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Preliminary Response of Soil Fauna to Simulated N Deposition in Three Typical Subtropical Forests*[1]

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ABSTRACT

A field-scale experiment arranged in a complete randomized block design with three N addition treatments including a control (no addition of N), a low N (5 g m^{-2} $year^{-1}$), and a medium N (10 g m^{-2} $year^{-1}$) was performed in each of the three typical forests, a pine (*Pinus massoniana* Lamb.) forest (PF), a pine-broadleaf mixed forest (MF) and a mature monsoon evergreen broadleaf forest (MEBF), of the Dinghushan Biosphere Reserve in subtropical China to study the response of soil fauna community to additions of N. Higher NH_4^+ and NO_3^- concentrations and a lower soil pH occurred in the medium N treatment of MEBF, whereas the NO_3^- concentration was the lowest in PF after the additions of N. The response of the density, group abundance and diversity index of soil fauna to addition of N varied with the forest type, and all these variables decreased with increasing N under MEBF but the trend was opposite under PF. The N treatments had no significant effects on these variables under MF. Compared with the control plots, the medium N treatment had significant negative effect on soil fauna under MEBF. The group abundance of soil fauna increased significantly with additions of higher N rates under PF. These results suggested that the response of soil fauna to N deposition varied with the forest type and N deposition rate, and soil N status is one of the important factors affecting the response of soil fauna to N deposition.

Key Words: Dinghushan Biosphere Reserve, N deposition, soil fauna, subtropical China

Atmospheric deposition of nitrogen is mainly resulted from fossil fuel burning and from production and use of fertilizers in industrial regions of the world, and it is being elevated in many developing regions. Concern about the ecological effects of elevated N deposition on the functioning of forest ecosystem has been increasing (Vitousek *et al.*, 1997; Moffat, 1998). In temperate areas N deposition has influenced the structure and function of the forest ecosystem, including biodiversity, primary productivity, N transformation and leaching, and resulted in emission of greenhouse gasses (CH_4, NO_2, *etc.*), soil and water acidification, and forest degradation (Moffat, 1998; Sharon and Pamela, 1999; Xu *et al.*, 2003a). However, the response of soil fauna community, one of the most important components of the forest ecosystem, to increased levels of N is still unclear.

Soil fauna community, the "engineer of the soil ecosystem", has shown astonishing quantity and biodiversity and has exhibited significant ecosystem functions (Xu *et al.*, 2003b; Bölter, 2004). Since most of the deposited N will go into the soil eventually, it will undoubtedly affect the soil fauna community. However, how and to what extent does N deposition affect soil fauna community are still unclear. Therefore, it is necessary to study the response of the soil fauna to N deposition of different levels and to comprehensively assess the effects of increased atmospheric N deposition.

In a previous nursery study, changes of soil fauna community to simulated N addition have been found (Xu *et al.*, 2004). As a further step, from October 2002 to July 2003, permanent plots were built

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in three major forests of the Dinghushan Biosphere Reserve in subtropical China to study the response of soil fauna community to additions of N, herein we report the preliminary results from this study.

MATERIALS AND METHODS

Site description and experimental design

This study was conducted in three typical forests: pine (*Pinus massoniana* Lamb.) forest (PF), pine-broadleaf mixed forest (MF) and mature monsoon evergreen broadleaf forest (MEBF) in the Dinghushan Biosphere Reserve, subtropical China. The soil in the region is lateritic red soil derived from sandstone, but the soil thickness varies in each site. In MEBF the soil is thicker than 60 cm; in MF it ranges from 30 to 60 cm; and in the pine forest it is generally less than 30 cm to the bedrock (Mo *et al.*, 2003). Soil properties of these three forests are shown in Table I.

