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Nitric Acid Rain Increased Bacterial Community Diversity in North Subtropical Forest Soil

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Abstract: Nitric acid rain (NAR) seriously affects the biogeochemical cycles of forest communities' ecosystems. However, the effects of NAR on the composition and diversity of the soil bacterial community remain unclear. In this study, a typical subtropical forest of *Quercus acutissima* was selected and simulated spraying of NAR at pH 2.5 (AR2.5), 3.5 (AR3.5), and 4.5 (AR4.5) was implemented to investigate the response of the forest soil bacterial communities to NAR. The results showed that the total number of OTUs of soil bacteria in AR2.5 and AR3.5 treatments was 1.11 and 1.23 times that in the control treatment without NAR (CK), respectively. Acidobacteria, Proteobacteria, and Actinobacteria were the dominant phyla in the subtropical forest, accounting for more than 80% of the community's relative abundance. Concurrently, simulated NAR changed the relative abundance of *Rhodanobacter* significantly, which could be an indicator of soil bacterial community structure under NAR stress. Moreover, the Chao1, Shannon, and Simpson indices of strong acid rain treatments (i.e., AR2.5 and AR3.5) increased by 9.55%–22.5%, 3.6%–7.43%, and 0.15%–0.26%, respectively, compared to CK. Redundancy and correlation analysis illustrated that the phylum level structure of the bacterial community was significantly affected by soil total carbon, total nitrogen, and ammonium nitrogen. Our findings contribute to a better understanding of the effects of NAR on soil microbial communities and potential soil element cycling in north subtropical forests.

Keywords: nitric acid rain; subtropical forest; soil; bacterial community; diversity

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1. Introduction

Acid rain (AR) pollution has been a global concern since the industrial revolution. Although the harm of AR to human health and ecosystem functions has been alleviated by strict control of SO_2, AR types are gradually changing with the increase in NO_X and NH_3 emissions, and the environmental pollution caused by AR is still serious [1,2]. The proportion of nitric acid rain (NAR) in the Yangtze River Delta region of China is gradually increasing and it has become one of the regions with the highest risk of being eroded by NAR in the world [3]. The increase in H^+ and NO_3^- contents in AR [4,5] leads to severe leaching of K, Ca, Na, and other base ions [6], which directly or indirectly causes changes in the soil microbial community [7]. Considering the increased risk of AR erosion, the impact on global forest ecosystems and soil microorganisms is worth studying.

Forest soil microorganisms are one of the most critical components of the ecosystem [8], and also a sensitive index of forest ecosystem response to environmental stress [9]. Soil bacteria grow rapidly and decompose vigorously. Studies have shown that the relative abundance of Acidobacteria, Actinobacteria, and Proteobacteria of soil bacteria in forest ecosystems is relatively high [10–12], and Acidobacteria and Actinobacteria have the ability to decompose lignin and cellulose [13]. Most studies [14,15] showed that the relative abundance of Acidobacteria was significantly negatively correlated with soil pH, but Jones et al. [16] found that some subgroups of Acidobacteria were significantly positively correlated with soil pH. Wang et al. [17] found that simulated AR treatment improved the

diversity of the bacterial community in early rice soil. Moreover, Liu et al. [18] found that AR with high acidity would increase the biomass of soil bacteria in the early stage. These studies indicate that the effects of AR on soil bacterial communities in a forest ecosystem are restricted by many factors.

The soil bacterial community is the highest proportion of soil microorganisms, and cycling drives all of the biogeochemical cycles in soils [19]. Bacteria mediate nitrogen (N) fixation in forest ecosystems [20], and N can be obtained from a series of organic compounds such as chitin in the polysaccharides of fungal mycelium in forest soil and amino acids and proteins in the dead organic matter [21]. Gao et al. [13] found that pH, total nitrogen (TN), and water content were the main factors affecting bacterial community structure by studying *Betula platyphylla*, a pioneer species in the succession process of the natural secondary forest ecosystem. Cong et al. [15] found a correlation between soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) and soil bacteria by studying forest soil microorganisms in different climatic zones. Horner-Devine et al. [22] found that changes in soil ammonium ion concentration (NH_4^+) affect bacterial community composition. Wang et al. [23] found that the C/N ratio, soil pH, and organic carbon (C) were the key environmental factors affecting soil bacterial diversity.

However, the effects of NAR on soil bacterial community diversity and structural composition, as well as its regulatory factors, remain unclear. We hypothesized that H^+ and NO_3^- carried by NAR into forest soil would reduce the pH value of forest soil, thus affecting the diversity and structural characteristics of the soil bacterial community. In order to test this hypothesis, a typical north subtropical forest of *Quercus acutissima* was selected, and three NAR treatments (AR2.5, AR3.5, and AR4.5) were set in the test site with pH gradients (pH = 2.5, 3.5, and 4.5). The goals of this study were: (1) to study the response of soil bacterial community structure to NAR, (2) to study the response of soil bacterial community diversity to NAR, and (3) to study the relationship between soil environmental factors and bacterial community under NAR stress. This study provides a theoretical basis for exploring the response of the soil microbial community to NAR stress in north subtropical forest ecosystems.

