



Response of soil fauna to simulated nitrogen deposition: A nursery experiment in subtropical China

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Abstract

We studied the responses of soil fauna to a simulated nitrogen deposition in nursery experimental plots in Subtropical China. Dissolved NH_4NO_3 was applied to the soil by spraying twice per month for 16 months, starting in January 2003 with treatments of 0, 5, 10, 15 and 30 $\text{gN}/(\text{m}^2\cdot\text{a})$. Soil fauna was sampled after 6, 9, 13 and 16 months of treatment in three soil depths (0–5 cm, 5–10 cm, 10–15 cm). Soil available N increased in correspondence with the increasing N treatment, whereas soil pH decreased. Bacterial and fungal densities were elevated by the N treatment. Soil fauna increased in the lower nitrogen treatments but decreased in the higher N treatments, which might indicate that there was a threshold around 10 $\text{gN}/(\text{m}^2\cdot\text{a})$ for the stimulating effects of N addition. The N effects were dependent on the soil depth and sampling time. The data also suggested that the effects of the different N treatments were related to the level of N saturation, especially the concentration of NO_3^- in the soil.

Key words: soil fauna; N deposition; response; subtropical China

Introduction

The emission of biologically available nitrogen has increased globally (Galloway and Cowling, 2002). In industrial Europe and North America, the amount of deposited N now is 20 times higher than before industrialization (Bartnicki and Alcamo, 1989). With the economic development, high N deposition also occurs in China. For example, the concentration of NH_4^+ in rain is 5–10 times higher than that found in North America (Chou and Wu, 1997). On average, 38.4 $\text{kgN}/(\text{hm}^2\cdot\text{a})$ is deposited in the Dinghushan area, West Guangdong (Zhou and Yan, 2001). Generally, China has become one of the three major atmospheric N deposition areas (Europe, America, and China) (Townsend *et al.*, 1996; Xu *et al.*, 2003). Increased N deposition causes diverse effects on the constitution and functions of forest ecosystems (Fenn *et al.*, 1998), and even leads to degradation of forests (Li *et al.*, 2003).

Most of the deposited N will eventually end up in the soil. Until now, only few studies on the effects of N deposition on soil fauna have been performed. From the NITREX project in Europe it was reported that the species richness and biomass of soil Collembola and Oribatid mites responded to N deposition in a litterbag experiment in July 1993 (Boxman *et al.*, 1998). But

one study of only two species is not sufficient to assess whole community responses. N inputs are beneficial for soil fauna in many agricultural fertilization experiments (Rodgers, 1997; Lindberg and Persson, 2004; Nkem *et al.*, 2002; Whalen *et al.*, 1998; Sarathchandra *et al.*, 2001), which provide indirect information about the effects of atmospheric N deposition on the soil fauna. However, in order to discover more about the effects of atmospheric N deposition on the soil fauna community, a simulated experiment designed for this purpose is necessary.

Current studies on the effects of N deposition on soil fauna come from temperate areas. Compared with temperate areas, there is an even more abundant soil fauna community in tropical conditions (Gonzalez and Seastedt, 2001). At the same time, because forest ecosystems in tropical areas are often not N limited (Pamela *et al.*, 2002), their responses to atmospheric N deposition could be stronger than in N limited temperate ecosystems. Thus a hypothesis is suggested that soil fauna in tropical areas may react more negatively to N deposition than in temperate areas.

We carried out this study in Dinghushan Biosphere Reserve (DHSBR) in subtropical China using a simulated experimental gradient of N deposition. The objectives were: (1) to study the effects of N deposition on soil fauna community characteristics; (2) to study the effects of increasing N deposition on soil fauna abundance and diversity; (3) to identify the mechanisms of N deposition effects on soil fauna.

