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Soil Organic Carbon and pH Dominate the Effects of Nitrogen Addition on Soil Microarthropods in a Poplar Plantation in Coastal Eastern China

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Abstract: Soil biodiversity and fuction have been altered by the increasing levels of nitrogen as a result of fertilization and atmospheric deposition. Although soil microarthropods are a crucial component of soil biodiversity and play a key role in a diverse range of soil functions, our understanding of the mechanisms by which N addition affects them remains limited. Using a long-term nitrogen addition experiment (2012–2016) in poplar plantations (*Populus deltoides* L. CL'35') located along the coast of Yellow Sea Forest Park in northern Jiangsu, eastern China (32°52′ N and 120°49′ E), where the soil was entisols, we examined the response of soil microarthropods across three soil depths (0–15 cm, 15–25 cm, 25–40 cm) to five N input levels (0, 5, 10, 15, 30 g N m^{-2} $year^{-1}$) over four seasons. We found that the number of microarthropods per unit area initially grew and then dropped as more nitrogen was added to soils. Soil organic carbon (positive correlation, R^2 = 0.53) and pH (negative correlation, R^2 = 0.19) were the two dominant factors driving the effects of nitrogen addition on soil microarthropod densities at all soil depths. These results suggest that nitrogen input enhances the density of soil microarthropods via the increase in fresh organic matter input. However, the increase in organic matter may be offset by an indirect increase in acidity under high levels of N addition, providing one possible explanation for the reduced density of microarthropods in heavily fertilized soils.

Keywords: nitrogen addition; soil microarthropods; soil organic carbon; pH; poplar plantation; liner mixed model

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1. Introduction

Soil microarthropods contribute to carbon turnover and maintain soil fertility. Common soil microarthropods, such as free-living Acari and Collembola, decompose fresh organic matter and increase the amount of inorganic N in soils, which commensurately improves primary productivity [1–3].

Forest ecosystems, especially in the plantations of coastal eastern China, have experienced increased nitrogen (N) additions resulting from fertilization and atmosphere deposition [4–6]. N pollution is likely to continue as fertilization practices and fossil-fuel use increase in industrialized countries. This crisis is further exacerbated by land clearing and burning regimes in developing regions [7–9]. Elevated levels of N deposition have only a small effect on aboveground carbon (C) sequestration, but they impact underground C processes significantly [10–12]. An extraordinarily high N concentration can reduce the feeding activity and fecundity of microarthropods, which may lead to reduced density and individual death [7,13]. Given our understanding of the important role that microarthropods play in the turnover of organic N, it is likely that their reduced abundance in high-N soils may impact the carbon storage potential of these ecosystems. Despite this, the exact role that N addition plays in microarthropod communities in forest soils is still poorly understood due to the complexity of the interactions between C and N cycles.

In their review, Nijssen et al. concluded that the main effects of increased N deposition on microarthropods were indirect [14], by altering food supply and abiotic conditions [15]. Although these mechanisms were not understood, they did observe an influence of N-deposition on microarthropod communities. Ochoa-Hueso et al. conducted a study on the shrub ecosystem in the semi-arid region of the Mediterranean Sea, indicating that nitrogen addition reduced the number of small and medium-sized arthropods by 44%. The addition of nitrogen has a significant impact on the individual number of Acari. The abundance of Acari first increases and then decreases with the increase in nitrogen application concentration. The addition of nitrogen has a promoting effect on the abundance of Oribatida, and different species of Oribatida have different responses to nitrogen addition. Ochoa-Hueso et al. also observed that the addition of 50 kg N ha^{-1} $year^{-1}$ nitrogen is beneficial to the growth of Pauropoda [16].

There are many reasons why N may influence microarthropod communities. First, N deposition affects the food supply of soil microarthropods. Extra N input influences plant productivity, both above- and below-ground, which then alters soil carbon accumulation [16,17]. N addition also causes a shift in soil microbial communities by removing N limitation [18] and changing soil chemical properties [7,19], which is specifically harmful to some soil microarthropods, including many species of Prostigmata [20]. The addition of N can also cause soil acidification, which affects various soil ecological processes [21]. Importantly, the abundance of soil microarthropods may be reduced in alkaline soils [22,23]. In addition, N deposition has been shown to facilitate a buffered microclimate (levelled temperatures and greater humidity) mainly by increasing vegetation density [24–26]. The dominant factors influencing soil microarthropods may differ between different ecosystem types.