2. Materials and Methods

2.1. Study Site

This study was conducted at the Yangtze River Delta Forest Ecological Station (32°7′49″ N, 119°12′7″ E) in Zhenjiang, Jiangsu Province, China. This area is home to a monsoon season, having an average yearly rainfall of 1184.3 mm at pH 5.15, with 15.1 °C as the average yearly temperature [24]. The soil texture is medium or heavy soil (Q_{3x}) [25], whereas the altitude of the experimental site is 180 m. The tree stands were mainly *Q. acutissima* with a mean age of ~70 years. The soil pH, total carbon (TC), TN, C/N, dissolved organic carbon (DOC), and alkali-hydrolyzable nitrogen (AN) were 4.53, 17.43 g·kg^{-1}, 1.53 g·kg^{-1}, 11.37, 85.93 mg·kg^{-1}, and 109.88 mg·kg^{-1}, respectively.

2.2. Experimental Treatments

In December 2020, 12 quadrats (3 m × 3 m) were randomly set in a *Q. acutissima* forest, designated as CK (pH = 6.5), AR4.5 (pH = 4.5), AR3.5 (pH = 3.5), and AR2.5 (pH = 2.5) treatments, with three replicates for each treatment. The NAR was prepared used using a master batch of 0.5 mol L^{-1} H_2SO_4 and 0.5 mol L^{-1} HNO_3 with a molar mass ratio of 1:5 [26]. Localized rainfall data for 2011–2021 quantified the average monthly precipitation, the intensity of which fluctuated occasionally (Figure 1). The contents of soil total N added to each plot under different treatments from December 2020 to November 2021 were shown in Table A1. Moreover, the configured NAR were sprayed onto the sample plots on the 15th day of each month from December 2020 to November 2021 (Figure 1). At the same time, the control area was sprayed with an identical amount of tap water (pH = 6.5).

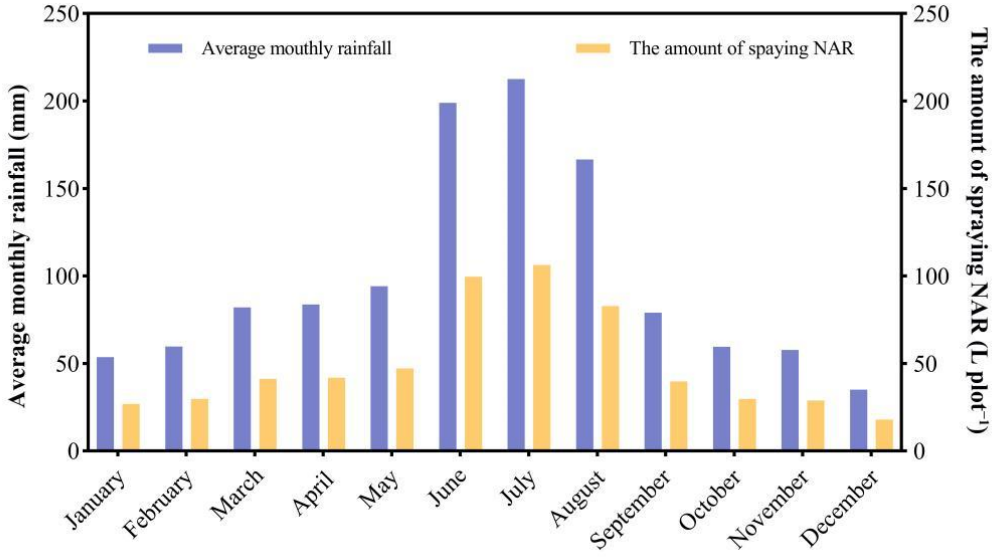


Figure 1. Average monthly rainfall and spraying amounts of NAR at the test plots. 2/3 of the average monthly rainfall from 2011 to 2021 was established as the total annual spraying amount per square meter, and 1/12 was taken as the monthly spraying amount per square meter. The amount of spraying at a given plot was calculated by the monthly spraying amount × 9 m^2 (the area of a single plot).

2.3. Soil Collection and Chemical Analysis

On 30 November 2021, topsoil samples (0~10 cm depth) were collected using the five-point sampling method. One portion of these samples was stored in a dry ice incubator (−80 °C) for later use to quantify the resident bacteria. The other portion was employed to quantify the fresh soil's chemical attributes. One-half was stored at 4 °C, whereas the other half (following the removal of debris such as stones and fallen leaves) was dried naturally and screened (2 mm) for the determination of soil nutrient indices. Soil pH was measured using a pH meter (Sartorius Gmb H: Gottingen, Germany). Soil TN and TC concentrations were determined with an elemental analyzer (Vario EL III, Elementar: Langenselbold, Germany). Soil DOC concentration was quantified using a K_2SO_4 extraction method. Soil AN concentration was measured via an alkaline hydrolysis diffusion technique. Soil NO_3^--N concentration was quantified by spectrophotometry, and the NH_4^+-N was measured via (indophenol blue) colorimetry.

