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Fine Root Biomass Mediates Soil Fauna Community in Response to Nitrogen Addition in Poplar Plantations (*Populus deltoids*) on the East Coast of China

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Abstract: Soil fauna is critical for maintaining ecosystem functioning, and its community could be significantly impacted by nitrogen (N) deposition. However, our knowledge of how soil-faunal community composition responds to N addition is still limited. In this study, we simulated N deposition (0, 50, 100, 150, and 300 kg N ha⁻¹ year⁻¹) to explore the effects of N addition on the total and the phytophagous soil fauna along the soil profile (0-10, 10-25, and 25-40 cm) in poplar plantations (Populus deltoids) on the east coast of China. Ammonium nitrate (NH4NO3) was dissolved in water and sprayed evenly under the canopy with a backpack sprayer to simulate N deposition. Our results showed that N addition either significantly increased or decreased the density (D) of both the total and the phytophagous soil fauna (D_{total} and D_p) at low or high N addition rates, respectively, indicating the existence of threshold effects over the range of N addition. However, N addition had no significant impacts on the number of groups (G) and diversity (H) of either the total or the phytophagous soil fauna (Gtotal, Gp and Htotal, Hp). With increasing soil depth, Dtotal, Dp, G_{total}, and G_p largely decreased, showing that the soil fauna have a propensity to aggregate at the soil surface. H_{total} and H_p did not significantly vary along the soil profile. Importantly, the threshold effects of N addition on D_{total} and D_p increased from 50 and 100 to 150 kg N ha⁻¹ year⁻¹ along the soil profile. Fine root biomass was the dominant factor mediating variations in D_{total} and D_p . Our results suggested that N addition may drive changes in soil-faunal community composition by altering belowground food resources in poplar plantations.

Keywords: soil fauna; N addition; soil profile; community structure; food resources; poplar plantations

1. Introduction

Nitrogen (N) deposition, which is predicted to continuously increase, has become a major global concern [1–3]. In terrestrial ecosystems, most of the deposited N eventually dissipates into soils [4]. N accumulation in soils could change the community structure of soil fauna and could thereby impact ecosystem functioning [5]. Collectively, the soil fauna is a major consumer and decomposer and is therefore an essential component of the forest ecosystem [6,7]. As the "engineer of the soil ecosystem", soil fauna plays an important role in dissolving residues and altering biogeochemical cycles [8,9]. However, our understanding of the responses of soil fauna community to increased levels of N input is still limited.

Increasing evidence has demonstrated that N addition that mimics natural N deposition can undoubtedly have profound impacts on soil fauna communities. Despite much research, however, no consensus exists regarding the impacts of N addition in soil fauna communities. For instance, N addition significantly increases the densities of both phytophagous soil fauna and total soil fauna, which could be attributed to enhanced ammonium (NH₄⁺) production and nitrification processes from increased root exudation and organic matter input to the system [10]. In addition, Raub, et al. [11] confirmed the positive effect of N-rich food resources on the abundance of soil fauna. Conversely, a long-term N addition study reports that continuous N addition significantly reduces the density and taxa richness of the soil fauna due to the decreased allocation of carbon (C) to leaf and fine root litter, especially C from the roots associated with changes in rhizodeposition [12-14]. Additionally, some studies find no significant effects of N addition on the density of soil fauna due to reduced competitive exclusion of soil fauna and a short observation period [15]. A lack of impacts of N addition on the diversity of soil fauna has also been reported; results from the demands for N are similar among various soil fauna [16,17] or elevated intraguild predation [11]. The different responses of soil fauna communities to N addition—whether positive, negative, or non-significant—may primarily depend on the N addition rate [4]. The impact of N addition on soil fauna communities could change directionally and dramatically at a critical concentration [18,19], suggesting a threshold effect. The study of soil fauna community responses to N addition at a range of rates is therefore of great importance.

N addition could affect the vertical distribution of soil fauna, leading to major variations in the soil fauna community with increasing soil depth [20]. The density of soil fauna, for example, could appreciably increase in the topsoil layer due to N addition while exhibiting no significant changes in deep soil layers [21]. Soil depth is one of the most important factors influencing soil fauna communities due to variations in the soil's physical and chemical properties along the depth gradient [22,23]. Soil fauna may be more abundant in the subsoil, where they take refuge to avoid the high N concentrations in the upper soil layers under N addition [24]. Moreover, thresholds, if present, could shift from high to low along the soil profile [24]. Although N addition is reported to affect the soil fauna community, field-based studies of the responses of a soil fauna community to soil depth are still lacking, making predictions about soil food web changes with N deposition in plantations difficult.

