilized plots, no forests showed consistent difference in soil extractable N between control and N addition treatments except for NH_4^+-N in the mature forest site (Fig.2). The reasons for lack of strong N treatment effect on soil extractable N may be attributed to sampling time (samples were collected at least 20 d after each N application in our study). A number of studies showed that inorganic N added disappeared rapidly in soils due to both microbial immobilization and abiotic fixation for NH_4^+-N and NO_3^--N (Stark and Hart, 1997; Berntson and Aber, 2000; Micks *et al.*, 2004). For example, in Harvard Forests of USA, extractable N levels in both pine and hardwood plots returned to pre-application levels within 2 weeks of the N addition (Micks *et al.*, 2004). Another possibility is that high rainfall events of tropical climate could quickly leach N out of the surface soil and causing elevated N concentrations in lysimeter solutions as we had observed (Fig.3). In tropical rain forests in the Hawaiian Islands, Hall and Matson (2003) similarly found highly variable soil inorganic N concentrations, yet no consistently significant difference among fertilized treatments, despite dramatic different response of N gas emission following N fertilization. In contrast, inorganic N in soil solution was remarkably elevated by N addition in this study, especially in 2004 (Fig.3). Soil solution chemistry may, thus, provide an early indication of the long-term changes in soils associated with a chronic N stress (McDowell *et al.*, 2004).

We hypothesized that following the N additions pine and mixed forests would proportionally retain more N added than mature forest. Our results appear to support this hypothesis. Increases in N flux of soil solution at the depth of 20 cm due to N addition accounted for 22%—50% of N added in the pine and mixed forests, while in the mature forest the increase accounted for 27% —100% of N added. In other words, 50%—78% of N added was retained by upper 20 cm soils in the pine and mixed forests and 0%—73% was retained in the mature forest if the gaseous N emission was not counted.

Difference in soil initial N status and soil response to N addition in our study forests could be explained by the different land-use history and forest age. Our mature site has been protected for 400 years as Fengshui forest and long-term N accumulation may have already eliminated any N limitation while elevated N deposition in the past half century could induce N saturation. Old-growth forest has long been considered approach “N saturation” status with higher NO_3^--N export compared to young, aggrading forest (Vitousek and Reiners, 1975). In contrast, both pine and mixed forests were originated from the 1930's clear-cut and pine plantation. In addition, in both forests understory plants and floor litter had been harvested several times a year until ten years ago for fuel need of local residents (Mo *et al.*, 1995, 2003).

Some experimental N addition studies in temperate forests have suggested that land-use history, soil N pool size, species composition, stand age, and growing season length were some of the important factors that determine differing forest responses to additional N because they influence the balance between N availability and demand (Aber *et al.*, 1998; Fenn *et al.*, 1998; Rueth *et al.*, 2003). For example, at Harvard Forest, the hardwood site received >1020 kgN/hm^2 input before significant increases in nitrification or N leaching losses were observed. On the other hand, McNulty and Aber *et al.* (1993) observed significant biogeochemical responses in a Vermont spruce-fir forest following just 3 years of N addition. The authors suggested prior land-use history, reaching back even 100—200 years, could play a significant role in preconditioning forest response to N deposition. Rueth *et al.* (2003) found two old-growth coniferous forests in Colorado with different initial organic horizon C/N ratios and organic N pools had different response patterns to 4 years of N additions.

4 Conclusions

In sum, the mature forest exhibited higher soil initial N status than pine and pine-broadleaf mixed forests, as evidenced by a greater extractable inorganic N pool, higher solution inorganic N leaching, lower N retention capacity, and lower soil C/N ratios. The distinction in soil N status resulted in different responses to experimental N addition, with larger fractions of N added retained by upper 20 cm soils in the pine and mixed forests (42%—74%) than in the mature forest (0%—70%). These results suggest that land-use history, forest age and species composition are likely to be some of the important factors that determine differing forest N retention to additional N input in the study region.

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