2.4. Extraction and Sequencing of Soil Bacterial DNA

Soil microbial DNA was extracted from the samples using primers from an E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek: Norcross, GA, USA). Soil bacterial V3–V4 region underwent PCR amplification. Amplicons were extracted using 2% agarose gel and then refined with an AxyPrep DNA Gel Extraction kit (Axygen Biosciences: Union City, CA, USA). The pure PCR products were then quantified via Qubit® 3.0 (Life Invitrogen: Carlsbad, CA, USA) software. According to Illumina genomic DNA library preparation procedures, the polymerized DNA products were integrated into Illumina peer libraries. Subsequently, the amplicon library was double-terminal sequenced using the Illumina Novaseq 6000 platform (GenePioneer Co. Ltd.: Nanjing, China) (2 × 250).

2.5. Analysis Methods

R (V3.6.2) (R Development Core Team, NZ) was utilized to develop the Venn diagram and count the number of shared and unique bacterial OTUs in the four samples. GraphPad Prism 9 was employed to plot the stacked histogram of the top 20 species abundance at the bacterial phyla and genus levels. MOTHUR software (V1.30.2) (University of Michigan, Stateof Michigan, MI, USA) was used to calculate α diversity (Chao1, Shannon, Simp-

son, and Goods_coverage indices). The effects of different treatments on soil bacterial communities were tested based on Principal Coordinates analysis (PCoA) of Bray–Curtis distance using the "Vegan" package. SPSS 21.0 (SPSS Inc., Chicago, IL, USA) was used for one-way ANOVA and multiple comparative analysis (LSD), with the significance level set as $p < 0.05$. Correlations between ecological factors and the α-diversity of soil resident bacterial populations were tested using Pearson correlation analysis. Soil ecological factor and bacterial community redundancy analysis (RDA) proceeded using Canoco 5 software (Microcomputer Power, Ithaca, NY, USA).

3. Results

3.1. Effects of NAR on Soil Nutrient Contents

Soil pH under the AR2.5 treatment significantly decreased by 0.17 units compared with the CK treatment (Table 1). In contrast to the CK, soil TC, TN, and NH_4^+-N contents under the AR3.5 and AR2.5 treatments significantly decreased by 10.3%–22.4%, 13.1%–19.6%, and 7.0%–18.2%, respectively. Conversely, soil DOC and AN contents under the AR2.5 treatment significantly increased by 31.8% and 17.4%, respectively, compared with the CK treatment. Moreover, there were negligible differences in the C/N under all the treatment groups compared with CK.

Table 1. Soil nutrient contents under different treatments.

Treatments	pH	TC g·kg^{-1}	TN g·kg^{-1}	C/N	DOC mg·kg^{-1}	AN mg·kg^{-1}	NO_3^--N mg·kg^{-1}	NH_4^+-N mg·kg^{-1}
CK	4.53 ± 0.11 a	1.74 ± 0.08 a	1.53 ± 0.06 a	11.4 ± 0.2 a	85.9 ± 10.8 b	110 ± 5 b	15.5 ± 1.1 ab	7.10 ± 0.16 a
AR2.50	4.36 ± 0.06 b	1.35 ± 0.07 c	1.23 ± 0.06 c	11.0 ± 0.2 a	113 ± 4.3 a	129 ± 9 a	17.2 ± 0.8 a	5.81 ± 0.19 c
AR3.50	4.45 ± 0.06 ab	1.56 ± 0.08 b	1.33 ± 0.15 bc	11.8 ± 0.9 a	99.9 ± 9.2 ab	118 ± 2 b	16.1 ± 0.9 a	6.60 ± 0.34 b
AR4.50	4.50 ± 0.08 ab	1.76 ± 0.07 a	1.47 ± 0.06 ab	11.9 ± 0.3 a	96.5 ± 10.9 ab	114 ± 2 b	14.2 ± 0.9 b	6.57 ± 0.24 b

Data are mean ± standard error. Lowercase letters represent significant differences in soil nutrient contents under different NAR treatments ($p < 0.05$). TC: total carbon; TN: total nitrogen; C/N: total carbon/total nitrogen ratio; DOC: dissolved organic carbon; AN: alkali-hydrolyzable nitrogen; NO_3^--N: nitrate nitrogen; NH_4^+-N: ammonium nitrogen. CK: control without acid rain; AR2.5: Acid rain at pH 2.5; AR3.5: Acid rain at pH 3.5; AR4.5: Acid rain at pH 4.5.

3.2. Changes in Soil Bacterial Community Composition

The overall number of bacterial OTUs increased with NAR inputs (Figure 2a). Specifically, the total number of bacterial OTUs was 4165 across all treatments. Among them, there were 1921 OTUs common to all treatments, which accounted for about 46.1% of the total. The distribution of fungal OTUs unique to CK was the lowest, which accounted for 2.88% of the overall OTUs. The dissemination of soil fungal OTUs exclusive of the AR3.5 treatment was the highest, accounting for ~9.24% of the total. Moreover, the total numbers of bacterial OTUs under the AR2.5 and AR3.5 treatments were ~1.11 and 1.23 times that of the CK treatment, respectively.