TABLE I

Soil properties of pine forest (PF), pine-broadleaf mixed forest (MF) and mature monsoon evergreen broadleaf forest (MEBF) in the Dinghushan Biosphere Reserve of subtropical China[a)]

Forest type	Soil depth	Organic matter	Total N	C/N	pH	Soil moisture
	cm	g kg^{-1}				g kg^{-1}
PF	0–20	27.3 (1.7)[b)]	0.9 (0.1)	16.79 (0.94)	4.03 (0.02)	249.0 (11.0)
MF	0–20	34.5 (3.5)	1.0 (0.1)	18.90 (0.97)	3.86 (0.02)	259.7 (9.1)
MEBF	0–20	53.5 (5.8)	1.9 (0.1)	15.91 (0.86)	3.79 (0.06)	385.7 (11.9)

[a)]From Mo *et al.* (2003); [b)]Standard errors of the means in parentheses ($n = 10$).

The experiment was arranged in a complete randomized design with three N addition treatments including a control (no addition of N), a low N (5 g m^{-2} year^{-1}), and a medium N (10 g m^{-2} year^{-1}) in each of the three forests (Magill *et al.*, 1997; Aber *et al.*, 1998; Wright and Rasmussen, 1998). There were 3 replicated plots for each treatment in each forest. All 27 plots (20 m $\times$ 10 m) were set up (9 plots in each forest site) and surrounded by a buffer strip (10 m in width). Each plot was further divided into 8 subplots (assigned numbers 1–8) of 5 m $\times$ 5 m for various field samplings. Beginning in July 2003, water (only in control plots) and NH_4NO_3 solution (in N addition plots) were sprayed monthly by hand onto the forest floor over these plots, 12 times of spraying over the course of the year. NH_4NO_3 fertilizer was weighed, and then dissolved in 20 L of water to make NH_4NO_3 solution. The control plots received 20 L of water without N addition.

Sampling and analysis

To collect soil solution, two square stainless steel devices (755.4 cm^2 in cross section) were set at the 20 cm soil depth in subplots 2 and 4 of each plot. The content of the solution from each collector was measured at first; then the solutions from the same plot were mixed. In the laboratory, the solution samples were filtered (2 mm), and then the NH_4^+, NO_3^- concentration was determined using the phenate and copper-coated cadmium reduction-diazotization methods while pH was determined with glass electrode (Liu *et al.*, 1996).

From May to July 2003 before the experimental treatments were initiated, a survey of the background soil fauna communities in all plots was conducted. In October 2003, after the treatments had been applied for three months, another survey was completed. In each plot, 25 litter samples were randomly collected using a square frame (10 cm $\times$ 10 cm) and all these samples were mixed to form one composite sample. Thus, 27 composite samples from all plots were collected for each survey. Samples were immediately taken back to laboratory and soil fauna were collected using dry Tullgren funnel method. Soil fauna specimens were sorted and counted using a dissecting microscope. According to Yin (1998) and Zhen and Gui (1999), most organisms were classified into taxonomic families (superfamilies), except for mites which only into orders. These levels of taxonomic identification were regarded as sufficient for characterizing the response patterns of the soil macroinvertebrate community (Kuperman, 1996). The diversity index (DI) was calculated as (Liao et al., 1997):

$$\mathrm{DI} = (g/G)\sum_{i=1}^{g}[(D_iC_i)/(D_{i\,\max}C)] \tag{1}$$

where "g" is the group number in a single soil fauna community (for example, for one type of forest or one type of N treatment); "G" is the sum of all groups; "D_i" are the individuals of the "ith" group in a single community; "C_i" are the frequencies of the "ith" group occurring among all of the communities; "$D_{i\,\max}$" is the maximum number of individuals of the "ith" group among the all communities; and "C" is the number of soil fauna communities.

ANOVA was performed to analyze the effects of N addition treatment and forest type on soil fauna community using SPSS 12.0 statistical analysis software. Differences at $P < 0.05$ were considered to be significant for all tests.

RESULTS

Under the medium N treatment, inorganic nitrogen concentrations of the soil solutions in MEBF were higher and pH values were lower than those in the other two forests. Overall, the NO_3^- concentration of the solution was the lowest in PF after the addition of N (Table II).