The soil fauna community's responses to N addition are mediated by the quantity and quality of the food resources available, such as roots, litter, and fungi [25,26]. Typically, soil fauna density is correlated with root biomass [23], though previous studies in various ecosystems have shown that N addition can have a variety of impacts on fine root biomass, either stimulating the production of fine root biomass [27] or retarding it [28]. Moreover, root chemical traits are key factors in shaping soil fauna communities, and do so via mediating species-specific interactions and affecting the soil fauna at multiple trophic levels [29]. It is well established that plant root-derived nutrition, such as N, may strongly influence a soil fauna community [30]. Litter quantity and quality are two additional factors that have a major influence on the activity and composition of soil fauna [31]. High quantities of litter can stimulate an increase in soil fauna density, mainly due to concomitant increases in the food supply and the habitat space of the ecosystem [11,32]. In addition, the density and community parameters of soil fauna also largely depend on the chemical and physical qualities of the litter [31]. A previous study showed that the density of soil fauna could increase as the litter C:N ratio decreased [33]. Furthermore, some soil fauna prefer feeding

on older, more decomposed litter, and this preference strongly depends on the structure of the litter, e.g., tensile strength and palatability [26]. Recent studies, however, have suggested that root-derived food resources can be much more effective in fueling belowground food webs than those that come from leaf litter (e.g., [30,34]). Roots as a food source for soil fauna are considered to be an essential component of the underground food web [35]. The quantity and quality of food resources are likely to change in response to N addition, mediating the effects of N addition on soil fauna communities.

China grows more than 7 million ha of poplar plantations. Biomass production and C fixation by poplars (*Populus*) thus play important roles in mediating global climate change [36]. Compared to the natural forest ecosystem, changes in the soil fauna community in response to N addition in poplar plantations are less studied, especially for the whole soil fauna community [19]. Over the decades, many studies have evaluated the impact of N addition on particular species of soil fauna [37,38], while few studies have reported whole soil fauna community responses to N addition. Studies focused on one or two soil fauna species are not sufficiently indicative of the changes in whole soil fauna community [39]. It is thus necessary to study the response of the whole soil fauna to N addition in poplar plantations. In this study, we aimed to: (1) explore the effects of N addition and soil depth on the whole soil fauna community, and (2) determine the primary factors controlling the responses of the whole soil fauna community across N treatments and the soil profile in a poplar plantation at the Dongtai Forest Farm, eastern China.

2. Materials and Methods

2.1. The Experimental Site and Design

Our experimental site is located at the Dongtai Forest Farm in Yancheng, Jiangsu Province, eastern China ($120^{\circ}49'$ E, $32^{\circ}52'$ N). The farm is close to the Yellow Sea State Forest Park (on the coast of the Yellow Sea) and has a climate classified as Cfa (Humid subtropical climate) according to Köppen [40]. The mean annual temperature (MAT) is 13.7 °C and the mean annual precipitation (MAP) is 1051 mm. The soil of the forest farm is a desalting meadow and sandy soil with a pH value ~8 [41].

Our N addition experiment was established in May 2012. We chose 12-year-old pure poplar plantations (*Populus deltoids cv. 'I-35'*) with uniform site conditions and management measures as our plots. We used a randomized block design with a gradient of five levels of N addition (0, 50, 100, 150, and 300 kg N ha⁻¹ year⁻¹) in three replicate blocks (25×190 m). Each N treatment subplot was 25×30 m with a 10 m buffer zone between any two adjacent subplots. The distance between any two adjacent blocks was at least 500 m. We chose a range of N addition rates because the ambient N deposition rate is about 50 kg N ha⁻¹ year⁻¹ for this area [42] and Liu et al. [18] estimated that the critical loads for N deposition in Jiangsu province could be more than 200 kg N ha⁻¹ year⁻¹. In each month of the growing season (May through October), one-sixth of the yearly amount of NH₄NO₃ was dissolved in 20 L water and sprayed evenly under the canopy by backpack sprayer to simulate natural N deposition. Each control subplot received 20 L of water.