There were 21 known phyla of bacteria identified among all treatments (Figure 2b). Among them, the relative abundances of Acidobacteria, Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Planctomycetes, Bacteroidetes, WPS-2, Armatimonadetes, and Elusimicrobia in CK were 43.8%, 33.1%, 7.05%, 4.40%, 2.07%, 2.38%, 1.65%, 1.07%, 1.81%, and 1.33%, respectively. These were the predominant bacterial phyla in the *Q. acutissima* forest soil (relative abundance > 1%). Notably, the cumulative relative abundances of Acidobacteria, Proteobacteria, and Actinobacteria were over 80%. In contrast to the CK, AR2.5 and AR3.5 treatments had lower relative abundances of Acidobacteria and Actinobacteria, but a higher relative abundance of Proteobacteria. Concurrently, the relative abundances of Gemmatimonadetes, Planctomycetes, and Bacteroidetes under the AR3.5 treatment significantly increased by 48.3, 18.9, and 51.4% compared to CK treatment.

As shown in Figure 2c, the relative abundance of *Acidibacter*, *Candidatus*, *Solibacter*, *Acidothermus*, and *Bryobacter* under the CK treatment were 2.70%, 1.98%, 1.86%, and 1.59%, respectively, which could be used as indicator genera for the north subtropical forest

(relative abundance > 1%). The spraying of NAR significantly altered the soil bacteria genera. Compared to CK, there was a comparative decrease in the relative abundances of *Acidibacter* and *Acidothermus*, albeit an increase in the relative abundances of *Rhodanobacter* and *Ellin6067* under AR2.5 and AR3.5 treatments. Specifically, the relative abundance of *Acidibacter* and *Acidothermus* under the AR2.5 and AR3.5 treatments were significantly decreased by 17.5%–23.2% and 25.9%–29.6% related to CK. The relative abundances of *Rhodanobacter* and *Ellin6067* in AR2.5 and AR3.5 were 2.3–2.9 and 3.5–2.6 times higher than those of the CK, respectively.