TABLE II

Inorganic nitrogen concentrations and pH of soil solutions (0–20 cm soil depth) in different treatments[a)] of pine forest (PF), pine-broadleaf mixed forest (MF) and mature monsoon evergreen broadleaf forest (MEBF)

Item	PF			MF			MEBF		
	C	L	M	C	L	M	C	L	M
NH_4^+ (mg L^{-1})	1.59 (0.55)[b)]	2.49	2.8	0.57	2.25	2.68	0.93 (0.16)	2.37 (0.53)	3.86 (0.45)
NO_3^- (mg L^{-1})	10.23 (1.34)	10.71	11.13	5.08	15.28	17	12.71 (1.28)	17.28 (2.44)	21.22 (3.22)
pH	4.01 (0.29)	3.88 (0.23)	3.86 (0.34)	3.98 (0.33)	3.8 (0.28)	3.95 (0.4)	3.82 (0.14)	4.11 (0.23)	3.68 (0.16)

[a)] C, L and M refer to control plot (no addition of N), low N treatment (5 g m^{-2} $year^{-1}$) and medium N treatment (10 g m^{-2} $year^{-1}$), respectively; [b)] Standard errors of the means in parentheses ($n = 3$).

After the additions of N, the greatest decreases of the density, group abundance and diversity index of soil fauna in MEBF and marked increases in PF occurred (Fig. 1), suggesting that N addition had negative effect on soil fauna in MEBF but positive effect in PF.

The negative effect of N addition on soil fauna in MEBF was further demonstrated in comparing the three soil fauna indices (density, group abundance and diversity index) before and after addition of N in different treatments (Fig. 2). After the addition of N, all the three soil fauna indices in the control plots showed no significant difference relative to those before addition of N, however, there was a significant decrease for all the three soil fauna indices under the medium treatment in MEBF ($P < 0.05$, Fig. 2). There were no significant differences in MF before or after receiving N (Fig. 2). However, after the N treatment the soil fauna density, group abundance and diversity significantly increased in PF ($P < 0.05$) compared to the control (Fig. 2), indicating the positive effect of N addition on soil fauna in PF.

As also shown in Fig. 2, the density, group abundance and diversity of soil fauna in all three forests did not change over the three-month experimental period under the control treatment. Although there were significant differences for all three indices between MEBF and PF at the start of the experiment, these differences disappeared after 3 months of low N treatment.

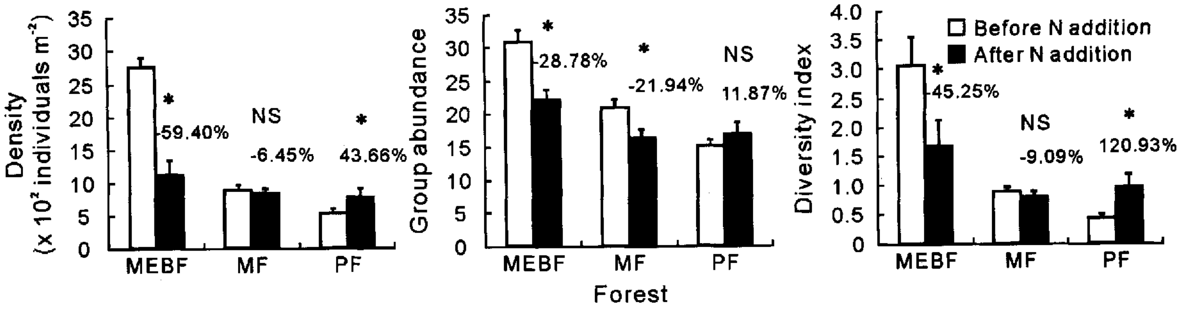

Fig. 1 Comparison of soil fauna density, group abundance and diversity index before and after addition of N in different forests. MEBF, MF and PF refer to mature monsoon evergreen broadleaf forest, pine-broadleaf mixed forest, and pine forest, respectively. Error bars indicate standard errors ($n = 3$). Percentages represent the changes after addition of N. * represents significant difference between before and after N addition at $P < 0.05$ and NS represents no significant difference.