1 Materials and methods

1.1 Site description

DHSBR is located near Zhaoqing City of southern China, 23°08'N, 112°35'E. The DHSBR occupies an area of more than 1155 hm². Because it is relatively close to the Pacific Ocean in the east and south, and close to Indian Ocean in the southwest, there are often warm and humid southeast winds and southwest winds in the summer, and often tropical storms including typhoons bring rich rainfall in the summer and fall; the climate belongs to lower subtropical monsoon humid, with solar radiation providing 4655 MJ/(m²·a) and sunshine duration averaging 1433 h/a. The average temperature is 20.9°C, and annual average rainfall is 1900 mm (Xia *et al.*, 1997; Hou *et al.*, 2002). The soils in this area mainly consist of lateritic red-earth, with yellow-earth and mountain shrubby-meadow soil also present; soil pH is 4.2–5.0 (Hou *et al.*, 2002).

1.2 Experimental design

The experimental design was a randomized complete block, consisting of five treatments, with three replications (plots) in each case. Soil in the research site was mixed to obtain a uniform mixture before the start of experiment in October 2002 and all weeds were removed during this soil treatment. Fifteen plots of dimensions 4 m by 5 m were established and each plot was surrounded by a 1-m wide buffer strip. One-year-old seedlings of three tree species (*Schima superba*, *Castanopsis chinensis*, and *Cryptocarya concinna*) were obtained from a nearby experimental field. A total of 1800 seedlings (600 seedlings per species) were prepared. On October 25, 2002, forty seedlings of each species were transplanted into each plot of the study site. Throughout the experiment, weeds were removed regularly by hand.

Five N addition treatments (in three replicates) were established: control (without N added), low (5 gN/(m²·a)), medium (10 gN/(m²·a)), high (15 gN/(m²·a)) and double high (30 gN/(m²·a)) nitrogen deposition. Fertilizer was weighed, mixed with 10 L of water, and applied to the plots using a backpack sprayer. NH₄NO₃ solution was sprayed by hand onto the soil in the middle and at the end of each month from January 2003 until April 2004. Two passes were made across each plot to ensure an even distribution of fertilizer. The control plots received 10 L water without N added.

1.3 Soil microorganism, fauna and soil sampling

The upper 10 cm mineral soils were sampled in November 2003, February 2004 and May 2004. In each plot, five soil cores (2.8 cm in diameter) were collected randomly and combined to one composite sample in the field, yielding a sample size of three for each N treatment. One 10 g subsample was used for soil property analysis and the remaining sample from each composite sample was used to investigate microbial abundance.

We performed soil fauna sampling in July and October 2003, and in February and May 2004 (6, 9, 13 and 16 months after treatment started). In each plot five sampling

points were selected by diagonal method and at each point, a soil core was taken by a cylindrical soil corer with an internal diameter of 60 mm and divided into three depth intervals (0–5, 5–10, 10–15 cm). In each plot, the samples were bulked to make one sample per layer, yielding a sample size of three for each N treatment and for each layer. We took these samples immediately back to the laboratory for fauna analysis.

1.4 Samples treatment

Extractable NH₄⁺-N was determined colorimetrically by the indophenol blue method and NO₃⁻-N was analyzed by copper-cadmium reduction method (Liu *et al.*, 1996). Soil pH was measured in distilled water suspension using the glass electrode, after shaking for 1 h at a ratio of 25 ml water to 10 g mineral soil (Liu *et al.*, 1996). Microbial community abundance was measured by the dilution plate method, with a beef extract and peptone culture medium (2–3 d) for bacteria and MD culture medium (6–7 d) for fungi (Institute of Soil Science, 1985). Soil fauna were collected with dry Tullgren funnels for 6 h (Liao *et al.*, 1997). We sorted and counted all of the soil fauna using a dissecting microscope and classified them to genera, families or superfamilies except for mites, which were classified to suborder, using key books of soil fauna (Yin, 1998; Zhen and Gui, 1999). These levels of taxonomic identification were regarded as sufficient for characterizing the response patterns of the soil macroinvertebrate community (Kuperman, 1996).

1.5 Data analysis

To determine soil fauna community diversity, the following diversity index (DG) (Liao *et al.*, 1997) was used:

$$DG = (g/G) \sum_{i=1}^g ((D_i C_i) / (D_{i \max} C)) \quad (1)$$

Where, g is the group number in a single soil fauna community (for example, for one type of N treatment); G is the summary of all groups; D_i is the individuals of i group in a single community; $D_{i \max}$ is the max individuals of i group among the all communities; C_i is the frequencies of i group occurring among all of the communities; C is the number of soil fauna communities.