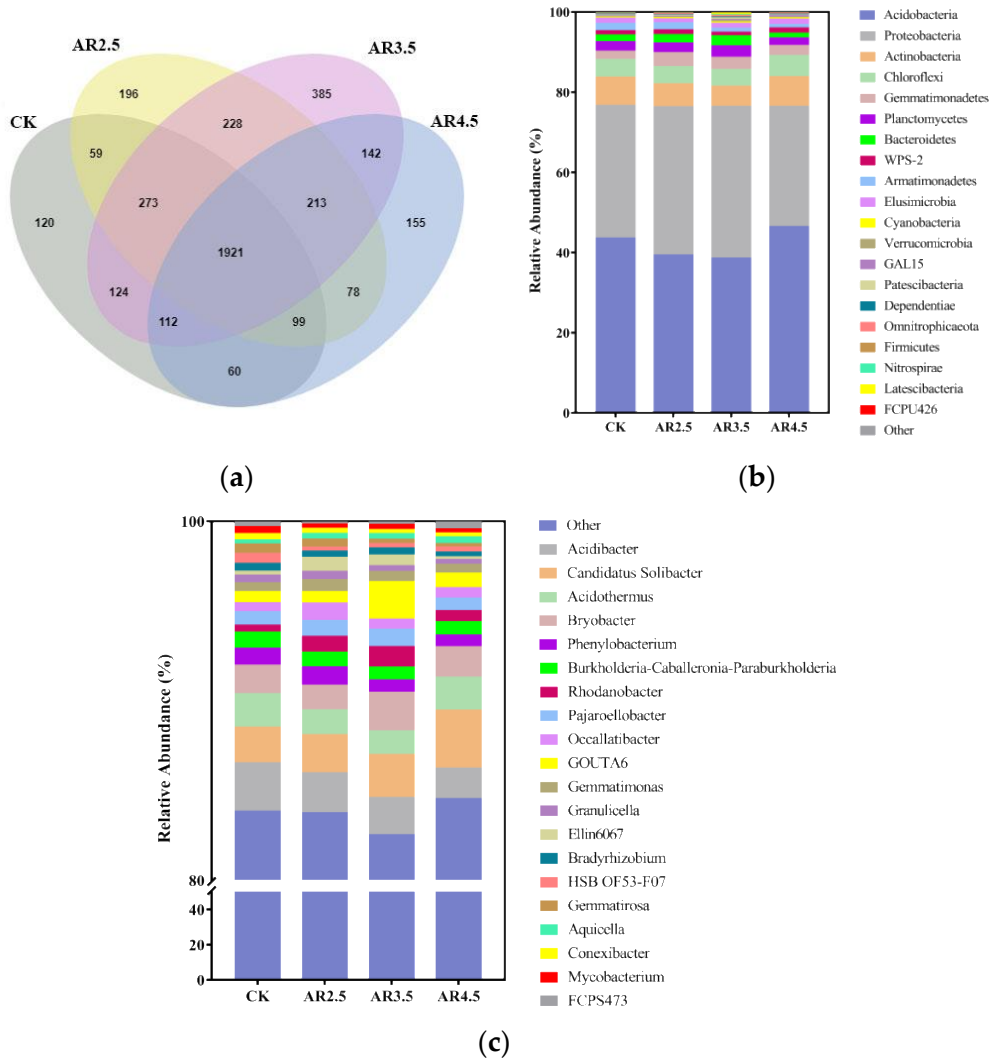


Figure 2. Venn of bacterial OTUs (**a**), community composition at the phyla of soil bacteria (**b**), and community composition at the genus of soil bacteria (**c**) of all treatments. Black dots exist in the set, black dots with a line between them represent intersections, and gray dots do not exist in the intersections. CK: control without acid rain; AR2.5: Acid rain at pH 2.5; AR3.5: Acid rain at pH 3.5; AR4.5: Acid rain at pH 4.5.

3.3. Changes in Soil Bacterial Community Diversity

As shown in Table 2, the α-diversity indexes of AR2.5 and AR3.5 treatments were significantly higher than those of CK. In particular, the Chao1, Shannon, and Simpson indices in AR2.5 and AR3.5 treatments increased by 9.55%–22.5%, 3.60%–7.43%, and 0.15%–0.26%, compared with CK treatment. However, the Goods_coverage index of AR3.5 treatments was less than that of CK and AR4.5 treatments.

Table 2. Soil bacterial community α-diversity indexes under different treatments.

	Chao1	Shannon	Simpson	Goods_Coverage
CK	2472 ± 73 c	8.61 ± 0.07 c	0.9930 ± 0.0006 c	0.9937 ± 0.0002 a
AR2.50	2733 ± 80 b	8.92 ± 0.05 b	0.9945 ± 0.0003 b	0.9931 ± 0.0003 ab
AR3.50	3027 ± 70 a	9.25 ± 0.06 a	0.9956 ± 0.0005 a	0.9928 ± 0.0004 b
AR4.50	2465 ± 96 c	8.56 ± 0.05 c	0.9920 ± 0.0003 d	0.9936 ± 0.0005 a

Data are mean ± standard error. Lowercase letters represent significant differences in soil bacterial community α-diversity indexes under different NAR treatments ($p < 0.05$). CK: control without acid rain; AR2.5: Acid rain at pH 2.5; AR3.5: Acid rain at pH 3.5; AR4.5: Acid rain at pH 4.5.

Principal coordinate (PCoA) analysis (Figure 3) based on Bray–Curtis distance showed that the first two axes of the principal coordinate explained the total variance contribution rate of 64.9%. These two principal components (PC1 and PC2) were the main contributors to the difference in bacterial community composition in forest soil under NAR. The first axis (PC1) and the second axis (PC2) explained 38.3% and 26.6%, respectively. The structure of the soil bacteria community in CK and AR4.5 treatments was concentrated in the positive half axis of the first axis, while that in AR2.5 and AR3.5 treatments was concentrated in the negative half axis of the first axis. At the same time, AR3.5 was concentrated in the positive half axis of the second axis, and AR2.5 was concentrated in the negative half axis of the second axis, indicating significant differences between groups.