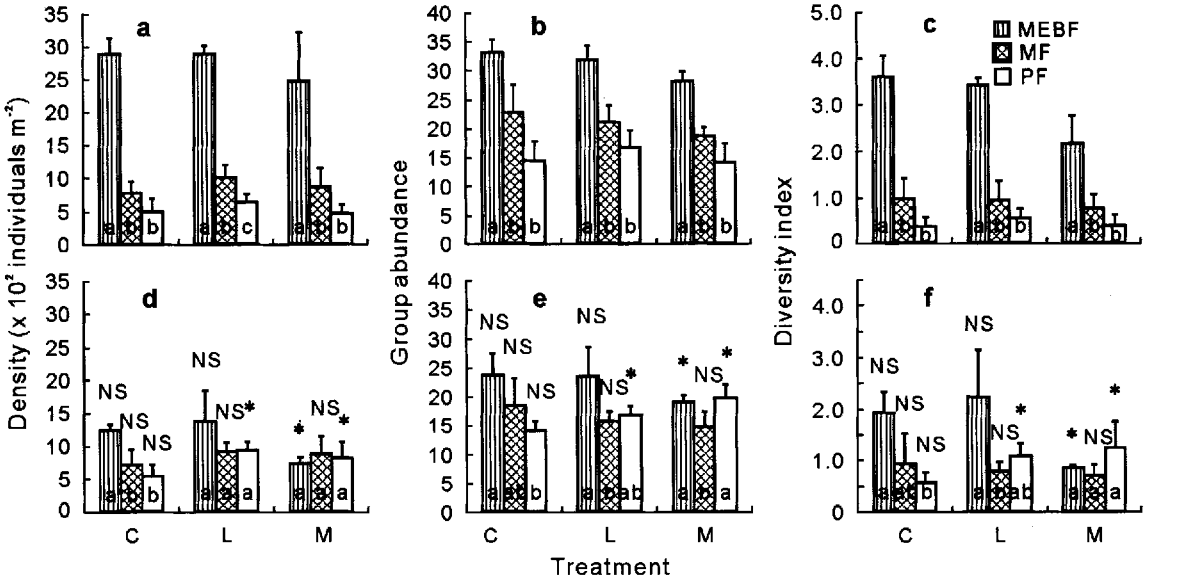

Fig. 2 Comparison of soil fauna density, group abundance and diversity index in different treatment plots before (a–c) and after (d–f) addition of N. Treatments C, L and M refer to control plot (no addition of N), low N treatment (5 g m^{-2} $year^{-1}$), and medium N treatment (10 g m^{-2} $year^{-1}$). MEBF, MF and PF refer to mature monsoon evergreen broadleaf forest, pine-broadleaf mixed forest, and pine forest, respectively. Error bars indicate standard errors ($n = 3$). * represents significant difference between before and after N addition at $P < 0.05$ and NS represents no significant difference. Bars marked by the same letter(s) within the same treatment are not significantly different at $P = 0.05$.

In the medium treatment plots, the changes before and after N addition were even greater. After the addition of N, soil fauna density of the three forests was not significantly different, group abundance under PF was significantly higher ($P < 0.05$) than MF and diversity index under PF exhibited the highest among the three forests (Fig. 2).

DISCUSSION

The response of soil fauna to addition of N in this study could lead to two important conclusions: 1) the response to N deposition varied with the forest type, and 2) the response to N deposition varied with the level of N addition. For the first conclusion, the response of soil fauna to additions of N exhibited opposite trends under MEBF and PF, negatively for the former and positively for the latter (Fig. 1). However, the N treatments had no obvious effect under MF. For the second conclusion, there were significant negative effects under the medium N treatment, but not under the low N treatment in MEBF (Fig. 2). In PF, the response of group abundance to addition of N increased significantly with greater additions of N (Fig. 2).

