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Long-Term Nitrogen Addition Exerts Minor Effects on Microbial Community but Alters Sensitive Microbial Species in a Subtropical Natural Forest

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Abstract: Increasing nitrogen (N) deposition profoundly affects nutrient cycling in soil, thereby influencing forest ecosystem productivity and function. Soil microorganisms are integral in driving nutrient turnover; the changes in microbial communities in response to N deposition and the associated soil nutrient availability, especially of limited nutrients, are far from clear. To explore the changes in soil bacterial and fungal communities and their key environmental drivers under N deposition, we conducted a multilevel field N addition experiment in a Castanopsis carlesii natural forest. Soil properties and bacterial and fungal communities were investigated. There were no significant changes in alpha diversities (presented as Chao1 and Shannon's indexes) and beta diversities of bacteria and fungi among the three treatments. Consistently, the relative abundances of dominant bacterial phyla (i.e., *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, and *Planctomycetes*) and fungal phyla (i.e., *Basidiomycota*, *Ascomycota*, and *Rozellomycota*) did not change following N addition. These results suggest that N deposition did not alter microbial community diversity and structure. In addition, the results of the Mantel test showed that soil pH, NO_3^--N, dissolved organic N (DON), and total phosphorus (TP) predominantly influenced the community diversity and structure in bacteria, but not in fungi. Meanwhile, the relative abundance of some sensitive microbial genera, such as *Bryobacter*, *Bradyrhizobium*, *Sorangium*, and *Archaeorhizomyces*, were significantly decreased. These results indicate a decreased microbial ability for N fixation and P mobilization induced by N deposition. Moreover, there were significant relationships between *Bryobacter*, *Bradyrhizobium*, and *Archaeorhizomyces* and NO_3^--N and available P (AP), suggesting that the responses of sensitive microbial groups to N deposition likely depend on the changes in available nutrients in soil, especially limited N or P. Collectively, 6 years of N addition had no significant influence on microbial communities, but some sensitive microbial groups were associated with N or P turnover. This finding emphasizes the critical roles of sensitive microbial species in meditating limited nutrient cycling in soil under climate change.

Keywords: microbial community structure; alpha diversity; species richness; available P; high-throughput sequencing

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1. Introduction

Nitrogen (N) deposition, due to the increased utilization of fossil fuels and N fertilizers, has been dramatically increasing globally [1]. The elevated N input has substantially altered the availability of soil nutrients, further influencing terrestrial ecosystem stability and function [2–4]. Soil microorganisms, with an irreplaceable role in driving soil nutrient cycling, are susceptible to environmental changes such as soil acidification and the imbalanced ratio of N to phosphorus (P) induced by N deposition [5–7]. Therefore, clarifying the response of soil microorganisms to N deposition, and its underlying mechanism, is

imperative for a future understanding of the microbial-driven nutrient cycle under ongoing N deposition.

It is generally acknowledged that the alteration of nutrient turnover mediated by microbes is primarily associated with changes in microbial community diversity, composition, and structure [8–10]. Mounting studies have investigated the influences of N application on soil microbial communities in various ecosystems, and the results were inconsistent [9,11–14]. In general, N deposition exerts a negative effect on microbial biomass and diversity, and such an effect was increased with the rate and duration of experimental N addition [15,16]. Nevertheless, several studies have reported no significant changes in microbial diversity or structure in response to N addition, regardless of the levels of it [17]. These contrasting findings indicate the high uncertainty of microbial communities encountering changed soil conditions. Narrowing down this variation requires further study. Furthermore, the sensitive groups of microorganisms have attracted great attention, given their rapid response to environmental changes and notable contribution to the improvement of limited nutrients in soil, particularly N or P [18,19]. However, which sensitive microbial groups respond to N deposition, how they do so, and what are their relationships with the limited nutrients in subtropical forest soil remains unclear.

N deposition affects soil microbial communities mainly by altering soil properties such as pH and nutrient availability [20–22]. Additional N input reduces soil pH, causing acidification and associated toxicity, consequently influencing microbial diversity and composition [20,23,24]. Fungi are more tolerant to the stress of low pH than bacteria [25], resulting in different responses of bacterial and fungal communities to N addition, with more sensitivity in bacteria than fungi [26]. In addition, changes in soil nutrients induced by N addition also affect the microbial community [27]. For instance, N addition enhances N availability in N-limited soil, thereby facilitating eutrophic microbe growth but decreasing the relative abundances of oligotrophic microorganisms [28]. In P-limited soils, N deposition might intensify the competition of plants and microbes for P, consequently increasing the abundance of P-mobilization microbes, and ultimately altering the microbial community [29]. However, the predominant factor in regulating the responses of the microbial community to N deposition in subtropical forest soil is far from clear.