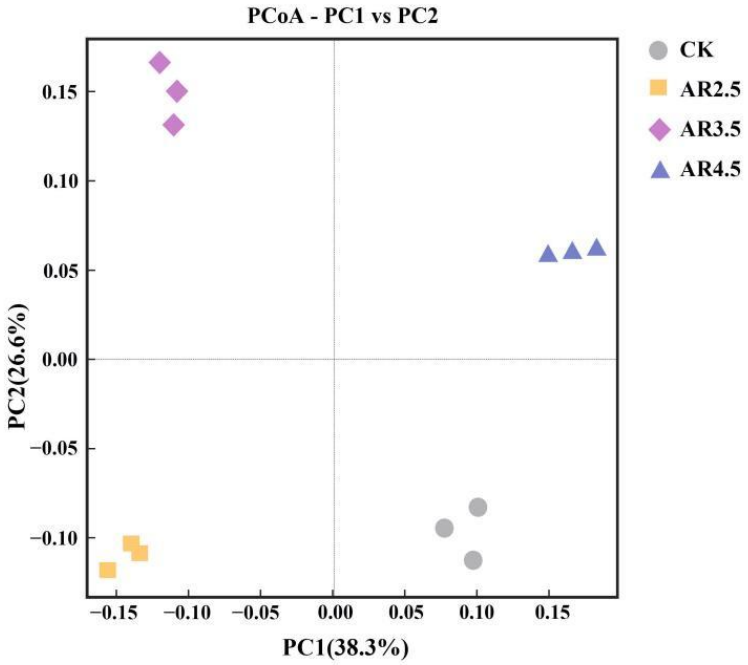


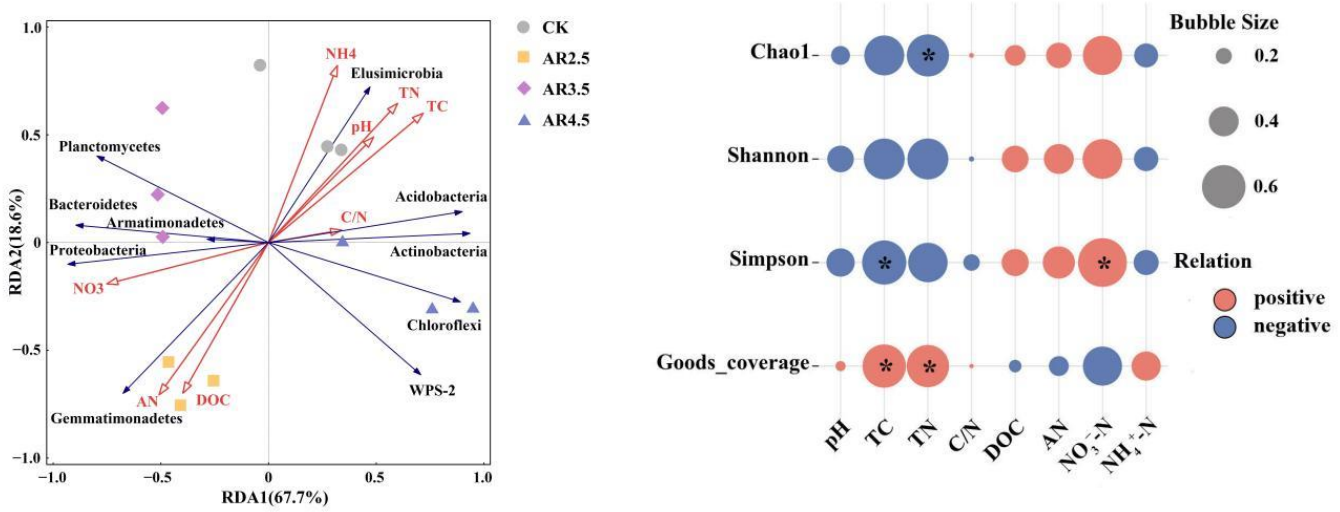
Figure 3. Principal Coordinate Analysis (PCoA) of structure of soil bacteria community. CK: control without acid rain; AR2.5: Acid rain at pH 2.5; AR3.5: Acid rain at pH 3.5; AR4.5: Acid rain at pH 4.5. Each point in the figure represents different treatments. The closer the point distance is, the more similar the bacterial community structure is. The farther the point distance is, the more obvious the difference in bacterial community structure is.

3.4. Response of Soil Bacterial Community to Soil Environmental Factors

According to the redundancy analysis of soil bacterial community structure and soil environmental factors of the *Q. acutisana* forest (Figure 4a), the explanation rate of the first two ranking axes reached 86.3%, the first axis explained 67.7% of the variables, and the second axis explained 18.6% of the variables. Soil TC ($F = 6$, $p = 0.01$) and NH_4^+-N ($F = 3.6$, $p = 0.026$) were the significant factors affecting soil bacterial community structure, with the explanatory rates of 37.6% and 19%, respectively. Gemmatimonadetes were positively

correlated with soil AN, DOC, and NO_3^--N. Concurrently, Acidobacteria, Actinobacteria, and Elusimicrobia were positively correlated with soil pH, TN, TC, and NH_4^+-N.

Correlation analysis results (Figure 4b) showed that the Chao1 index was significantly negatively correlated with soil TN (R = 0.580, $p < 0.05$). The Simpson index was negatively correlated with soil TC (R = 0.610, $p < 0.05$) but was positively correlated with NO_3^--N (R = 0.677, $p < 0.\ 05$). The goods_coverage index was positively correlated with TC (R = 0.598) and TN (R = 0.583, $p < 0.\ 05$).



(**a**) (**b**)

Figure 4. (**a**) Redundancy Analysis (RDA) of the effects on the soil bacterial community and soil environmental factors. (**b**) Bubble plot: Correlation analysis of soil chemical properties and bacterial community α-diversity indexes. The size of the circle indicates the size of the correlation coefficient. * indicate significant difference at $p < 0.05$. TC: total organic carbon; TN: total nitrogen; C/N: total carbon/total nitrogen ratio; DOC: dissolved organic carbon; AN: alkali-hydrolyzable nitrogen; NO3: nitrate nitrogen; NH4: ammonium nitrogen. CK: control without acid rain; AR2.5: Acid rain at pH 2.5; AR3.5: Acid rain at pH 3.5; AR4.5: Acid rain at pH 4.5.