2.2. Microclimate

Soil temperature (0–15 cm) and moisture (0–20 cm) were measured once or twice a month using a Delta-T WET-2 (UK) from January to December 2016. Litter was collected in each subplot from November to December 2016 with a 2 m \times 2 m litter trap constructed of 2 mm mesh nylon cloth. We oven-dried the litter at 65 °C for 48 h and weighed it to calculate litter mass.

2.3. Measurement of C and N in Litter and Soil Samples

Litter samples for measuring C and N content were collected in October 2016, oven-dried, ground, filtered by sieving (0.5 mm), and analyzed with an elemental analyzer (PerkinElmer 2400 II, Waltham, MA, USA). Soil cores of 4 cm in diameter were taken in October 2016 from the 0–15 cm soil layer in each subplot. Soil samples were transferred to our lab, air-dried, ground, and dipped into 0.5 M

hydrochloric acid (HCl) to remove carbonates [43,44]. We then measured soil organic C (SOC) and total N (TN) by combustion with an elemental analyzer (Elementar, Vario EL III, Elementar Analysen Systeme GmbH, Elementar Analysensysteme GmbH, Hanau, Germany).

2.4. Estimation of Fine Root Biomass

Soil cores were taken from the top 45 cm of soil in each subplot with polyvinyl chloride (PVC) tubes (with an inside diameter of 4.6 cm) in June 2016 to estimate the fine root biomass [45–47]. Soil cores were numbered and then transported to our laboratory at Nanjing Forestry University, where they were frozen at $-20\,^{\circ}\text{C}$ before analyzing. The cores were separated into three sections by depth (0–10, 10–25, and 25–40 cm), then carefully washed by wet sieving (0.5 mm) under gently flowing water to remove attached soil and dark-brown/black debris. Collected root samples were separated into coarse (>2 mm in diameter) and fine (\leq 2 mm in diameter, live and dead) roots, oven-dried at 65 $^{\circ}\text{C}$ for 48 h and weighed to calculate the live and dead fine root biomass.

2.5. Soil Fauna Sampling and Identification

Soil samples were collected from the 0–10, 10–25, and 25–40 cm soil layers using a soil coring with a diameter of 4 cm. Different layers represent organic horizon (O horizon, 0–10 cm), eluvial horizon (A horizon, 10–25 cm), and deposition horizon (B horizon, 25–40 cm), respectively [48,49]. In June 2016, four soil cores were collected from each 25×30 m subplot and pooled together as a replicate sample. The soil samples were immediately shipped back to our laboratory. The soil fauna was then collected from each soil sample using Tullgren extractors (Tullgren Funnel Unit, BURKARD, BURKARD SCIENTIFIC Ltd., Uxbridge, UK) [50–53]. All collected fauna samples were preserved in 75% ethanol and then sorted under a dissecting microscope (Nikon Eclipse E200, Nikon Instech Co., Ltd., Tokyo, Japan). Soil fauna was identified according to Yin [54,55].

2.6. Statistical Analysis

To determine the structure of the soil fauna in our study site, we chose density, the number of groups, and the Shannon-Wiener diversity index to describe the characteristics and composition of the soil fauna of each sample. The soil fauna density (*D*) was calculated as the following:

$$D = \frac{N}{V};\tag{1}$$

where D is the density of soil fauna, N is the total number of individuals, and V is the volume of the soil samples. The number of groups (G) was estimated as the number of groups of the fauna in the same order. The Shannon-Wiener diversity index (H) of each sample was estimated according to Whittaker [56]:

$$H = -\sum_{i=1}^{n} P_i \ln P_i \tag{2}$$

where H is the Shannon-Wiener diversity index of each sample, n is the number of groups of soil fauna, and P_i is the proportion of the number of individuals in the ith order to the total number of individuals, which could be calculated with the following equation:

$$P_{\rm i} = \frac{N_{\rm i}}{N} \tag{3}$$

where N_i is the number of individuals in the ith order.