In this study, we conducted a multilevel field N addition experiment in a Castanopsis carlesii natural forest. A previous study reported that N addition stimulated plant growth and induced increases in fine root biomass [30]. We aimed to explore the changes in soil bacterial and fungal communities, the relative abundances of sensitive microbial groups, and their key environmental drivers following 6 years of experimental N addition. We hypothesized that (i) N addition alters bacterial communities but not fungal communities; (ii) N addition decreases the relative abundances of the sensitive microbial groups that are involved in N turnover; and (iii) the availability of soil nutrients such as N and P act as a dominant driver for the responses of microbial communities to N application.

2. Materials and Methods

2.1. Site Description

The study site is located in Sanming City, Fujian Province, Southeast China (26°11′ N, 117°28′ E). This region is characterized by a subtropical monsoon climate, with mean annual temperature, mean annual precipitation, and relative humidity of 19.4 °C, 1700 mm, and 79%, respectively. The annual frost-free period is about 300 days. The soil is an oxisol formed from sandstone and equivalent to an oxisol in USDA soil taxonomy. Castanopsis carlesii is the dominant canopy species in this subtropical natural forest ecosystem.

In November 2012, a permanent plot of simulated N deposition was established in a natural forest of Castanopsis carlesii in the Castanopsis kawakamii Nature Reserve. Three treatments, including control without N addition (CT), low N addition (LN, 40 kg hm^{-2} a^{-1}), and high N addition (HN, 80 kg hm^{-2} a^{-1}), as well as four replications for each treatment, were set. The size of each plot was 20 m × 20 m, and a 10 m buffer zone was set up to prevent mutual interference. N application started in November 2012; 20 L of NH_4NO_3

solution was evenly sprayed to each plot with LN (381 g NH_4NO_3) and HN (762 g NH_4NO_3) treatments, and the same amount of deionized water was applied for CT at the beginning of each month.

2.2. Soil Sample Collection

Sampling was completed in April 2018. After removing the litter layer, soil samples were taken at 0–10 cm and 10–20 cm soil layers, and five soil cores (diameter of corer was 5 cm) were randomly sampled within each plot. Samples from the same layer in each plot were mixed into a sample. A total of 24 samples (3 treatments × 2 soil layers × 4 replicates) were brought back to the laboratory. After picking out the stones and roots, each fresh sample was sieved through a 2 mm sieve and divided into two subsamples: one was air-dried for determination of physical and chemical properties, and another was refrigerated at 4 °C for analysis of high-throughput sequencing and NH_4^+-N and NO_3^--N.

2.3. Measurement of Soil Properties

Soil pH was measured with a glass electrode pH meter (STARTER300; OHAUS, Parsippany, NJ, USA) at a water-to-soil ratio of 2.5:1; soil organic carbon (SOC) and total N (TN) were measured with an elemental analyzer (Elementarvario EL III; Langenselbod, Germany). The contents of NH_4^+-N and NO_3^--N were determined by a continuous flow analyzer (Skalar san++; Skalar, The Netherlands). A total of 5 g of fresh soil was weighed into 20 mL of deionized water, shaken and centrifuged, then filtered through a 0.45 μm filter membrane, and the content of dissolved organic carbon (DOC) was determined using a total organic C analyzer (TOC-VCPH/CPN, Shimadezu, Japan) and the content of dissolved organic N (DON) was determined using a continuous flow analyzer (Skalar san++; Skalar, Breda, The Netherlands).

Total P (TP) and available P (AP) were extracted in 4:1 $H_2SO_4/HClO_4$ and 0.5 mol·L^{-1} $NaHCO_3$, respectively; then, the P content was determined using a continuous flow analyzer (Skalar san++; Skalar, Breda, The Netherlands).

2.4. DNA Extraction and Sequencing

U.S. MOBIO Soil Microbial DNA Powerful Extraction Kit (PowerSoil® DNA Isolation Kit, MoBio Laboratories, Carlsbad, CA, USA) was used to extract DNA from soil microorganisms following the manufacturer's recommendations. Briefly, a total of 0.25 g of fresh soil was weighed into PowerBead Tubes, reagents were added, then it was placed on a vortexer equipped with a 24-well vortex adapter and vortexed for 15–20 min. Then, C1 solution was added, the sample briefly vortexed. Soil humus was removed with the reagents of C2 solution provided in the kit. All steps after this were conducted as in the manual provided with the Mobio Powersoil kit. Samples were eluted in 100 μL solution C6 and stored at −20 °C in a refrigerator. A total of 1 μL extracted DNA samples was taken to detect the concentration by ultra-microspectrophotometer (Therme NanoDrop 2000, Massachusetts, USA), and 3 μL of DNA samples was mixed with 1 μL of 6 × loading buffer; then, the purity was checked by 1% agarose gel electrophoresis. The quality-checked samples were sent to the sequencing company (Majorbio, Shanghai, China) in ice packs and insulated boxes for subsequent experiments.