4. Discussion

4.1. Effects of NAR Stress on Soil Bacterial Community Structure

Bacterial communities are extensively dispersed in forest soils and have vital roles in forest ecosystems and soil nutrient cycling [27]. Xia et al. [27] studied bacterial populations in various types of forest soils in Eastern China and found that Actinobacteria and Acidobacteria phyla were the most prevalent. Fierer et al. [28] studied different ecological communities, such as forests, grasslands, tundra, and deserts, and observed that Acidobacteria, Actinobacteria, Bacteroidetes, and Proteobacteria were prevalent across all communities. Other studies [10–12] revealed relatively high Acidobacteria, Actinobacteria, and Proteobacteria populations in forest ecosystems. Acidobacteria can degrade complex lignin and cellulose. In the case of cellulose, it facilitates the generation of acetic acid and hydrogen under hypoxic conditions [29], thereby improving soil nutrient status [30]. Actinobacteria are a group of Gram-positive bacteria, some of which secrete enzymes that decompose lignin and cellulose [13]. Moreover, most of the autogenous and symbiotic nitrogen-fixing microorganisms are derived from Proteobacteria [31]. Our results revealed that phyla with relatively high populations in the CK and simulated NAR groups included Acidobacteria, Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Planctomycetes, Bacteroidetes, WPS-2, Armatimonadetes, and Elusimicrobia (Figure 2b).

Among them, the relative abundances of Acidobacteria, Proteobacteria, and Actinobacteria accounted for more than 80%, indicating that they might be the dominant soil bacteria phylum in the north subtropical forest. These findings are consistent with the results of Lauber et al. [32] and Liu et al. [33], who reported that the most abundant phylum was Acidobacteria. It may be due to the fact that Acidobacteria, Proteobacteria, and Actinobacteria are highly adaptable to diverse environments with more extensive ecological niches [13]. Additionally, potent NAR treatments (i.e., AR2.5 and AR3.5) reduced Acidobacteria and Actinobacteria populations (Figure 2b). This was in contrast to Jones et al. [16], who reported that the populations of soil residing in Acidobacteria were augmented at a lower soil pH [14,15]. This may be related to the differences in the responses of various Acidobacteria subclasses to environmental soil attributes [34]. For instance, the comparative population of Acidobacteria increased with a higher soil N content [35]. Moreover, the reduced population of Actinobacteria might be related to soil pH and it might generally decrease at lower pH [28,36]. Further, the population of Proteobacteria and Gemmatimonadetes treated with AR2.5 and AR3.5 increased at lower pH, which is partially consistent with the results of Wang et al. [17].

The results of this study at the genus level (Figure 2c) revealed that *Acidibacter*, *Candidatus*, *Solibacter*, *Acidothermus*, and *Bryobacter* under the CK treatment could be used as indicator genera of north subtropical forest soil (relative abundance > 1%). However, simulated NAR altered the soil bacteria genus structure. Compared with CK, the AR2.5 and AR3.5 treatments were observed to decrease the *Acidibacter* and *Acidothermus* populations yet enhance the population of *Rhodanobacter* and *Ellin6067*. Among them, the relative abundance of *Rhodanobacter* changed significantly under NAR spraying, which may be employed as a marker for changes in the structure of soil bacterial communities. *Rhodanobacter* is an acid-tolerant genus that can maintain the pH within its cells by adjusting its functions under the influence of AR to maintain its biological activities [17]. This may be one of the reasons behind the increased relative abundance of *Rhodanobacter* in soil treated with NAR in this study.

4.2. Effects of NAR Stress on Bacterial Community Diversity in Forest Soil

The potency and variety of bacterial communities have vital roles in the cycling of nutrients and the ecological balance of forest soils [37,38]. The simulated NAR treatments altered the variety of soil resident bacterial communities in *Q. acutissima* forests. The results indicated (Table 2) that strong NAR treatments (AR2.5 and AR3.5) substantially improved the Chao1, Shannon, and Simpson indices of the bacterial communities in forest soils. Particularly, the AR3.5 treatment induced the greatest richness and evenness in bacterial communities. This was similar to the research results of Wang et al. [17] and Liu et al. [18], contrary to the findings of Wang et al. [39]. Wang et al. [39] found that the growth of bacterial communities was inhibited with greater AR treatment intensity, thereby reducing soil bacterial diversity. However, Wang et al. [17] observed that simulated AR treatments improved the diversity of soil bacterial communities in the early rice season, and the community diversity under the pH 3.5 treatment group was the highest. Liu et al. [18] found that AR with high acidity increased the biomass of soil bacteria in the early stage. This might be due to the fact that the stimulating effects of NAR fertilization were higher than the inhibiting effects of acidity, which improved the soil's bacterial diversity and richness. Moreover, the evenness of soil bacteria was significantly impacted by strong AR, which may have been due to the inhibition of the growth of some bacteria under AR stress, which reduced the richness of the bacteria. However, most bacteria adapted to the acidic environment grew rapidly, which impacted the soil bacterial community even considerably in the *Q. acutissima* forest [39].