Soil microarthropods also exhibit temporal and spatial heterogeneity [27,28]. The seasonal dynamics of temperature and plant species composition regulate litter decomposition and soil microarthropod density [29–31]. Because of this, the effects of N addition on soil biological processes may be magnified during the growing season [32]. The food source of microarthropods is typically some form of vertically declining biomass of bacteria, fungi and saprophagous soil fauna. The changes that occur with soil depth may also lead to a decrease in predatory soil microarthropods [33]. Furthermore, the impact of N application is expected to weaken with increased soil depth [34]. Plantations are important contributors to global forest function and diversity, particularly in China. China has the largest area of poplar (*Populus*) plantation (more than 7×10^4 km^2) in the world, concentrated in a vast area along the coast [35,36]. The rapidly growing poplars have multiple commercial uses and can also be used to fix carbon dioxide to combat global climate change [37]. Previous research conducted in a costal agroecosystem illustrated that total SOC in topsoil increased by about 14% under the treatment of reduced tilliage with green manure when compared to the no-tillage treatment [38]. Moreover, the afforestation of poplar benefits saline–alkali coastal areas as it can tolerate high-salinity environmental conditions and enhances the aggregation and enrichment of soil organic carbon (SOC) [6].

The objectives of this study were to: (1) examine the effects of inorganic N addition on soil microarthropods in poplar plantations in coastal soils, and (2) evaluate any relationships between soil moisture content and pH (representing the abiotic conditions important to soil microarthropods), between SOC and soil microbial biomass carbon (SMBC) (representing the food supply of soil microarthropods), and the density of Acari and Collembola, the dominant orders of microarthropods in the study area, as shown in our previous research [23,39,40].

2. Materials and Methods

2.1. Site Description

The research site is a coastal area of Yellow Sea Forest Park in northern Jiangsu, eastern China (32°52′ N and 120°49′ E). It lies between the subtropical zone and warm temperate zone, experiencing a conventional monsoon climate with distinct seasons and concentrated rainfall. Temperatures reach a mean of 14.6 °C, and the area has approximately 1050 mm of rainfall each year. The total annual duration of solar radiation is 2200 h [41].

Soils were predominantly entisols and had been desalinated before the reseach area was converted into farmlands or forests. Originally, the soil was allunial soil. The soil is now a sandy loam with high porosity and compressibility, with alkaline pH and a conductivity of 2.68 ± 0.58 ds/m [6]. Farmland covers a total area of 3000 hm^2, over 80% of which is occupied with forests which takes up 2500 hm^2. The terrain is relatively flat, the stratum is mudstone interbedded with frequent siltstone, and the geological structure could be categorized as an overlying loose layer. The main species of the forest farm include *Populus deltoides* L., *Metasequoia glyptostroboides* Hu et Cheng, *Ginkgo biloba* L., *Cinnamomum camphora* (L.) Presl, *Robinia pseudoacacia* L. with understory as *Imperata cylindrica* (L.) Beauv., *Apocynum venetum* L., *Rosa multiflora* Thunb., etc.

2.2. Experimental Design

Treatments were conducted in a 12-year-old pure poplar plantation (Populus deltoids L. '35') with understory vegetation mainly comprising *Erigeron annuus*, *Artemisia argyi* and *Oplismenus undulatifolius*. The planting density of poplar trees was 333 stand ha^{-1}. The canopy coverage was 60% in the study area, and the average tree height was 21.2 m with a mean diameter at breast height (DBH) of 23.2 cm. N was applied six times in the liquid form of NH_4NO_3 throughout each growing season (approximately once per month from May to October). The amount of water added to the soil through this N application was equivalent to 1.2 mm rainfall, which is negligible.

2.3. Experimental Setup

We established three blocks in 2012, each 30 m × 190 m, spaced > 1 km. Within each block, we established five plots (25 m × 30 m), with 10 m spacing between plots. The N addition treatments were 0, 5, 10, 15, 30 g N m^{-2} $year^{-1}$. Soil samples were taken from the 0–15, 15–25 and 25–40 cm soil layer in each of the 15 plots in March, June, September and December 2016. Five soil cores (2.5 cm diameter) were randomly taken in each of the three soil layers in four sampling periods in all fifteen plots. All 5 cores were then homogenized into 1 composite sample, leading to a total of 180 observations.

2.4. Sample Measurements

2.4.1. Soil Microarthropods Identification

We extracted soil microarthropods from 100 g of soil (fresh weight) with modified Tullgren extractors [42]. The soil microarthropod density was calculated as the number found per 100 g dry soil. This collection strategy depended on the efficiency of the extraction technique. All extracted microarthropod samples were preserved in 75% ethanol before they were sorted under a dissecting microscope (LeicaMZ 125, Leica Microsystems, Wetzlar, Germany). Soil microarthropod community biodiversity was classified according to taxonomic group, e.g., Oribatid, Mesostigmatid and Prostigmata, Collembola and Hymenoptera [43]. Our results showed that the Collembola, Oribatida and Prostigmata account for 12.36%, 46.38% and 19.25% of soil fauna, respectively, which is the dominant group of soil fauna, accounting for 77.99% of the total. As a result, we only used data on the communities of Oribatida, Prostigmata and Collembola in this research.