We performed a three-way ANOVA to examine the effects of main factors, N treatment, sampling date and soil depth, and their interactions on soil fauna abundance of individuals, group richness (the number of taxonomic groups), and DG diversity index. We examined the effects of N treatment on microbial density and soil parameter responses to N treatment with one-way ANOVA. An F -test was used to identify the effects of main factors and their interactions. Differences among levels of one factor were identified by a Duncan test, and paired sample t -test were used to identify the differences between the values of 2004 and values of 2003 for soil fauna density and richness during the sampling period. All tests were considered to be significant at the 0.05 level unless otherwise stated. SPSS 11.5 was used for all analyses.

2 Results

2.1 Responses of microbial densities and soil properties to N additions

Generally, the abundance of soil bacteria and fungi increased significantly with increasing levels of N addition ($P < 0.05$, Table 1). Abundance of bacteria and fungi were significantly lower in control plots ($P < 0.05$, Table 1).

Similarly, soil available N, especially soil NO_3^- -N, increased with increasing concentration of N treatment (Table 1). The soil NO_3^- -N was significantly lower in the control plots than in all N treated plots ($P < 0.05$), with the highest soil NO_3^- -N in the plots treated with 30 g N (Table 1). However, the soil pH decreased with increasing levels of N additions and was significantly lower than control plots in 15 g N plots and 30 g N plots ($P < 0.05$, Table 1).

2.2 Effects of sampling date

Sampling date affected significantly the abundance of individuals, group richness and DG index of soil fauna community (Table 2, $P < 0.001$). Overall, the soil fauna density and richness increased during the sampling period; the values of 2004 were much higher than those of 2003 ($P < 0.05$, Fig.1). The interactions between sampling date and N treatment, and between sampling date and soil depth on soil fauna, were also significant (Table 2).

2.3 Effects of soil layer

The vertical distribution of soil fauna was obvious (Table 2, Fig.2), because soil fauna abundance of individuals, group richness and DG index in layer I were significantly higher than in the two deeper soil layers (layer I: 611.67, 112.71 and 6.88; layer II: 391.45, 87.86 and 3.20; layer III: 288.92, 76.33 and 2.37, respectively) ($P < 0.001$, Fig.2). All parameters of soil fauna were in the order from highest to lowest: I>II>III in all N treated plots except those treated with 30 g N (Fig.2). In the plots treated with 30 g N, soil

fauna group richness and DG index in layer III (85.36 and 3.13) increased over layer II (77.12 and 2.62), significantly so for group richness ($P < 0.05$) (Fig.2).

2.4 Effects of N treatments

The group richness and DG index but not abundance of individuals of soil animals were significantly affected by N addition ($P < 0.05$, Table 2). Generally, in 10 g N plots, the group richness (78.40) was significantly higher than in control (60.49), 15 g N (67.82) and 30 g N (66.10) plots by 29.60%, 15.6% and 18.60%, respectively ($P < 0.05$), and the DG indices were significantly higher in 10 g N plots (3.73) than that in control (2.63) and 30 g N (2.82) plots by 41.38% and 32.26%, respectively ($P < 0.05$) (Figs.1b and 1c).

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The differences between N treated plots and control plots increased with sampling date (Fig.1). For abundance of soil fauna individuals, there was no significant difference between any treatments after 6 months, but the abundance was significantly lower in control (148.83) than in 15 g N (216.62) and 30 g N (212.33) treatments after 9 months ($P < 0.05$); this difference had increased after 16 months ($P < 0.05$) (Fig.1a). Group richness was significantly lower in the controls (36.78) than in 10 g

Table 1 Effects of nitrogen treatments on soil microorganisms and soil properties