2.5. Data Analysis

To evaluate the changes in bacterial and fungal communities in response to N addition, the Chao1 and Shannon indexes (OTUs) were used to present the microbial species richness and diversity, respectively. Nonmetric multidimensional scaling (NMDS) analysis of bacteria and fungi was performed using UniFrac distance based on OTU levels. One-way ANOVA was used to test the influences of N addition on soil properties, microbial diversity, and the relative abundances of dominant microbial species at phylum and genus levels. All statistical analyses were performed using SPSS 21.0 software. $p < 0.05$ was considered to indicate a significant difference among treatments. The Mantel test was used to evaluate the

effects of different environmental factors on the bacteria and fungi communities' diversities and structures. Pearson correlation analysis was used to examine the correlations between microbial species and environmental factors. All plots were constructed using Origin 9.1 and R 4.2.1 software.

3. Results

3.1. Soil Properties

N application significantly increased the concentrations of NO_3^--N in both the 0–10 and 10–20 cm soil layers ($p < 0.01$) but significantly decreased the AP content in the 0–10 cm soil layer ($p < 0.01$) (Table 1). There were no significant changes in DOC, DON, NH_4^+-N, TP, SOC, and TN in either soil layer among the three treatments ($p > 0.05$) (Table 1).

Table 1. Effects of N addition on soil properties in subtropical natural forest soil. DOC, dissolved organic carbon; DON, dissolved organic nitrogen; AP, available phosphorus; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus. CT, control; LN, low nitrogen addition; HN, high nitrogen addition. Values indicate mean ± SE ($n = 4$). Different letters indicate significant difference at $\alpha = 0.05$.

Soil Properties	0–10 cm Layer			10–20 cm Layer		
	CT	LN	HN	CT	LN	HN
pH	4.03 ± 0.05 a	3.88 ± 0.18 a	3.88 ± 0.01 a	4.14 ± 0.08 a	4.10 ± 0.11 a	4.08 ± 0.05 a
NH_4^+ (mg kg^{-1})	24.15 ± 3.57 a	27.79 ± 8.82 a	23.21 ± 7.39 a	18.50 ± 3.41 a	16.62 ± 4.63 a	24.84 ± 9.91 a
NO_3^- (mg kg^{-1})	1.67 ± 0.46 c	5.70 ± 1.09 b	7.91 ± 1.69 a	1.22 ± 0.20 c	3.32 ± 0.50 b	6.91 ± 0.61 a
DOC (mg kg^{-1})	92.04 ± 34.23 a	96.76 ± 25.84 a	75.68 ± 24.50 a	42.22 ± 7.84 a	31.34 ± 12.39 a	33.93 ± 8.79 a
DON (mg kg^{-1})	18.48 ± 4.86 a	15.68 ± 4.41 a	15.03 ± 6.63 a	4.11 ± 2.93 a	3.08 ± 8.03 a	12.15 ± 4.76 a
AP (mg kg^{-1})	6.07 ± 1.05 a	3.61 ± 0.75 b	2.43 ± 0.41 b	2.43 ± 0.98 a	2.66 ± 0.77 a	2.26 ± 0.77 a
SOC (g kg^{-1})	33.46 ± 7.70 a	35.41 ± 5.25 a	31.37 ± 4.14 a	15.26 ± 1.54 a	15.38 ± 4.72 a	14.37 ± 2.67 a
TN (g kg^{-1})	2.36 ± 0.31 a	2.41 ± 0.51 a	2.34 ± 0.40 a	1.09 ± 0.07 a	1.06 ± 0.18 a	1.07 ± 0.13 a
TP (g kg^{-1})	0.22 ± 0.02 a	0.20 ± 0.02 a	0.19 ± 0.02 a	0.15 ± 0.02 a	0.14 ± 0.02 a	0.14 ± 0.02 a

3.2. Microbial Community Diversity

The bacterial and fungal species richness and Shannon's diversity indexes ranged from 1 424–1 677 to 440.25–680.28, and from 5.48–5.74 to 1.25–3.35, respectively (Table 2). Compared with the CT, no statistical changes in Chao 1 and Shannon's indexes in bacteria and fungi were observed under LN and HN treatments in either soil layer ($p > 0.05$, Table 2). However, the values of Chao 1 and Shannon's indexes in bacteria and fungi in LN treatment were higher than those in HN treatment in the 0–10 cm soil layer ($p > 0.05$, Table 2).

Table 2. Effects of N addition on the alpha diversities of soil bacteria and fungi in subtropical natural forest soil. CT, control; LN, low nitrogen addition; HN, high nitrogen addition. Values indicate mean ± SE ($n = 4$). Different letters indicate significant difference at $\alpha = 0.05$.