2.4.2. Soil Analysis

To calculate soil moisture content, we baked 10 grams of the fresh soil samples in the oven at 105 °C for 12–24 h until achieving a constant weight. To determine SOC, we hydrolyzed the air-dried samples with HCL and then heated them at 60 °C to dry the samples, as described previously by Chen et al. and Marin et al. [44,45]. Total C and N were measured from the original air-dried samples with an elemental analyzer (Elementar Vario EL, Hanau, Germany). C, N elements in samples were burned, producing CO_2 and NO_2. The produced gas passed through a sensor that determines the elemental composition of the gas based on its adsorption spectra. Soil pH was determined using a glass electrode in a 1:2.5 soil: water solution (w/v). We used the fumigation–extraction method, which was first described by Vance et al. for soil microbial biomass carbon (SMBC) measured by TOC − VCPH + TNM − 1 (Shimazu Inc., Kyoto, Japan) [6,46]. The microbial cell contents were released into soil after chloroform fumigation, which would greatly increase the extractable carbon, nitrogen, phosphorus and sulfur in the soil. The contents of total carbon and nitrogen in the extractive solution from the soil were extracted by potassium bisulfate. Then, the the contents of microbial biomass carbon and nitrogen were calculated by the comparison of total carbon and nitrogen in both extractive solutions, with or without fumigation.

2.5. Statistical Analysis

To analyse the impacts of the addition to N to pH and SOC, and the abundance of the three microarthropod orders as a whole and individually across four sampling dates and three soil depths, the following linear mixed model was applied:

$$\mathrm{Y_{ijklm} = N_i + L_j + D_k + N_i \times L_j + N_i \times D_k + L_j \times D_k + N_i \times L_j \times D_k + \pi|B_l + \varepsilon_{m(ijkl)}} \quad (1)$$

where $\mathrm{Y_{ijklm}}$ is soil microarthropod density, SOC or pH; $\mathrm{N_i}$ (i = 0, 1, 2, 3, 4) is the level of N addition (0, 5, 10, 15, 30 g N m^{-2} $year^{-1}$); $\mathrm{L_j}$ (j = 1, 2, 3) is soil layer (0–15, 15–25, 25–40 cm); $\mathrm{D_k}$ (k = 1, 2, 3, 4) is sample date (March, June, September and December); $\pi|\mathrm{B_l}$ represents random plot effect (l = 1, 2, ... 9) nested in the three random blocks; and $\varepsilon_{\mathrm{m(ijkl)}}$ (m = 1, 2, 3) is sampling error. We conducted the linear mixed effect analysis using the restricted maximum likelihood estimation within the 'lme4' package [47].

To further study the mechanisms associated with changes in soil microarthropod density, we tested how SOC, soil pH, soil moisture and SMBC responded to N application rate, soil layer and sampling date using Equation 1. We then used Pearson correlation analysis, performed using the 'PerformanceAnalysis' package [48], to examine the association between soil microarthropod density and these variables. All analyses were performed using R Statistical Software [49].

3. Results

The density of soil microarthropods significantly varied with N application rate, soil layer and sampling season (Table 1, Figure 1). Microarthropod density increased with increases in N application up to a rate of 15 g N m^{-2} $year^{-1}$. Beyond that, the addition of N decreased soil microarthropod density (Figure 1A). Microarthropod density decreased sharply from the topsoil to deep soil layers (Figure 1B). Across the four sampling dates, microarthropod density increased from March to September and then decreased in December (Figure 1C). Oribatida were most common, followed by Prostigmata and Collembola. The response to N application rate, soil layer and sampling date was similar in each group (Figure 1).

The effects of N application on soil microarthropod density was dependent on soil depth and sampling season, as we identified a significant interaction among the three factors (Table 1). In the topsoil, microarthropod density was insensitive or increased with low N application rates and declined with high N application rates across all sampling dates, although we identified that the point of change in N rate was dependent on the sampling date (Figure 2). In both March and September, topsoil microarthropod density peaked at an N application rate of 15 g N m^{-2} $year^{-1}$, while it peaked at 10 g N m^{-2} $year^{-1}$ in June and 15 g N m^{-2} $year^{-1}$ in December. Moreover, the seasonal patterns in the responses to N application rate differed among the three soil layers (Figure 2).

Table 1. The effects of nitrogen application rate (N), soil layer (L), sampling date (D) and their interactions on soil microarthropod density. The linear mixed-effects model used the Kenward–Roger method as the denominator of degrees of freedom.