Two-way ANOVA was used to examine the impact of N addition, soil layers, and their interactions on the fine root biomass (including live, dead, and total fine roots), density, number of groups, and biodiversity of phytophagous and total soil fauna. Repeated-measures ANOVA was performed to test the responses of litter mass, soil temperature, and moisture to N addition. One-way ANOVA

was used to examine the responses of the litter C:N ratio, soil TN, and SOC to N addition. Linear regression analyses were performed to examine the relationships between soil fauna and fine root biomass (live, dead, and total), SOC, TN, mean annual soil temperature and between moisture and the soil temperature in June. All statistical analyses were performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Microclimate, Litter C and N, Soil Samples, and Fine Root Biomass

Soil temperature and moisture showed remarkable seasonal variations. Soil temperature increased from January to August and then decreased from August to December (Table 1, Figure S1a) and soil moisture was low in summer and high in spring and fall (Table 1, Figure S1b). N addition did not affect the C:N ratio of the litter, SOC, TN, soil temperature or moisture (all p > 0.05, Tables 1 and 2). While N addition had no significant effect on live, dead, or total fine root biomass (all p > 0.05), root biomass in general decreased with increasing soil depth (all p < 0.01, Table 3, Figure S2).

Table 1. Results of repeated-measures ANOVA for seasonal responses of litter mass, soil temperature, and moisture to nitrogen (N) addition (0, 50, 100, 150, and 300 kg N ha⁻¹ year⁻¹). Statistical analysis was performed on log-transformed data. p levels are indicated beside the F values by the following: *: <0.05, **: <0.01, ***: <0.001, and no asterisks indicate p > 0.05. df represents degree of freedom.

Variables	N Addition (N)		7	Time (T)	$\overline{N \times T}$		
	df	F , р	df	Г , р	df	Г , р	
Litter mass	4	0.41	1	311.77 ***	4	0.58	
Soil temperature	4	0.49	15	1582.24 ***	60	0.29	
Soil moisture	4	0.67	15	233.25 ***	60	0.38	

Table 2. Results of one-way ANOVA for responses of litter carbon (C): N ratio, soil N (TN), and soil organic C (SOC) to N addition. Statistical analysis was performed on log-transformed data. p levels are indicated beside the F values by the following: *: <0.05, **: <0.01, ***: <0.001, and no asterisks indicate p > 0.05.

Variables	N Addition (N)		
	df	F, p	
Litter C:N ratio	4	1.68	
TN	4	0.96	
SOC	4	1.67	

Table 3. Results of two-way ANOVA for responses of the density (D), number of groups (G), Shannon-Wiener diversity index (H) of soil fauna and fine root biomass (live root biomass, dead root biomass, and total root biomass) to nitrogen (N) addition and soil depth (0–10, 10–25, and 25–40 cm). Statistical analysis was performed on log-transformed data. P levels are indicated beside the F values by the following: *: <0.05, **: <0.01, ***: <0.001, and no asterisks indicate P > 0.05.

Variables		N Add	N Addition (N)		Soil Depth (depth)		N × Depth	
		df	Г , р	df	Г , р	df	F, p	
	D _{total}	4	8.11 **	2	308.52 ***	8	2.77 *	
Total soil fauna	G_{total}	4	1.91	2	10.63 *	8	2.19	
	H_{total}	4	1.33	2	6.35	8	1.78	

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Variables		N Addition (N)		Soil Depth (depth)		N × Depth	
		df	Г , р	df	Г , р	df	Г , р
Phytophagous soil fauna	D_p	4	5.91 *	2	118.38 ***	8	1.75
	$G_p^{'}$	4	1.74	2	8.13 *	8	2.79*
	H_p	4	2.28	2	4.94	8	2.81
Fine root biomass	Live root biomass	4	1.84	2	13.42 ***	8	0.72
	Dead root biomass	4	2.54	2	8.9 **	8	1.39
	Total root biomass	4	1.64	2	17.32 ***	8	0.88

3.2. Soil Fauna Community as Affected by N Addition

In general, N addition and soil depth both played important roles in altering the density of the soil fauna. With increasing N addition rate, the density of the total soil fauna (D_{total}) significantly increased first, then declined to the levels of the controls (p < 0.01, Table 3, Figure 1a). In the 25–40 cm soil layer, for example, D_{total} increased from N_0 to N_3 and then declined from N_3 to N_4 (Figure 1a). In addition, D_{total} significantly decreased with increasing soil depth (p < 0.001, Table 3, Figure 1a). The interaction between N addition and soil depth had a significant impact on D_{total} (p < 0.05, Table 3). D_{total} increased from N_0 to N_1 and then declined with higher N addition (from N_1 to N_4) in the topsoil layer (0–10 cm). In the deep soil layers (10–25 and 25–40 cm), however, declines in D_{total} started at N_2 and N_3 , respectively, compared to the topsoil layer. The density of phytophagous soil fauna (D_p) showed similar responses to N addition along the soil profile (Table 3, Figure 1b).