Soil Layer	Treatment	Bacteria		Fungi	
		Chao 1	Shannon	Chao 1	Shannon
0–10 cm	CT	1601.16 ± 149.40 a	5.71 ± 0.12 ab	519.47 ± 146.66 ab	2.06 ± 0.72 ab
	LN	1598.81 ± 139.02 a	5.74 ± 0.14 a	680.28 ± 42.48 a	3.07 ± 0.65 a
	HN	1424.23 ± 30.98 a	5.51 ± 0.09 b	440.25 ± 123.83 b	1.25 ± 0.53 b
10–20 cm	CT	1677.31 ± 80.70 a	5.51 ± 0.22 a	594.92 ± 108.37 a	2.48 ± 0.82 a
	LN	1605.78 ± 84.45 ab	5.50 ± 0.12 a	514.40 ± 166.28 a	2.27 ± 0.85 a
	HN	1528.32 ± 36.59 b	5.48 ± 0.22 a	605.96 ± 76.50 a	3.35 ± 0.84 a

3.3. Microbial Community Composition and Structure

Proteobacteria, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, and *Planctomycetes* acted as the dominant bacterial phyla (average relative abundance > 5%) and accounted for 92.33% of the total sequences (Figure 1). *Basidiomycota*, *Ascomycota*, and *Rozellomycota* were the dominant fungal phyla (Figure 1). In addition, N application had no significant influence

on the bacterial and fungal community composition and structure at the phylum level in either soil layer ($p > 0.05$, Figures 1 and 2).

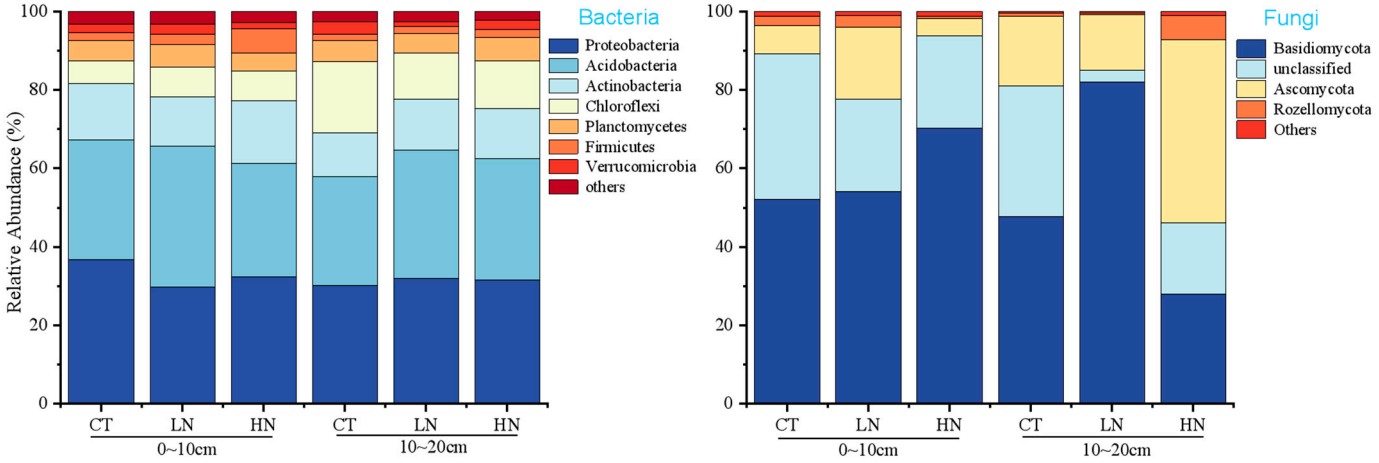

Figure 1. Effects of N deposition on the relative abundances of bacterial and fungal species at the level of phylum in subtropical natural forest soil. CT, control; LN, low nitrogen addition; HN, high nitrogen addition.

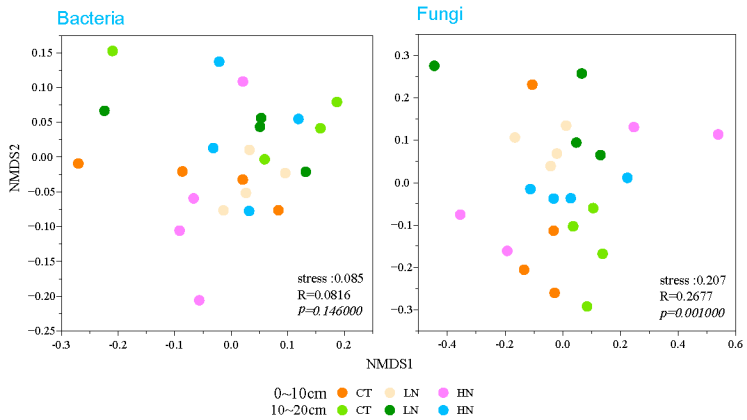

Figure 2. The unweighted nonmetric multidimensional scaling (NMDS) analysis of UniFrac community distances in a subtropical *Castanopsis* forest under N addition. CT, control; LN, low nitrogen addition; HN, high nitrogen addition.