Source	df	Sum Squares ($\times 10^3$)	F	*p*
N	4, 8	**12.4**	**8.3**	**0.006**
L	2, 110	**144.7**	**194.0**	**<0.001**
D	3, 110	**26.8**	**24.0**	**<0.001**
N × L	8, 110	1.4	0.5	0.877
N × D	12, 110	**8.5**	**1.9**	**0.042**
D × L	6, 110	**10.8**	**4.8**	**<0.001**
N × D × L	24, 110	4.8	0.5	0.958

Bold font indicates statistical significance (α = 0.05). The columns provide the degree of freedom (df), the sum squares, F and *p* values.

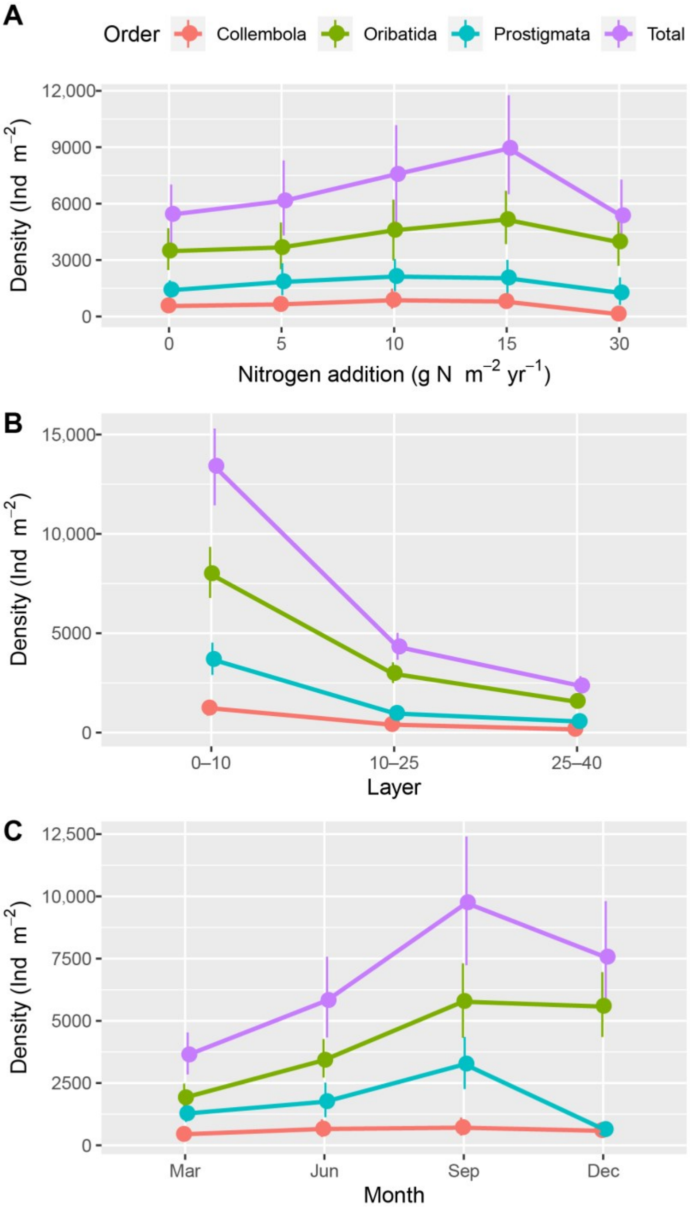

Figure 1. The response of soil microarthropod density to nitrogen application rate (**A**) at three soil depths (**B**) over four sampling dates (**C**). Values are means with bootstrapped 95% confidence intervals (CI). Differences are significant at α = 0.05 when the CIs do not overlap with the means of other groups.