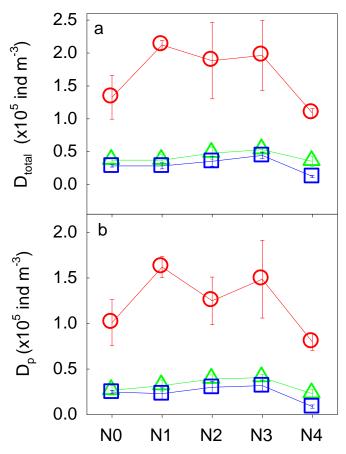


Figure 1. The density of total soil fauna (D_{total} , **a**) and phytophagous soil fauna (D_p , **b**) along the N addition gradient and the soil profile (red for 0–10 cm, green for 10–25 cm, and blue for 25–40 cm). N_0 , N_1 , N_2 , N_3 , N_4 indicate the gradient of N additions (N_0 : 0 kg N ha⁻¹ year⁻¹; N_1 : 50 kg N ha⁻¹ year⁻¹; N_2 : 100 kg N ha⁻¹ year⁻¹; N_3 : 150 kg N ha⁻¹ year⁻¹; N_4 : 300 kg N ha⁻¹ year⁻¹).

N addition had no significant impact on either the number of groups of total soil fauna (G_{total}) or the number of groups of phytophagous fauna (G_p) (all p > 0.05, Table 3, Figure 2). With increasing soil depth, in general, we found significant decreases in G_{total} and G_p (all p < 0.05, Table 3, Figure 2). Abrupt decreases in G_{total} and G_p at N_2 were observed in the topsoil layer (0–10 cm). We also found interactive effects between N addition and soil layer on G_p (p < 0.05, Table 3, Figure 2b). Along the soil profile, for instance, G_p reached its highest values at N_1 , N_2 , and N_3 . For the Shannon-Wiener diversity index of the soil fauna, neither N addition nor soil depth had significant effects on the diversity of total soil fauna (H_{total}) or the diversity of phytophagous soil fauna (H_p) (all p > 0.05, Table 3, Figure 3).

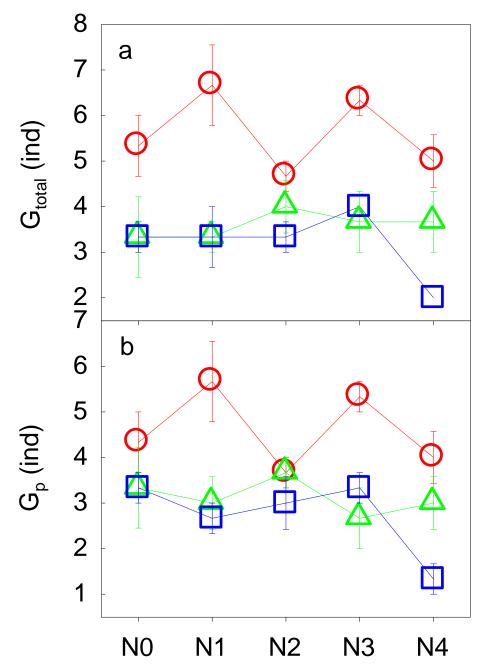


Figure 2. The number of groups of total soil fauna (G_{total} , \mathbf{a}) and phytophagous soil fauna (G_p , \mathbf{b}) along the N addition gradient and the soil profile. See Figure 1 for abbreviations.