3.4. Sensitive Microbial Groups

We further detected the changes in sensitive microbial groups at the genus level in response to N application (Figure 3). For bacteria, N application significantly reduced the relative abundance of *Bryobacter*, *Bradyrhizobium*, *Sorangium*, and *unclassified_p_Proteobacteria* in the 0–10 cm soil layer ($p < 0.05$, Figure 3). In the 10–20 cm soil layer, N application significantly reduced the relative abundance of *Rhizobium*, *Rudaea*, *unclassified_p_Chloroflexi*, and *Bradyrhizobium*, but increased *Coxiella* by 55.56% ($p < 0.05$, Figure 3). For fungi in the 0–10 cm soil layer, N application induced a significant decrease in the relative abundance of *Archaeorhizomyces* ($p < 0.05$, Figure 3). Compared with CT, the relative abundances of *unclassified_p_Ascomycota*, *unclassified_p_Basidiomycota*, and *Arachnopeziza* were significantly increased in LN treatment, but had no significant changes in HN treatment ($p < 0.05$, Figure 3). In the 10–20 cm soil layer, LN and HN application decreased the relative abundance of *Metarhizium*, while HN treatment significantly increased the relative abundance of *Trichoderma* ($p < 0.05$, Figure 3).

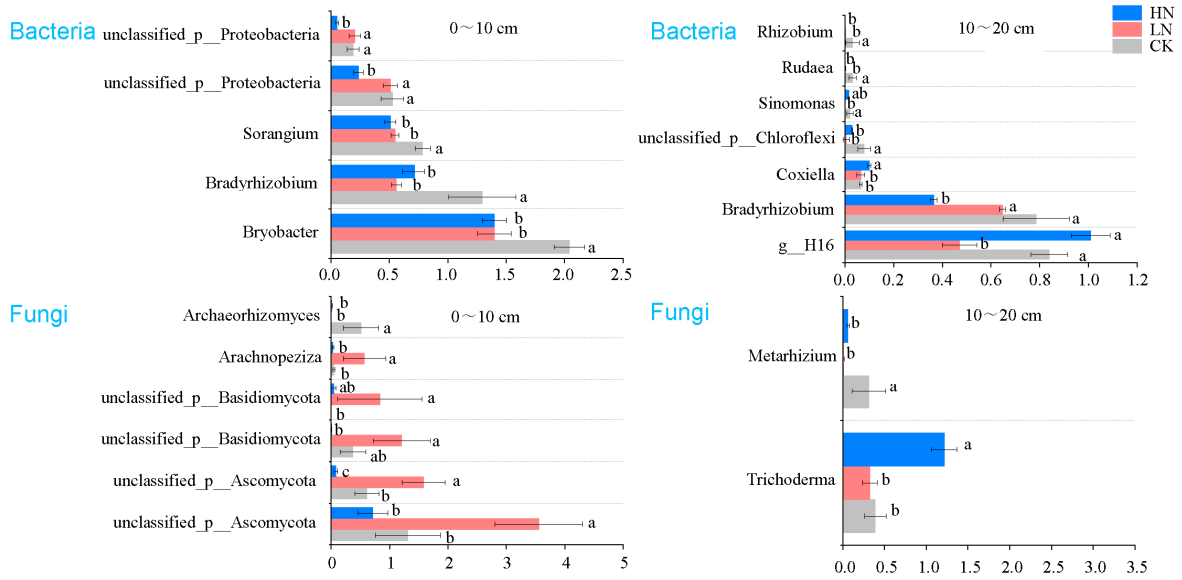

Figure 3. Effects of N deposition on the relative abundances of sensitive microbial species at the level of genera in subtropical natural forest soil. CT, control; LN, low nitrogen addition; HN, high nitrogen addition. Different letters indicate significant difference at $\alpha = 0.05$ ($n = 4$).

3.5. Relationships between Microbial Community Diversity and Structure and Soil Properties

The Mantel test was performed to identify the critical factors influencing bacterial and fungal community diversity and structure after N application (Figure 4). The results showed that bacterial community diversity was positively related with soil pH and NO_3^--N, but negatively related to DOC ($p < 0.05$, Figure 4). NO_3^--N, DON, SOC, TN, and TP were the predominant factors responsible for the changes in bacterial community structure (Figure 4). There are no significant relationships between fungal community diversity and structure and soil properties ($p > 0.05$, Figure 4).