4.3. Interactions between Soil Bacterial Communities and Soil Environmental Factors under NAR Stress

Ecological factors have potent influences on the distribution of soil-residing bacterial communities [40], where soil nitrogen is closely related to bacterial community diversity and structural changes [41,42]. Gao et al. [13] observed that TN was the primary factor that affected the structures of bacterial communities, which was strongly correlated with Acidobacteria, Gemmatimonadetes, Actinobacteria, Proteobacteria, and Planctomycetes populations. Cong et al. [15] reported on the relationship between the soil NH_4^+-N and NO_3^--N and soil bacteria by studying forest soil microorganisms in different climatic zones. Horner-Devine et al. [22] found that modified soil NH_4^+ affected the compositions of bacterial communities. Redundancy analysis in this study (Figure 4a) revealed that NH_4^+-N was a significant driver that impacted the structures of bacterial communities in the soil. Correlation analysis (Figures 4b and A1) found that TN was strongly associated with the comparative richness of the Chao1 index, Acidobacteria, Proteobacteria, Actinobacteria, Gemmatimonadetes, and Elusimicrobia. This suggested that TN was the primary driver that impacted the diversity and phylum level structures of bacterial communities. Concurrently, NH_4^+-N was significantly correlated with Gemmatimonadetes and Elusimicrobia, while NO_3^--N was negatively correlated with Actinobacteria, which aligned with earlier studies. This may have been due to bacteria serving as the main facilitator of nitrogen fixation [20] and mineral weathering, which led to the leaching of inorganic nutrients within forest ecosystems [43]. Previous studies found that there were denitrification genes in certain strains of Acidobacteria, Actinobacteria, and other bacteria [44,45]. The addition of NAR inhibits the relative abundance of Acidobacteria and Actinobacteria, which may have affected the denitrification process. Furthermore, NO_3^- carried by NAR is a substrate for denitrification and an inhibitor of N_2O reductase [46], which can also affect the soil nitrogen cycle.

Forest soil stores two-thirds of all terrestrial C [47], where the degradation of lignin and cellulose by soil microorganisms is a key process that regulates the C cycle in the soil system, which influences the ratio between C mineralization and immobilization [47]. Acidobacteria and Actinobacteria play critical ecological roles in the degradation of plant biomass polysaccharides in acidic forest soils [48,49]. The results of redundancy analysis in this study (Figure 4a) indicated that TC was the main influencing factor that drove community structure and diversity patterns. The results of correlation analysis (Figure A1) showed that TC was significantly positively associated with Acidobacteria, whereas TC and DOC were strongly associated with Gemmatimonadetes and Elusimicrobia, which was similar to the research results of Zhao et al. [50]. Therefore, TC is the main influencing factor that drives the diversity and structural distribution of soil bacterial communities [40].

5. Conclusions

In this study, simulated nitric acid rain (NAR) increased the Chao1, Shannon, and Simpson indices, and altered the structure of soil bacterial communities in a north subtropical typical forest (*Quercus acutissima*). The strong acid rain treatments (i.e., AR2.5 and AR3.5) reduced the relative abundances of Acidobacteria and Actinobacteria, whereas enhanced the relative abundances of Proteobacteria and Gemmatimonadetes. At the same time, *Rhodanobacter* can be used as an indicator of soil bacterial community structure under NAR stress. Moreover, the diversity and structural compositions of soil bacterial communities were strongly impacted by the soil's total carbon, total nitrogen, and ammonium nitrogen under NAR stress. This research provides a hypothetical foundation for exploring the reactions of communities of soil bacteria under NAR stress in typical forest ecosystems in north subtropical regions. It is suggested that future research should focus more intently on the long-term effects of NAR on soil microorganisms.

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

Appendix A

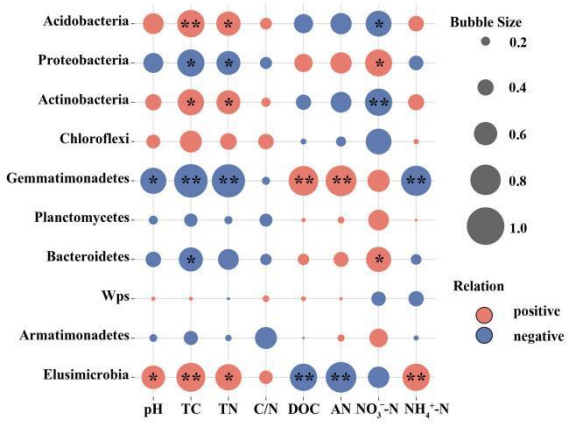

Figure A1. Correlation analysis of soil bacterial community structure characteristics and soil chemical properties. ** and * indicate significant difference at $p < 0.01$ and $p < 0.05$. TC: total organic carbon; TN: total nitrogen; C/N: total carbon/total nitrogen ratio; DOC: dissolved organic carbon; AN: alkali-hydrolyzable nitrogen; NO_3^--N: nitrate nitrogen; NH_4^+-N: ammonium nitrogen.

Table A1. The contents of soil total N added to each plot under different treatments from December 2020 to November 2021.

	January	February	March	April	May	June	July	August	September	October	November	December
CK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AR2.5	0.42	0.47	0.65	0.66	0.74	1.57	1.67	1.30	0.63	0.47	0.45	0.28
AR3.5	0.28	0.31	0.43	0.44	0.49	1.05	1.12	0.87	0.42	0.31	0.30	0.19
AR4.5	0.14	0.16	0.22	0.22	0.25	0.52	0.56	0.43	0.21	0.16	0.15	0.09

Note: the unit of N content is g/plot. When NAR was applied, fine adjustments would be made according to the actual situation in the field.

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