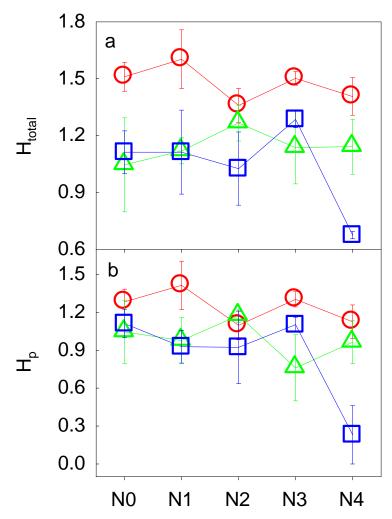


Figure 3. The Shannon-Wiener diversity index of total soil fauna (H_{total} , **a**) and phytophagous soil fauna (H_p , **b**) along the N addition gradient and the soil profile. See Figure 1 for abbreviations.

3.3. Fine Root Biomass Regulation of Soil Fauna Density

Variations in soil fauna densities (both D_p and D_{total}) were consistently regulated by fine root biomass (Table 4). We found that variations in D_p and D_{total} across N addition treatments and soil profile were positively correlated with the amount of live, dead, and total fine roots (all p < 0.01, Figures 4 and 5). The positive relationships remained even without the "outliers" — the very small values (both in fine root biomass on the X-axis as well as in the soil fauna density on the Y axis, all p < 0.01, Figures S3 and S4). Multifactor linear regression showed that the dominant factors controlling variations in D_p and D_{total} were the total and the dead fine root biomass across N addition treatments (all p < 0.001, Table 4).

Table 4. Results of multi-regression analysis of the density of phytophagous soil fauna (D_p) and total soil fauna (D_{total}) with fine root biomass (live, dead, and total), SOC, TN, mean annual soil temperature and moisture, and the soil temperature and moisture in June across N addition treatments. ***: p < 0.001.

Dependent	Model	Variables	Regression	r^2 and p
D _p	A-1 A-2	Total root Total root, dead root		0.48 *** 0.53 ***
D _{total}	B-1 B-2	Total root Total root, dead root	$D_{total} = 3.03 + 0.67 \text{ total root}$ $D_{total} = 3.20 + 0.58 \text{ total root} + 0.12 \text{ dead root}$	0.42 *** 0.49 ***

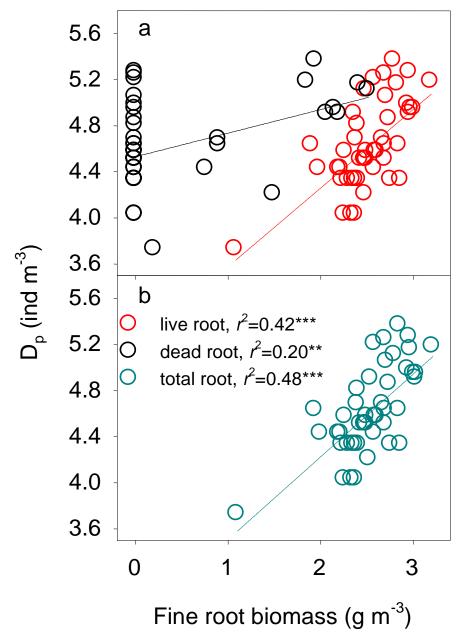


Figure 4. Log-log regulation of fine root biomass ((a) live and dead root biomass; (b) total root biomass) on the density of phytophagous soil fauna (D_p). **: p < 0.01, ***: p < 0.001.

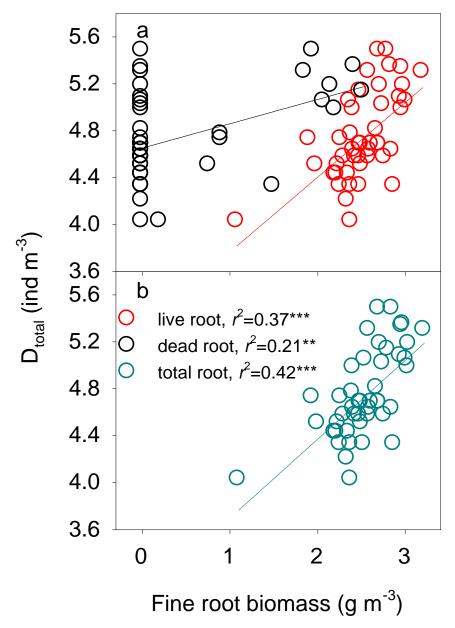


Figure 5. Log-log regulation of fine root biomass ((**a**) live and dead root biomass; (**b**) total root biomass) on the density of total soil fauna (D_{total}). **: p < 0.01, ***: p < 0.001.