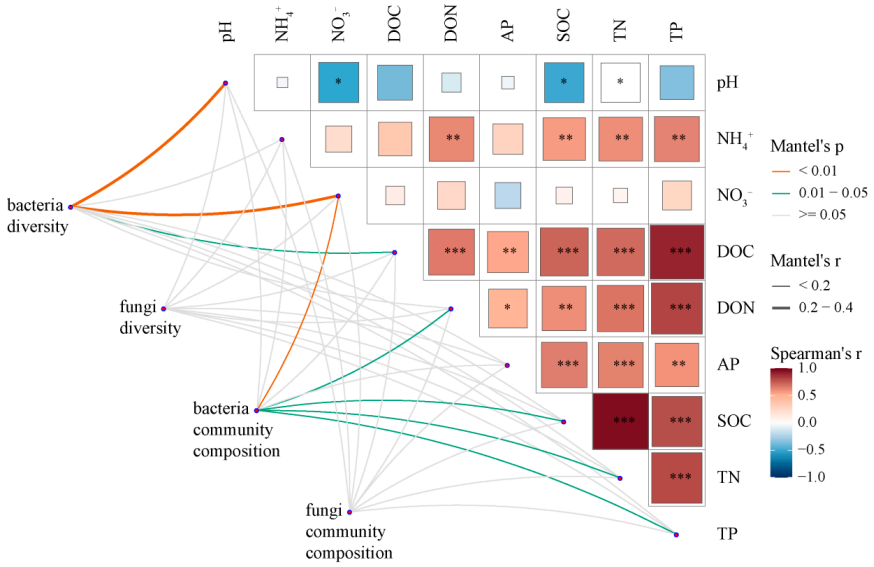

Figure 4. The dominant factors influencing microbial community diversity and structure based on the Mantel test. DOC, dissolved organic carbon; DON, dissolved organic nitrogen; AP, available phosphorus; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus. *indicates signiffcant correlations at the level of 0.05; ** indicates signiffcant correlations at the level of 0.01; *** indicates signiffcant correlations at the level of 0.001.

Consistently, soil pH, DOC, DON, AP, SOC, TN, and TP act as the predominant factors contributing to the changes in dominant bacterial phyla, but not fungal phyla (Figure 5). Specifically, soil pH was negatively related to Actinobacteria and Firmicutes ($p < 0.05$) but positively related to Chloroflexi ($p < 0.01$). Chloroflexi was negatively correlated with the content of DOC, DON, AP, TC, TN, and TP in soil. Firmicutes was positively correlated with NO_3^--N, DOC, DON, and TP content (Figure 5). There were no significant correlations between major fungal phyla and environmental factors (Figure 5).

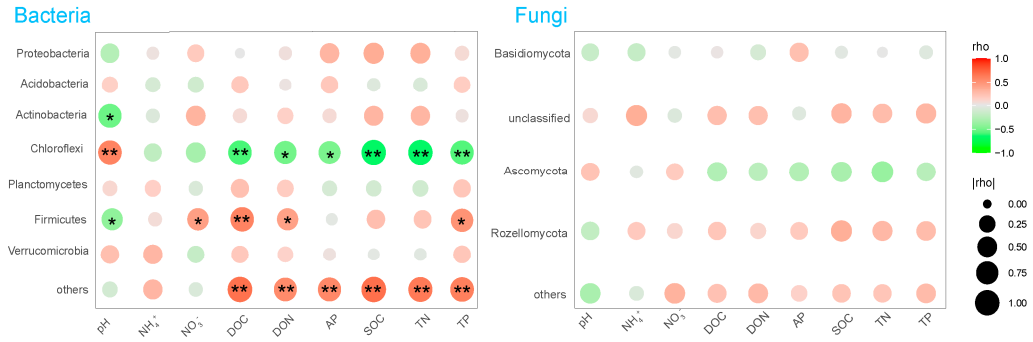

Figure 5. Correlations between major bacterial and fungal phyla and soil properties. DOC, dissolved organic carbon; DON, dissolved organic nitrogen; AP, available phosphorus; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus. *indicates signiffcant correlations at the level of 0.05; ** indicates signiffcant correlations at the level of 0.01.

Pearson correlation analysis was used to examine the correlations between sensitive microbial genera and soil properties (Figure 6). In the 0–10 cm soil layer, Bryobacter and Sorangium were negatively related to NO_3^--N but positively related to AP (Figures 6 and S1). Unclassified_p_Proteobacteria was positively correlated with NO_3^--N, TC, and TN (Figure S1). In the 10–20 cm soil layer, Coxiella was positively correlated with NO_3^--N (Figure S1). In addition, the fungal genera of Archaeorhizomyces were positively correlated with AP in the 0–10 cm soil layer (Figures 6 and S1).

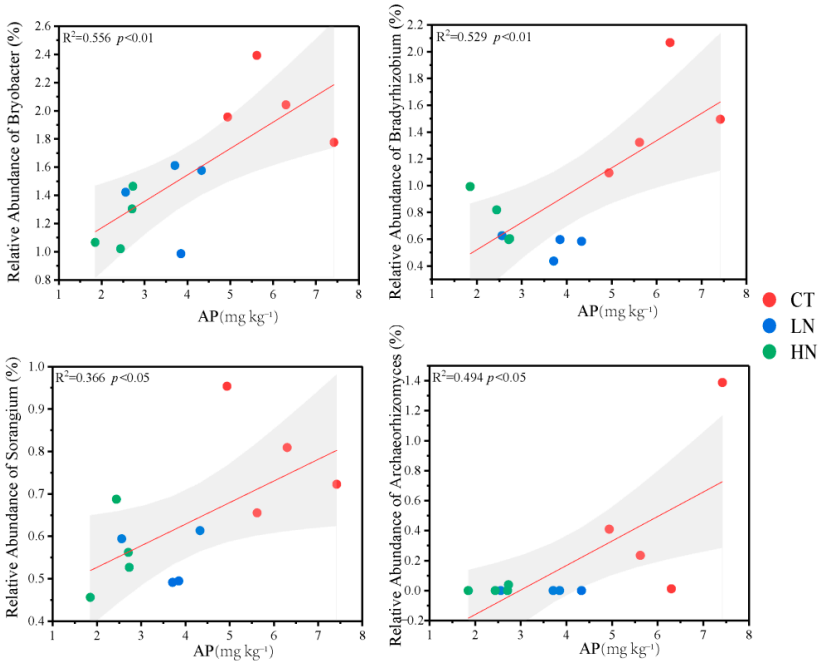

Figure 6. Relationships between sensitive microbial species and available phosphorus. AP: available phosphorus.