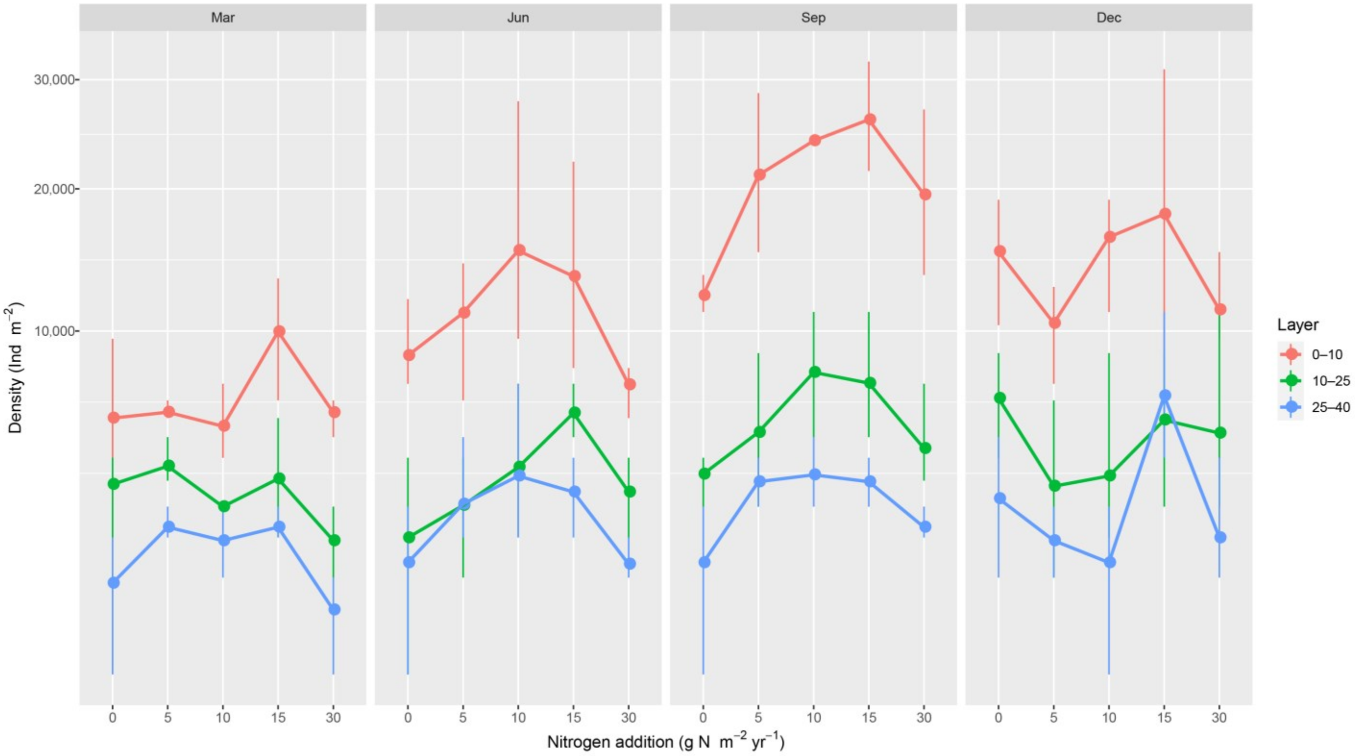

Figure 2. Soil fauna density in response to nitrogen application rate by soil layers and sampling dates. Values are means with bootstrapped 95% confidence intervals (CI). Differences are significant at $\alpha = 0.05$ when the CIs do not overlap with the means of other groups.

Soil organic carbon and soil pH showed a significant response to N application rate, soil depth and sampling date, with multiple significant interaction terms (Table 2). N application accounted for the majority of the variation in SOC and pH, but did not affect soil's relative moisture content or soil's microbial biomass carbon (Table 2). Soil organic carbon increased with N application rate, decreased with soil depth, and was higher in September and December than in March and June, whereas soil pH showed contrasting responses (Figure 3).

Table 2. The effects of nitrogen application rate (N), soil layer (L), sampling date (D) and their interactions on soil organic carbon (SOC), pH, humidity and microbial biomass carbon (SMBC). The linear mixed-effects model used the Kenward–Roger method as the denominator of degrees of freedom.

Effects	SOC			pH			Humidity			SMBC		
	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS ($\times 10^3$)	F	*p*
N	**316.0**	**83.0**	**<0.001**	**0.7**	**33.9**	**<0.001**	0.006	1.3	0.350	20.3	0.3	0.866
L	**2656.7**	**1396.3**	**<0.001**	**3.3**	**341.9**	**<0.001**	**0.1**	**59.2**	**<0.001**	**390.6**	**11.7**	**<0.001**
D	**242.5**	**85.0**	**<0.001**	**6.7**	**460.6**	**<0.001**	**0.1**	**33.1**	**<0.001**	**6936.6**	**139.0**	**<0.001**
N × L	**38.6**	**5.1**	**<0.001**	0.1	1.3	0.236	0.007	0.8	0.645	78.2	0.6	0.786
N × D	**42.0**	**3.7**	**<0.001**	**0.4**	**6.2**	**<0.001**	0.01	1.1	0.403	214.1	1.1	0.390
D × L	**38.8**	**6.8**	**<0.001**	**0.1**	**4.8**	**<0.001**	**0.03**	**4.5**	**<0.001**	**244.1**	**2.4**	**0.029**
N × D × L	**81.5**	**3.6**	**<0.001**	**0.3**	**2.4**	**0.001**	0.03	1.0	0.425	334.9	0.8	0.680

Bold font indicates statistical significance ($\alpha = 0.05$). The columns provide the sum squares (SS), F and *p* values.

Pearson correlation analysis showed that soil microarthropod density was positively related to SOC and negatively related to soil pH (Figure 4). Soil moisture was positively related to SOC but negatively related to soil pH. Soil microarthropod density was not significantly related to soil microbial biomass carbon, although microbial soil carbon was positively correlated with soil pH.