	Control	5 gN/(m ² ·a)	10 gN/(m ² ·a)	15 gN/(m ² ·a)	30 gN/(m ² ·a)
Bacteria (10 ⁵ /g dry soil)	261.92 (50.20) ^c	365.06 (87.70) ^b	339.96 (30.20) ^b	440.19 (69.19) ^a	457.56 (14.40) ^a
Fungi (10 ⁴ /g dry soil)	10.70 (0.67) ^d	11.79 (1.76) ^{cd}	13.50 (1.01) ^c	16.27 (2.19) ^b	24.69 (1.53) ^a
NO_3^- -N (mg/kg)	4.29 (1.34) ^c	7.00 (1.71) ^b	8.75 (1.02) ^b	8.83 (2.43) ^b	12.92 (1.57) ^a
NH_4^+ -N (mg/kg)	4.93 (1.42) ^b	3.40 (0.43) ^b	4.20 (0.42) ^b	4.93 (1.50) ^b	6.91 (1.25) ^a
pH	5.2 (0.03) ^a	4.9 (0.14) ^{ab}	4.9 (0.09) ^{ab}	4.8 (0.14) ^b	4.7 (0.15) ^b

Data are means of three samplings (November 2003, February 2004 and May 2004); data in parenthesis are SE; means followed by different letter within a row differ significantly at $P < 0.05$.

Table 2 F-Values of three-way ANOVA

	Density F-value	Group richness F-value	DG index F-value
Sampling date	98.67***	64.31***	44.52***
Nitrogen	0.9 ^{ns}	4.73***	2.75*
Soil depth	44.72***	32.62***	63.59***
Sampling date × nitrogen	1.91*	2.06*	2.42**
Sampling date × soil depth	20.60***	27.94***	33.19***
Nitrogen × soil depth	0.96 ^{ns}	1.51 ^{ns}	2.15*
Sampling date × nitrogen × soil depth	1.22 ^{ns}	0.68 ^{ns}	1.58 ^{ns}

Nitrogen (0, 5, 10, 15 and 30 (gN/(m²·a)); soil depth (0–5, 5–10, 10–15 cm); and sampling date (6, 9, 13, 16 months later) *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ^{ns} $P > 0.05$.

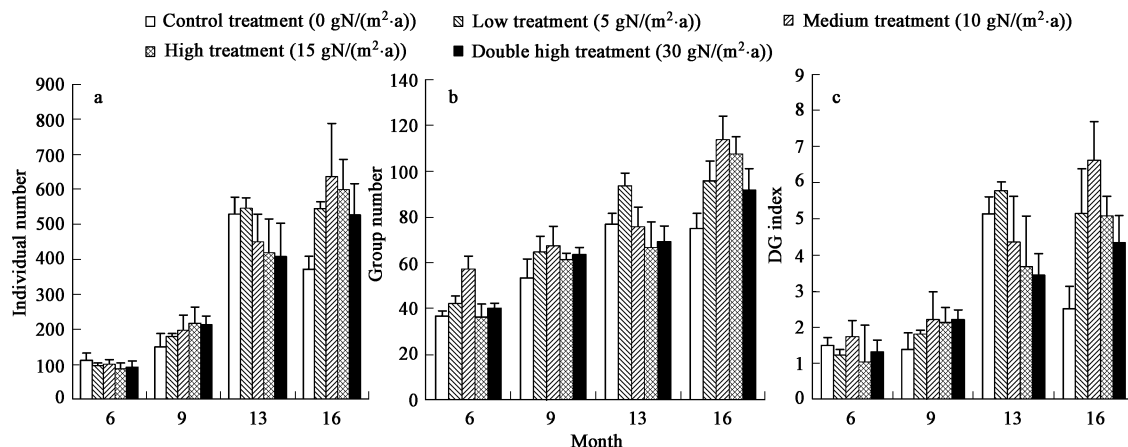


Fig. 1 Effects of nitrogen treatment and sampling date on soil fauna individual number (a), group number (b) and DG index (c). X-axis represents months after the experiment beginning. $P < 0.05$.