4. Discussions

4.1. Effects of N Deposition on Soil Properties

Changed soil properties, such as pH and the availabilities of N and P nutrients, have been demonstrated as a result of N deposition in forest ecosystems at different latitudes [31,32]. In this study, N addition did not significantly alter the concentrations of SOC, TN, or TP in soil ($p > 0.05$, Table 1). Such results are in line with previous findings, where there were no statistical changes in total C, N, or P in response to N deposition in the same studied sites [33,34], suggesting that SOC, TN, and TP are insensitive to 6 years of exogenous N input. This might be a result of strong physical protection [35] due to the plentiful Fe and Al oxides in subtropical soil [36], which is beneficial to the stability of SOC, TN, and TP in the studied soil. In addition, it is generally acknowledged that N addition leads to soil acidification, since the loss of exchangeable base cations is caused by the release of protons due to reactive N input [37,38]. We did observe a general decrease in soil pH, with no significant changes among the three treatments ($p > 0.05$, Table 1), indicating that the pH in subtropical acid soil is less sensitive to environmental changes caused by N deposition than in alkaline soil.

Inconsistent with SOC, TN, and TP, the concentrations of NO_3^--N were significantly increased after N addition ($p < 0.05$, Table 1). Similar results have been reported in subtropical forest soils [33,34]. Nevertheless, no significant changes in NH_4^+-N were obtained after N addition ($p > 0.05$, Table 1). Possible reasons are that nitrification reduces the accumulation of NH_4^+-N induced by N addition, or that increased N utilization for plants due to N deposition might stimulate their growth, as evidenced by the root biomass increasing by 55.8% and 27.5% in LN and HN treatment, respectively [39]. Furthermore, N addition significantly decreased soil AP ($p < 0.05$, Table 1). This can be partly attributed to the enhanced insoluble phosphate precipitation, formed by AP and cations in the soil (e.g., Fe^{3+}, Al^{3+}, Mn^{2+}) under N enrichment [40]. Meanwhile, the increased P concentration in plant leaves and roots implied more AP being uptaken by plants, which thus decreased the AP in soil [41,42]. We observed an increase in fresh leaf P concentration due to N addition (unpublished data). The reduction in AP suggests that N deposition may aggravate P limitation for plants and microbes, especially in subtropical, highly weathered soil, where P is deficient.

4.2. Effects of N Deposition on Soil Microbial Community Diversity, Composition, and Structure

In this study, no significant changes in microbial community diversity, composition, or structure were observed after 6 years of N addition ($p > 0.05$, Table 2 and Figures 1 and 2). Such results differ from the results of meta-analysis that reported that N deposition reduced microbial diversity at a global scale [15,16]. One possible reason may be attributed to the difference in the duration of N application [43]. A previous study determined that the negative effects of N deposition on the microbial community were enhanced with the duration of experimental N input [15]. Moreover, the levels of N input also influence the dynamics of the microbial community in response to N deposition [15]. We also discovered that the bacterial and fungal diversity in LN treatment were higher than those in HN treatment ($p > 0.05$, Table 2), suggesting that LN application is beneficial to microbial growth in the studied forest soils, but HN is unfavorable.

In addition, soil properties such as pH and available C, N, and P play critical roles in influencing the soil microbial community [21,43–45]. In fact, the Mantel test showed that pH predominantly affects bacterial diversity (Figure 4), which is consistent with the notion that pH changes the growth and activity of microorganisms, thus affecting microbial biomass and community structure [46]. In this study, unchanged soil pH creates a relatively stable condition that can help microbes avoid the environmental stress induced by low pH, thus contributing to the lack of significant changes in bacterial diversity after N addition. Nevertheless, no significant correlations between fungal community diversity and soil properties (e.g., available C, N, and P) were observed (Figure 4). This may partly be attributed to the high variability of the fungal community in response to N application

(Figures 1 and 2). Such a result implies that the fungal community in this subtropical forest soil is vulnerable to ongoing N deposition in the future.

Bacterial diversity was positively related to NO_3^--N (Figure 4), indicating that N deposition may indirectly affect the bacterial community through altering N availability in soils [44]. Compared with fungi, bacteria are generally considered to be more sensitive to N addition, especially copiotrophic and oligotrophic microorganisms [47]. According to the "eutrophic hypothesis", the relative abundance of oligotrophic bacteria decreases when N is rich in soil caused by N input, but that of eutrophic bacteria increases [48]. Numerous studies have reported that experimental N addition promoted growth in *Proteobacteria*, *Actinomycetes*, and *Firmicutes* while inhibiting oligotrophic bacteria such as *Chloroflexi* [49,50]. Therefore, the increases in the relative abundance of Firmicutes might be a result of the improved N availability caused by N addition (Figure 2).