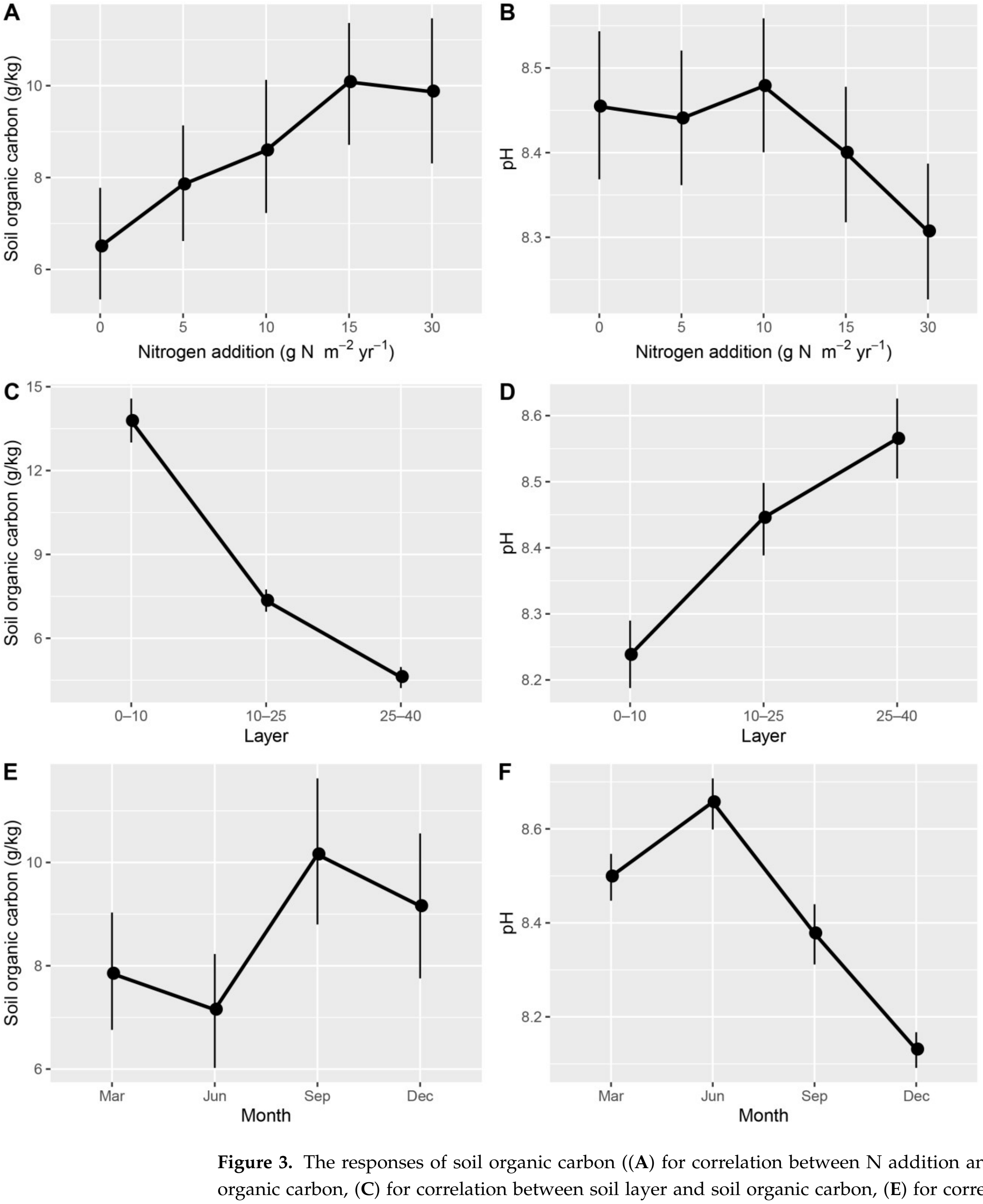

Figure 3. The responses of soil organic carbon ((**A**) for correlation between N addition and soil organic carbon, (**C**) for correlation between soil layer and soil organic carbon, (**E**) for correlation between sampling date and soil organic carbon) and pH ((**B**) for correlation between N addition and soil pH, (**D**) for correlation between soil layer and soil pH, (**F**) for correlation between sampling date and soil pH) to nitrogen application rate in three soil depths over four sampling dates. Values are means with bootstrapped 95% confidence intervals (CI). Differences are significant at $\alpha = 0.05$ when the CIs do not overlap with the means of other groups.