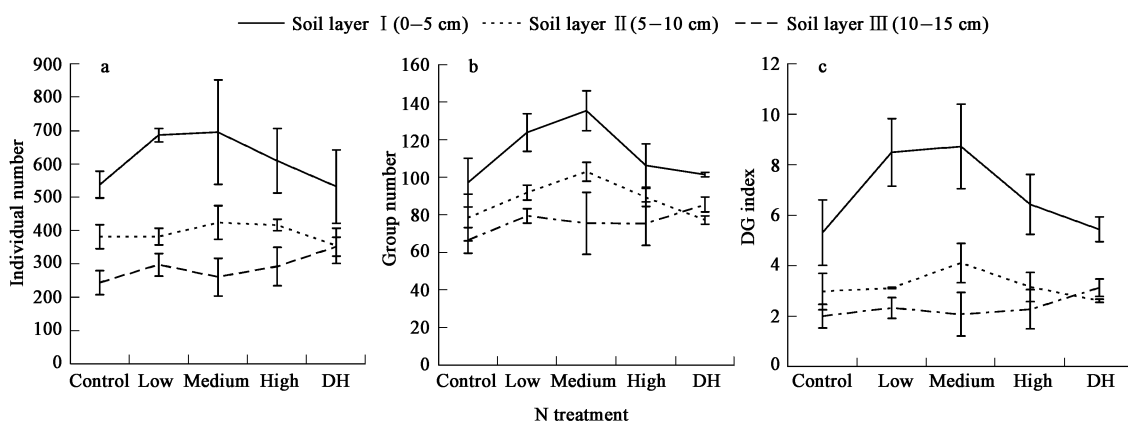


Fig. 2 Effects of nitrogen and soil depth on soil fauna individual number (a), group number (b) and DG index (c). Control: 0 gN/(m²·a); low: 5 gN/(m²·a); medium: 10 gN/(m²·a); high: 15 gN/(m²·a); DH (double high): 30 gN/(m²·a).

N (57.14) and 30 g N (40.13) treatments after 6 months ($P < 0.05$), and significantly lower (53.38) than 5 g N (64.67) and 10 g N (67.35) plots after 9 months; again, this difference increased over the 16 months of the study (Fig. 1b). After 6 months the community diversity was only significantly lower in control (1.50) than in 10 g N (1.73) plots ($P < 0.05$), but after 9 months and 16 months, the control was significantly lower (1.38 and 2.58) than in 10 g N (2.21 and 6.61), 15 g N (2.13 and 5.08) and 30 g N (2.21 and 4.33) plots ($P < 0.05$); it was the lowest in control (2.51) than other treatments (5.15, 6.61, 5.08 and 4.33) after 16 months ($P < 0.05$) (Fig. 1c). Additionally, from July 2003 to May 2004 (Fig. 1), soil fauna community increased from July 2003 to February 2004 and then declined in May 2004 in control plot, but increased constantly over time in the N treated plots, especially for the individuals under 10 g N, 15 g N and 30 g N treatments (Fig. 1a), the developments were all significant ($P < 0.05$).

There was also an interaction between N inputs and soil depth and a threshold of N treatment across different treatments was visible at soil layer I (Table 2; Fig. 2). Two opposite trends occurred with the increased N inputs (Fig. 2). In layer I, soil fauna community increased from control to 10 g N treatment but decreased thereafter; the diversity and group abundance in 10 g N (8.72 and 135.32) and 5 g N (8.49 and 123.65) plots were significantly higher

compared with those in control (5.31 and 97.11), 15 g N (6.43 and 106.15), and 30 g N (5.44 and 101.33) plots ($P < 0.05$) (Fig. 2). In layer II, soil fauna community also increased from control plots to 10 g N plots and then decreased from 10 g N plots to 30 g N plots, and values from 10 g N plots were also significantly higher than other treatments ($P < 0.05$) (Fig. 2). In layer III, soil fauna was relatively scarce in all treatments (Fig. 2). However, in the 30 g N treatment, soil fauna group richness and DG index and, in particular, group richness were greater in layer III (85.36 and 3.13) than in layer II (77.12 and 2.62) by the end of the experiment (Figs. 2b and 2c).

