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Nitric Acid Rain Decreases Soil Bacterial Diversity and Alters Bacterial Community Structure in Farmland Soils

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Abstract: Being regarded as one of the environmental problems endangering biodiversity and ecosystem health, acid rain has attracted wide attention. Here, we studied the effects of nitric acid rain (NAR) on the structure and diversity of microbial communities in agricultural soils by laboratory incubation experiments and greenhouse experiments. Our results indicated that NAR had an inhibitory effect on soil microorganisms, showing a significant reduction in the Chao1 index and Shannon index of soil bacteria. Proteobacteria, Acidobacteriota, Actinobacteriota, and Chloroflexi were the dominant bacterial phyla under NAR stress in this study. NAR significantly reduced the relative abundance of Proteobacteria and Actinobacteria, but significantly increased the relative abundance of Acidobacteriota and Chloroflexi, suggesting that NAR was unfavorable to the survival of Proteobacteria, and Actinobacteria. It is worth noting that the inhibitory or promoting effect of NAR on the dominant bacterial phyla gradually increased with increasing NAR acidity and treatment time. In addition, the study observed that the change in soil pH caused by NAR was the main reason for the change in soil bacterial community structure. In summary, the effects of NAR on soil microorganisms cannot be underestimated from the perspective of sustainable agricultural development.

Keywords: nitric acid rain; bacterial community composition; bacterial community structure; microbial diversity

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1. Introduction

Acid rain (AR) refers to atmospheric precipitation with a pH less than 5.6, which is caused by the transfer of sulfur (S) and nitrogen (N) emissions between different countries and regions [1], and it is a regional atmospheric pollution. In China, coal is the main energy source, and the emission of sulfur dioxide (SO_2) caused by burning coal has been high for a long time [2]. Therefore, sulfuric acid rain (SAR) has been the main type of AR in China. However, in recent years, with the widespread use of desulfurization equipment in China, the emission of SO_2 has gradually decreased [3]. At the same time, the emissions of nitrogen oxides from industrial and agricultural production and vehicle exhaust are on the rise [4]. This phenomenon has led to a shift in the type of AR in China from SAR to nitric acid rain (NAR).

Guangdong province is located in the south of the Chinese mainland. It belongs to a typical subtropical monsoon climate zone, with a meso-subtropical, south-subtropical, and tropical climate from north to south respectively, and it is one of the richest regions in China in terms of light, heat and water resources. Over the past 40 years, Guangdong province has made remarkable achievements in economic development, with its gross domestic product

(GDP) ranking first in the country for 30 consecutive years. However, the continuous socio-economic growth and the high speed of urbanization have also brought about more serious atmospheric pollution problems. Guangdong Province is one of the regions with severe AR pollution in China. Except for Maoming, Yangjiang, Heyuan, and Meizhou, its other cities are all listed as national AR control areas [5]. To alleviate the pressure of air pollution in the province, the government has implemented a series of policies and achieved remarkable results [6]. Nevertheless, some cities in the province still experienced AR in 2022. Therefore, Studying AR is beneficial for the sustainable development of the environment.

Soil microorganisms are an important part of the ecosystem and play an important role in maintaining soil ecological functions [7,8]. Microbial community structure can reflect the quality and health of soil to a certain extent, which is of great value in exploring the functional characteristics of soil and the structural properties of the ecosystem [9]. Previous studies have shown that AR can lead to soil acidification [10–12], which can promote an increase in the concentration of hydrogen ions in soil and cause changes in the composition and structure of soil microbial communities [13]. In addition, soil microbial diversity in farmland soil is closely related to soil quality [14]. Higher microbial diversity can promote soil nutrient cycling [15], improve crop yield and quality [16], and enhance soil resistance and stability [17]. Therefore, the protection and promotion of microbial diversity in farmland is important for maintaining soil quality and achieving sustainable agricultural development. However, at present, there are no uniform conclusions regarding the pattern of soil microbial diversity in response to acid rain. Wang et al. [18] found that AR at pH 5.5 increased soil microbial diversity and richness, while AR at pH 3.5 and 4.5 had no significant effects on soil microbial diversity. However, differently, Zhou et al. [19] showed that Chao1, Shannon, and Simpson indices increased by 9.6–22.5, 3.6–7.4, and 0.15–0.26% under strong AR treatments (i.e., pH 2.5 and pH 3.5), respectively, compared to the control. In addition, another study showed that the soil Chao 1 index decreased by 14.8 and 7.9% after heavy and moderate AR leaching, respectively [20]. Such inconsistent results may be related to the different changes in environmental factors occurring in different soil types.