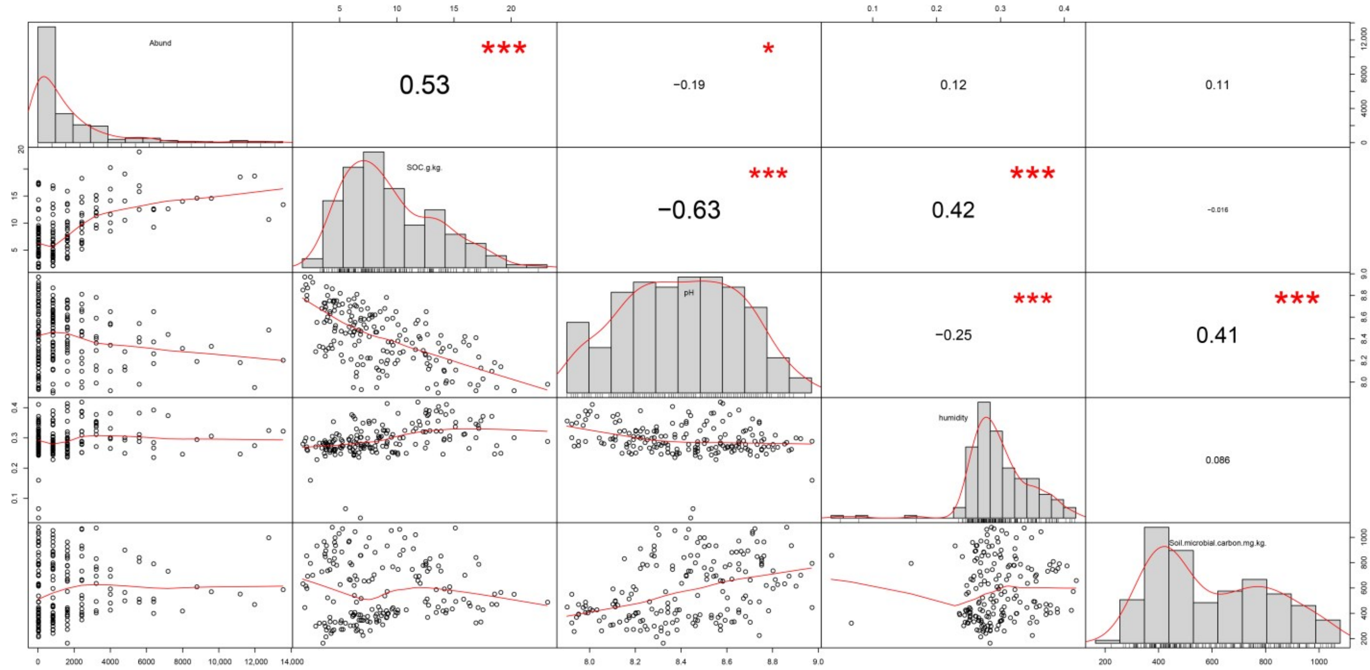

Figure 4. Pearson correlations between soil microarthropod density (Abund), soil organic carbon (SOC), soil pH, soil moisture (humidity) and soil microbial biomass carbon (SMBC). Below the diagonal are the bivariate scatter plots with a smooth line. Above the diagonal, the correlation coefficients and the significance level (* $p < 0.05$, *** $p < 0.001$) are shown. Units associated with variables are shown in Figures 1–3.

4. Discussion

We found that the density of soil microarthropods increased at low N application rates. Soil microarthropod communities are expected to be affected by N-driven ecosystem changes [50]. The addition of N was shown to enhance microarthropod density at first, due to the additional litter input and improved litter quality [25]. Soil microarthropod density was shown to vary in its response to N addition, depending on the duration of the experiment and the intensity of the N addition. In the short term (addition for fewer than 3 years), the impacts of N addition were varied, possibly leading to positive linear increases in soil microarthropod biomass and density or the lack of any relationship [23,51,52]. However, our data showed that soil microarthropod density decreased after only five years of heavy N addition (30 g N m^{-2} $year^{-1}$). This finding suggests that, under high-N conditions, the decrease in soil pH may affect soil's physical and chemical properties, potentially leading to a change in the osmotic potential of a number of soil ions [53], which may create an environment that is toxic to soil microarthropods [54,55]. The decrease in soil pH may also restrict predation and increase the incidence of epidermal burns on microarthropods. We observed that the moderate addition of nitrogen is beneficial for the growth of Oribatida. The density of Oribatida first increases and then decreases with the increase in nitrogen concentration, which is consistent with the trend of changes in the total density of soil fauna (Figure 1). Origami mites can serve as indicator organisms to reflect the impact of nitrogen addition on soil animals.