4. Discussion

4.1. N-addition Impacts on Soil Fauna Density

Although N addition, up to a certain rate, could have positive impacts on soil fauna, high rates of N input appears to negatively affect the fauna community. The increases in D_{total} at low N addition rates are in line with previous findings, which show that N addition, to a certain extent, can be beneficial to soil fauna (e.g., [57–59]). The increases in D_{total} under N addition may have resulted from (1) increases in available N in the soil [60]; (2) increases in the food sources (soil bacteria and fungi) for the soil fauna [5,61]; (3) increases in rhizodeposition-C accessible to soil fauna [14,62]; and (4) more hospitable soil conditions for soil fauna (e.g., decreases in soil pH associated with N addition) [63]. Interestingly, D_{total} dramatically decreased from N_3 to N_4 , exhibiting a negative response to high N addition. Decreases in the density of soil fauna at high levels of N addition may be attributed to at least two reasons. First, excessive acidic substances resulted from N addition may negatively

affect soil fauna [64,65]. For instance, soil acidification [66] significantly restricts the number of soil nematodes and could thus decrease D_{total} [67,68]. Second, N may cause soil eutrophication due to the increase in nitrate (NO_3^-) and NH_4^+ concentrations in soil, and thereby has a negative influence on soil fauna [19,67]. Similar to the variations observed in D_{total} , D_p increased from N_0 to N_3 and decreased from N_3 to N_4 . The decline in D_p is consistent with previous studies, which found that N addition that increases NH_4^+ concentration significantly decreases D_p , as the NH_4^+ suppresses phytophagous soil fauna [13,67,69]. Additionally, we found that D_p positively responds to low N addition rates, which is similar to the trend observed for D_{total} , probably due to the large proportion (~75% across all treatments) of phytophagous soil fauna to total soil fauna in our study. Since few studies have reported any positive impacts of N addition on D_p , the mechanisms remain to be explored.

A threshold effect of N addition on soil fauna is usually widely found [19,70]. In our study, we detected a threshold in the effects of N addition on soil fauna at approximately 150 kg N ha $^{-1}$ year $^{-1}$ for both D_{total} and D_p . N addition within a certain concentration range enables competitive soil fauna to coexist with less competitive soil fauna, leading to an increase in density, while excessive N addition is a threat to the less competitive soil fauna, as it negatively alters the soil's physical and chemical properties [2]. When an ecosystem reaches N saturation, excessive exogenous N might increase leaching of some basic ions, such as calcium ion (Ca^{2+}) , aluminum ion (Al^{3+}) , and magnesium ion (Mg^{2+}) , from the system [24], consequently destroying the structure and function of the ecosystem, causing soil acidification and nutrient imbalances, breaking intraspecific competition dynamics, and eroding the system's resistance to external disturbances [71]. Differences in N thresholds among ecosystems probably result from a quantitative variation in the primary available N. N addition may increase the quality and quantity of organic matter in young, developing ecosystems while having a toxic effect on the soil fauna in old ecosystems that reached N saturation from N addition [12]. Thus, the threshold effects of N deposition in different ecosystems and their potential impacts on soil fauna are worthy of further investigation from a community structure perspective.

4.2. N-addition Impacts on the Number of Groups and Diversity of Soil Fauna

N addition has either positive or negative effects on the number of groups and Shannon-Wiener diversity index of soil fauna in various ecosystems (e.g., [58,59,72,73]). However, a non-significant impact of N addition on those variables has been widely reported (e.g., [16,17,19]), supporting our findings in this study. The lack of N addition effects may be explained as follows: (1) N addition did not significantly alter the proportion of soil fauna in each group to the total number. Statistically, we found that the number of soil fauna in each group increased evenly with N addition (Table S1, Figures S5 and S6); (2) litter, as one of the main food sources for soil fauna [26], had no significant response to N addition either in terms of quantity (litter mass) or quality (C:N ratio) (all p > 0.05, Tables 1 and 2); (3) N addition had no significant influence on soil microclimate (soil temperature and moisture) (all p > 0.05, Table 1). The abrupt decreases in G_{total} and G_p at N_2 may have been related to a decrease in food resources, e.g., fine root biomass, at N_2 . Additionally, the discrepancy in the effects of N addition on the number of groups and the diversity of soil fauna between our study and the previous studies may have resulted from the differences in soils, as our experimental site is located in an alkaline coastal area.