During the process of N deposition, the forest ecosystem could use suitable inputs of NO_3^-, however excessive NO_3^- would leach out or partially accumulate in the soil. When the flux of nitrogen (mineralization and inputs) equaled the absorption ability of the soil, the ecosystem became "N saturated". Many studies on plants, microorganisms and soil fauna have proven that a certain level of added N could be beneficial for organisms, but excessive additions were unfavorable (Aber *et al.*, 1998; Magill *et al.*, 2000). This was because excessive nitrogen additions in a N-saturated ecosystem enhanced the nitrification of NH_4^+, the leaching of NO_3^-, and the increase of soil acidification (Minami, 2005). In addition, the pH and the Al^{3+}/Ca^{2+} ratio in the rhizosphere were considered to be indicative of soil acidification and potential negative effects on forests (Kros *et al.*, 1993). It has also been suggested that leaching of base cations (Ca^{2+}, Mg^{2+}, *etc.*) in the soil increased with an excess of NO_3^-, accelerating acidification of the soil (Foster *et al.*, 1989). As a consequence, the Al, Mn, and Pb fluxes increased strongly (Foster *et al.*, 1989; Guo *et al.*, 2003; Li *et al.*, 2004; Xu and Ji, 2004). Accordingly, the increase of soil acidity and concentration of Al following release of NO_3^-, directly or indirectly, led to forest degradation (Tomlinson, 1993). So, NO_3^- leaching at N saturation was very harmful for soil organisms.

At the study sites, MEBF was rich in N, containing twice as much total N as MF and PF. N in PF was the least (Table I; Mo *et al.*, 2003). The different N status in these three forests was also supported by a recent study on litter decomposition in the same forests, in which MEBF had relatively higher litter decomposition rate and exhibited no significant positive and even some negative response to nitrogen additions due to both long-term high N deposition in the region and the age of the ecosystem, however, both PF and MF forests exhibited slower litter decomposition rates with significant positive effects from nitrogen additions due to previous land use history, suggesting that MEBF had likely been N saturated but PF and MF were still N limited (Mo *et al.*, 2004). Following addition of N, leaching was more readily in N rich than in N poor ecosystems (Aber *et al.*, 1998; Gundersen *et al.*, 1998; Wright and Rasmussen, 1998; Matson *et al.*, 1999). In the present study, after the N treatments (Table II), the NO_3^- concentrations of the soil solution in MEBF with the medium treatment were higher and pH values for the medium treatment were lower than those in the other two forests, which was possibly due to NO_3^- leaching. Thus, the soil fauna community showed negative response to added N in MEBF relative to MF and PF as well as under the higher N treatment in MEBF relative to the lower N treatment. On the contrary, NO_3^- concentration of the soil solution was the lowest in PF with less N leaching and acidification. Consequently, positive effects were characteristic.

Our results were consistent with findings from other experiments. Along the Ohio River Valley, soil fauna in three sites exposed to different amounts of acidic deposition for several decades were studied, revealing that the density of soil fauna in the low dose site (with the lightest acidification) is significantly higher than that of the other two high dose sites (Kuperman, 1996). The large differences are significantly correlated with soil parameters, such as pH and exchangeable Al (Kuperman, 1996). In another case, in the Nitrogen Saturation Experiments Project in Europe, the composition of Collembola species also showed high diversity in Sweden due to low ambient N deposition (Boxman *et al.*, 1998).

In summary, our results suggested that the response of soil fauna in forest ecosystems of the study region to N addition varied with the forest type and N deposition rate, which might be due to the fact that the level of N saturation could control the response of the soil fauna community to N deposition. Perhaps N saturation had occurred in the medium N treatment plot in MEBF, but not in PF. What would happen as the experiment continues and N is being accumulated becomes an open question. Would the response of soil fauna become even more negative under MEBF? Would the direction of the response of soil fauna change from positive to negative under PF? To answer these, further studies are necessary.

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