Soil fauna community in layer I was sensitive to N deposition for the direct effect; where, soil fauna community obviously increased from control plots to 10 g N plots and then declined, and the highest point or inflexion appeared in 10 g N treatment (Fig. 2). Under the most N treatment, 30 g N, soil fauna seemed concentrated in deeper soils (Fig. 2).

3 Discussion

3.1 Positive effects of N treatments on soil fauna

In the present study, soil fauna density in N treated plots was obviously greater than that in control plots and the difference generally increased over time. The abundance

of soil bacteria and fungi increased significantly with increasing N inputs, which might have contributed to the increase of soil fauna because soil microbes are important food resources for soil fauna. Similarly, soil available N increased with increasing N inputs, which might also have contributed to the increase of soil fauna (Zhang, 1995; Su *et al.*, 1995; Zhang, 2002). Previous studies have reported that when soil available N increased from 69.8 to 84.7 mg/kg, soil fauna increased from 5375 m⁻² to 11725 m⁻² (Huang and Sheng, 1996) and the abundance of Collembola in N treated plots was twice the abundance of that in control plots (Rodgers, 1997). It has also been reported that soil available N is the most important factor affecting Acarina and the total number of soil fauna community (Su *et al.*, 2001). In addition, the content of soil ammonium is significantly correlated to the biomass of soil Enchytraeids (Sulkava *et al.*, 1996). In fact, nitrogen is one of the major nutrients for organisms, and additional N inputs to a certain extent are usually beneficial to organisms. Previous studies have reported that the abundance of Enchytraeid in all fertilized plots increased by up to 400% compared with that in controls (Abrahamsen and Thompson, 1979). In NITREX, Boxman *et al.* (1998) found that Collembola was more diverse under low ambient N deposition. Along the acidic Ohio River Valley, the total abundance and the number of decomposers and predators were much higher in a site receiving low doses of N (Kuperman, 1996). These are consistent with our results, with the N inputs having positive effects on soil fauna density and group richness during the experimental period.

3.2 Threshold of N treatments

Although low and medium doses of N had great positive effects on soil fauna in this study, higher N deposition treatments appeared to have some negative effects. Generally soil fauna group richness and diversity were significantly higher in 10 g N plots. In soil layer I, soil fauna increased significantly from the control to the 10 g N treatment and then decreased with increasing N inputs, and the highest point or inflexion was observed in 10 g N treatment. This indicated that there might be a threshold at around 10 g N treatment in the effects of N deposition on soil fauna.

Threshold effects of N deposition have been observed in many studies on plants and microbes. For example, Magill *et al.* (2000) found that the biomass of all forests increased to different extents with N deposition additions for nine years compared with control, but woody biomass of pine decreased with N inputs and woody biomass under the highest N treatment was significantly lower than the control nine years later (Magill *et al.*, 2000). Although the effect of N additions at a certain level was positive to soil fauna as described above, the negative effect developed under high level of N input. Fertilization as high as 12 gN/(m²·a) in a cotton field decreased the number of soil fauna (Nkem *et al.*, 2002). In a corn field, fertilization (NH₄NO₃, 15 gN/(m²·a)) caused significant decreases of earthworm abundance and biomass after 6 years (Whalen *et al.*, 1998). Under high dose (40 gN/(m²·a)) treatments, the nematode maturity index decreases drastically

(Sarithchandra *et al.*, 2001). This negative effect of N deposition can explain our findings, that soil fauna were more abundant in deep soil under the high N deposition (Fig.2). Perhaps they took refuge there to avoid the high N concentrations in the upper layers.