We found a strong effect of soil layer on soil microarthropod density. Soil depth stratification was observed, likely due to the soil compaction and lower biological activity in the lower layer [56]. Soil animals have previously been reported to gradually decrease in number and diversity in deep soils [57]. Correspondingly, we observed that soil fauna living in the 0–10, 10–25, 25–40 cm layer account for 60.71%, 24.18% and 15.12% of the total, respectively. This may be related to the decrease in root biomass, soil organic matter, temperature and water at these lower depths [58–60]. Our analysis provides direct evidence that SOC decreased while soil pH increased with depth, and we also observed that soil microarthropod density was strongly related to both soil organic matter and soil pH, although we acknowledge that the correlation between pH and soil microarthropods density was weaker than the influence of SOC (Figure 4). We observed that both SOC and soil microarthopod density were extremely variable.

Unsurprisingly, we found strong seasonal variations in soil microarthropod density. In winter, the change in soil microarthropods with the increasing amounts of nitrogen application was not as obvious as in other seasons (Figure 2), likely due to the increase in environmental stress, such as the lower temperature, precipitation [61] and the loss of food availability [62].

As Nijssen et al. reviewed, microarthropods are most affected by how an increased N deposition alters their environmental stressors and habitat suitability, including changes in fresh organic matter input and competitive predator–prey relationships [14]. Our results show that SOC and pH were the two most dominant factors driving soil microarthropod density with N addition. It has been reported that short-term N addition enhances SOC via extra litter input. Nitrogen input can promote plant growth and facilitate the net accumulation of plant biomass, thereby increasing the input of SOC through plant litter. This is because nitrogen is an important nutrient, which is necessary to produce the chlorophyll and enzymes responsible for photosynthesis. Because of this, nitrogen input directly impacts photosynthesis and, thus, the plant's carbon sequestration ability [19,63]. In our study, we found that SOC reached its peak with the addition of 15 g N m^{-2} $year^{-1}$, but a further rise in N led to either no increase or a slight decline in SOC. This finding corroborates that long-term and excessive N addition decreases SOC by changing the biomass allocation of plants [64]. The three types of soil fauna involved in this research were as follows: the Oribatida is saprophagous; most of the Prostigmata feed on microorganisms; there are both plant-eating, saprophagous and fungivorous soil microarthropods in the order Collembola. The increase in vegetation biomass and SOC caused by the addition of nitrogen, as well as the increase in soil microbial biomass caused by increased organic matter input, increased the food source for these three types of soil microarthropods, thus increasing their density.

Our results showed that N-induced acidification reduced soil microarthropod density, mitigating the positive effects of N-induced increases in SOC. However, soil was alkaline in our plantations, and even under the highest level of N addition, the soil was still alkaline (Figure 3). Because of this buffered system, the direct effect of reduced pH on soil microarthropods in our sites may be minimal. Instead, we suggest that the effect of N addition on pH could be mediated by two mechanisms: First, when soils are saturated with N, the excess NO_3^- then induces the leachate of Ca^{2+}, Mg^{2+} and other base cations, reducing the pH of the soil and increasing the flux of toxic cations such as Al^{3+}, Mn^{2+} and Rb^{+} [65]. Secondly, the additional N input could lead to an increase in free acidic soil solution due to the enhanced decomposition of litter [66], which was supported by the correlation analysis in our results (Figure 4). These can be taken up by soil microarthropods directly, but they are toxic [67].

Soil moisture was positively correlated with SOC and negatively correlated with pH in our study, although no significant correlation between soil moisture and microarthropod density was detected. N-addition-induced increases in SOC may enhance the increase in soil moisture, mainly via changing the physical structure of the soil [68]. In turn, this wetter soil could provide a more suitable habitat for soil biota [69]. We suggest that, given time, wetter soils may enhance soil microarthopod density, although this was not observed over the course of this experiment. The effects of soil moisture on pH depended on the initial pH [70]. In our experimental site, soil pH was alkaline (>8.38 ± 0.26). Leaching rates are higher in moist soils, leading to a decrease in pH and soil neutralization [71], as shown in our results.

5. Conclusions

In summary, we found that the densities of soil microarthropods (Oribatid, Prostigmata and Collembola) in poplar plantations in coastal soil first increased and then decreased with the addition of inorganic N. The dominant driving forces for these changes were SOC and pH, although SOC played a much more important role. The results indicated that the increased input of fresh organic matter, which was mainly caused by the increase in plant litter, dominated the effect of N addition on soil microarthropods in these young and sandy plantation soils.

Author Contributions: Z.G., H.X., Y.P. and H.R. designed the project. Z.G. and H.R. provided the funding. Z.G., Y.P. and L.M. performed the bioinformatics and statistical analysis. H.X. collected the samples and conducted the laboratory analysis. Z.G. wrote the original draft of the paper. S.P., Y.P. and H.R. revised the manuscript and contributed to the conception. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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