4.3. Increasing N-Addition Thresholds along Soil Profile

N addition did not affect the "surface aggregation" of soil fauna but increased the thresholds with increasing soil depth. The density and the number of groups of soil fauna usually decrease along soil profile [48,74], and this holds true under N addition in our study. Surface soil is more hospitable to soil fauna, as it always has a higher substrate content and air circulation rate [53,75]. Higher N addition thresholds for soil fauna may be attributed to the fact that topsoil could be more sensitive to environmental stress (e.g., N addition) than deeper soil layers [75]. Soil fauna move from the topsoil

downwards to the deeper soil layers when high amounts of N are added to escape the depression of high N addition in the topsoil [76].

4.4. Regulation of Fine Root Biomass on Soil Fauna

Responses of soil fauna to N addition at the site level may be influenced by many factors, such as soil temperature and moisture [77], pH value [78], and resource supply [15]. Based on the multifactor regression analyses, we found that fine root biomass was the dominant factor controlling variations in the density of soil fauna for both D_{total} and D_p . Fine roots and their exudates may positively influence soil biota through stimulated hyphal growth with increasing quantities of fine root biomass [79]. A growing body of evidence suggests that soil food webs rely heavily on "root C" because fine roots can be a source of readily available C for both soil microorganisms and phytophagous soil fauna [80]. Likewise, many studies have shown that phytophagous soil fauna are largely controlled by root-derived nutrition [30,81], just as aboveground herbivore populations are strongly linked to net primary production [82].

5. Conclusions

Our study in poplar plantations on the east coast of China suggests that N addition could have substantial effects on soil fauna along the soil profile. Across the N addition treatments, low N addition rates had positive effects on soil fauna densities, likely due to improved physical and chemical soil conditions. With increasing N addition rates, responses of soil fauna community returned to the control level. Soil fauna responded differently to N addition in different soil layers, which may largely relate to changes in the soil substrate content with N addition. Multifactor linear regressions showed that fine root biomass mediated N induced changes in the soil fauna density. The global N deposition rate is projected to continuously increase in the future [3,83], which will undoubtedly affect soil fauna and further the biogeochemical cycles. Therefore, it is imperative to explore impacts of N addition on soil fauna communities to understand the way an ecosystem functions under the global N deposition.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/10/2/122/s1, Figure S1: Variations in soil temperature (a) and moisture (b) from January to December along the nitrogen (N) addition gradient in 2016. N0, N1, N2, N3, N4 indicate different N additions (N0: 0 kg N ha⁻¹ year⁻¹; N1: 50 kg N ha⁻¹ year⁻¹; N2: 100 kg N ha⁻¹ year⁻¹; N3: 150 kg N ha⁻¹ year⁻¹; N4: 300 kg N ha⁻¹ year⁻¹), Figure S2: The live (a), dead (b), and total (c) fine root biomass along the N addition gradient and the soil profile (red for 0–10 cm, green for 10–25 cm, and blue for 25–40 cm). See Figure S1 for abbreviations, Figure S3: Regulation of fine root biomass (a: live and dead root biomass; b: total root biomass) on the density of phytophagous soil fauna (Dp), without the very small points (circled in the panels). **: p < 0.01, ***: p < 0.001, Figure S5: The proportion of each individual soil fauna over total soil fauna along the nitrogen (N) addition gradient and the soil profile. See Figure S1 for abbreviations, Figure S6: The proportions of macrofauna, mesofauna, and microfauna over total soil fauna along the nitrogen (N) addition gradient and the soil profile. See Figure S1 for abbreviations. Table S1: Results of two-way ANOVA for the responses of each individual soil fauna's proportion over total soil fauna to nitrogen (N) addition (0, 50, 100, 150, and 300 kg N ha⁻¹ year⁻¹) and soil depth (0–10, 10–25, and 25–40 cm). P levels are indicated beside the F values by the following: *: <0.05, **: <0.01, ***: <0.001, and no asterisks indicate p > 0.05.

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