The occurrence of a threshold may closely related to soil N saturation, especially regarding NO₃⁻. During the process of N deposition, a certain amount of NO₃⁻ can be used by forest ecosystem, but excessive NO₃⁻ will leach out or accumulate partially in the soil. When fluxes of nitrogen (mineralization and inputs) are equaled to the absorption ability by soil, the ecosystem becomes N-saturated (Xiao, 2001). Excessive nitrogen addition in an N-saturated ecosystem will significantly accelerate soil acidification (Xiao, 2001). It was reported that leaching of base cations, such as Ca, Al and Mn increased with excessive NO₃⁻ (Foster *et al.*, 1989; Bergkvist and Folkesson, 1992; Watmough *et al.*, 1999). Ca²⁺ leaching will further increase the acidification of soil (Foster *et al.*, 1989). The excessive concentration of NO₃⁻ may increase the soil acidification and the ratio of Al³⁺ to Ca²⁺, which will directly or indirectly damage the ecosystem (Kros *et al.*, 1993). In a conclusion, NO₃⁻ inputs might be positive until saturation is reached and negative beyond saturation levels. In the present study, NO₃⁻ content in all of N treated plots was significantly higher than that in control plots, and it was the highest in 30 g N plots among all treatments; and pH in 30 g N plots decreased compared with that in control plots. This might indicate an N saturation in soil. The changes in NO₃⁻ content and pH might lead to the responses of soil fauna. The number of individuals, group abundance and diversity in N treated plots increased consistently compared with that in control plots, but in 30 g N plots, soil fauna seemed concentrated into deeper soil layers.

3.3 DG index

The Shannon-weaver index (H') is a widely used diversity index in the study of soil fauna in the past. H' diversity is suggested to be closely related to even characteristics of the community but not richness (Fu *et al.*, 2002); and it will reach the largest value if the individual number within every group is equal. Thus, it is adapted to communities with little taxonomic variation (Liao *et al.*, 1997). However, soil fauna groups are extremely diverse in both abundance and biomass. Therefore H' is not suitable for the analysis of complex community richness. It is clear that there are large differences between H' index (Fig.3) and actual individual and group richness characteristics in the study (Figs.1 and 2). In the present study, we used DG index for the diversity analysis of soil fauna. DG index was introduced in 1990, and amended in 1997 by Liao *et al.* (1997). The advantages of the DG index lie in the better feasibility in the diversity analysis among various communities. However, the disadvantage of DG index is that it can not demonstrate the relationships within each group. Soil fauna are generally polyphagous, thus the relationships among different groups may be relatively less important compared with the effects of external factors.

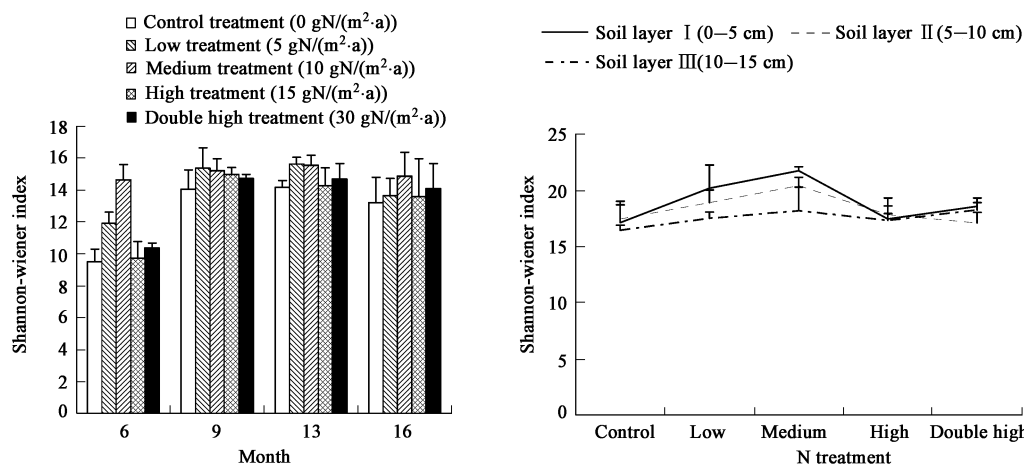


Fig. 3 Shannon-Wiener indexes of soil fauna under the effects of nitrogen, sampling date and soil depth. X-axis of left figure represents months after the experiment beginning.

In the present study, DG index clearly illustrated the characteristics of soil fauna individuals and group richness (Figs. 1 and 2), and it was a useful index to indicate the diversity of complex community of soil fauna.

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