The bacterial community diversity and composition were negatively related to the DOC and SOC (Figure 4). These results suggest an apparent C limitation for bacteria under N addition, which is in line with the conclusion that soil microbes are more limited by C rather than by nutrients [51]. As an important environmental factor for structuring soil microbial communities, SOC serves as the integral energy and C source for most microorganisms (e.g., *Actinobacteria*, *Firmicutes*, and *Ascomycota*). A previous study reported that changes in available C predominantly altered the microbial community structure in C-limited soil [17]. Thus, a lack of significant changes in DOC and SOC might partly account for the unchanged microbial community diversity and composition under N addition in this study.

4.3. Effects of N Deposition on Microbial Sensitive Groups

Contrary to the lack of significant changes in microbial community diversity at the phylum level, some sensitive microbial genera were significantly affected by N application (Figures 3 and 6). For example, the relative abundance of *Bradyrhizobium* was decreased after N addition ($p < 0.05$, Figure 3). This may be because *Bradyrhizobium* is an important nitrogen-fixing bacteria in soil [52], so the increases in available N, due to N input, might inhibit the reproduction of *Bradyrhizobium* [52]. Furthermore, there is a positive relationship between *Bradyrhizobium* and soil AP ($p < 0.01$, Figure 6). In addition to *Bradyrhizobium* improving P availability through solubilizing unavailable phosphate in soil [53], its decreased levels might be unfavorable to the mobilization of mineral-bound P, which provides another explanation for the reduction in AP in the studied forest soil.

Meanwhile, N addition significantly decreased the relative abundance of *Bryobacter* ($p < 0.05$, Figure 3). It was determined that *Bryobacter* is capable of reducing nitrate [54]; the decrease in *Bryobacter* means a weakened nitrate reduction, which is thus favorable to the accumulation of nitrate in soil. This can be further supported by the negative relationship between *Bryobacter* and NO_3^--N in soil ($p < 0.01$, Figure S1).

Several fungal genera were also determined to be affected by N application, such as the decreased relative abundances of *Archaeorhizomyces* and *Metarhizium* ($p < 0.05$, Figure 3). Considering that these two genera are associated with plant roots to acquire their needed energy or nutrients [55–57], the reduction in their relative abundances after N addition might be due to the intensified competition between plants and microbes for nutrients such as P [33,42]. This can be further evidenced by the significant relationship between *Archaeorhizomyces* and AP in soil (Figure S1). Moreover, the relative abundance of *Trichoderma* was significantly increased with N application ($p < 0.05$, Figure 3). Previous studies have demonstrated that *Trichoderma* could be involved in decomposing organic N compounds and releasing P [58,59]; thus, the increased *Trichoderma* might be a strategy of microbial adaptation for ameliorating P limitation in this P-deficient forest soil.

Collectively, the changed bacterial and fungal genera and their correlations with soil available C, N, and P suggest that N deposition might alter some sensitive microbial species that are involved in soil nutrient turnover, which, in turn, regulates the changes in C, N, or P availability. Considering that N addition stimulates plant growth and thus assimilates

more nutrients (N or P) from soil, plants might increase their underground C allocation to sensitive microbial species, influencing available N and P. This finding highlights the need for more attention towards sensitive microbial groups, for their responses to environmental changes may improve the mechanistic understanding of microbial-driven nutrient cycling under climate change in the future.

5. Conclusions

N addition did not significantly alter the contents of SOC, TN, or TP, but decreased AP and increased NO_3^--N in soil, suggesting that 7 years of N deposition mainly affected available nutrients rather than the total C, N, and P in the studied forest soil. There were no significant changes in alpha diversities (presented as Chao1 and Shannon's indexes) or beta diversities of bacteria and fungi among the three treatments following N addition, which implies that N deposition did not alter microbial community diversity and structure. In contrast, the relative abundance of some sensitive microbial genera such as *Bryobacter*, *Bradyrhizobium*, *Sorangium*, and *Archaeorhizomyces* was decreased. These results, combined with the positive correlations of *Bryobacter*, *Bradyrhizobium*, and *Archaeorhizomyces* with NO_3^--N and available P (AP), suggest that the responses of sensitive microbial groups to N deposition are likely associated with the changes in available N and P. Our study implies that the changes in some sensitive microbial groups might meditate the availability of limited nutrients (e.g., N or P), even when there are no significant changes in microbial communities under N deposition. Attention should be paid to the contribution of sensitive microbial species when evaluating the influences of climate change on nutrient cycling in soil in future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14050928/s1. Figure S1: Correlations between soil properties and microorganisms differing at the genus level. * indicates that the association between indicators reached the level of significance ($p < 0.05$). ** indicates that the association between indicators reached a highly significant level ($p < 0.01$).

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