The effects of acid rain on soil bacterial community structure have also been reported. Zhou et al. [19] found that strong NAR (pH 2.5 and pH 3.5) decreased the relative abundance of Acidobacteria and Actinobacteria. In another study, AR significantly increased the relative abundance of Lysobacter and Rhodanobacter, while significantly decreasing the relative abundance of Massilia [21]. In the past decades, researchers have made many efforts to explore the effects of AR on soil microbial communities, but most of the studies have focused on forest soils, and research on agricultural soils has been more limited. Therefore, the present study was conducted to investigate the effects of NAR on soil microbial diversity and community structure in agricultural soils. In this study, we aimed to (1) investigate how NAR affects the composition of soil bacterial communities, and (2) study how NAR affects the diversity of soil bacterial communities. We hypothesized that NAR would decrease the α-diversity of soil microbial communities and change microbial community structure.

2. Materials and Methods

2.1. Experimental Material

NAR was added as nitric acid (HNO_3) with two gradients (i.e., pH 3.0 and pH 5.0). HNO_3 (AR) was purchased from Guangzhou Congyuan Instrument Ltd., Guangzhou, China. The soils were collected from an ecological farm (23°14′ N, 113°38′ E) in Zengcheng District, Guangzhou City, Guangdong Province, China, with a depth of 0 to 20 cm. The soil was classified as Rhodudult [22] with clay, silt, and sand contents of 48.2%, 29.3%, and 22.5%, respectively. A portion of the soil was sieved through 2 mm and then mixed for laboratory incubation experiments, and the other portion of the soil was laid flat on the cement floor of the greenhouse after removing stones and leaves. The pH of the soil used in the experiments was 5.02, and the contents of soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) were 7.87, 0.97, and 0.64 $g \cdot kg^{-1}$, respectively.

2.2. Leaching Volume of NAR

The leaching amount of NAR was selected from the annual AR amount of 460 mm in 2019 in Guangdong Province published in the Environmental Status Bulletin. Additionally, according to the weather forecast of Guangzhou in 2019, the rainfall days were 152 days, including 25 days in May, 27 days in June, and 26 days in July, and the longest continuous rainfall was 7 days. Therefore, the leaching frequency of this study was designed as once a day, and the leaching rate was 2 $mm \cdot h^{-1}$ [23].

2.3. Experimental Design

In this study, laboratory incubation experiments and greenhouse experiments were conducted. Each experiment consisted of three treatments, namely blank control (CK), HNO_3 at pH 3.0 was considered a strong acid (T_{SA}), and HNO_3 at pH 5.0 was considered a weak acid (T_{WA}). In addition, to ensure the consistency of the leaching volume of AR, the soil weight in the greenhouse experiment was calculated according to the soil bulk density of 1.5 $g \cdot cm^{-3}$.

The greenhouse was completely enclosed to prevent natural precipitation and falling leaves. The plots in the greenhouse experiment were randomly distributed with three replicates per treatment, for a total of 9 plots. The plots were not exposed to NAR prior to the start of the experiment and had no vegetation cover. The area of each plot was 1 m^2, and the plots were separated by PVC expansion sheets with a thickness of 3 cm, and each sheet was spaced 50 cm apart to reduce the interference between each plot. Three automatic sprinkler irrigation devices were installed in each plot, and 5 L of treatment solution was added to each plot daily.

The laboratory incubation experiment was placed in climate-controlled chambers. Each plastic pot (d = 10 cm, h = 9 cm) contained 600 g of soil. Five replicates of each treatment were used for a total of 45. Here, three times for exposure to AR (30, 60, and 90 days) were independently established. All samples were incubated in the dark at 25 ± 1 °C (SD), and 10 mL of treatment solution was added to each pot daily.

The experiments were conducted in three sampling sessions, for 30, 60, and 90 days. At the end of sampling, a portion of the soil samples were frozen in liquid nitrogen and stored at −80 °C for subsequent microbiological analysis. Another portion was stored at 4 °C for soil property determination.

2.4. Soil Physicochemical Analysis

The pH of the soil samples was determined using a pH meter with a ratio of 1:2.5 (w/v) of soil to deionized water [24]. The content of SOC was determined by the potassium dichromate volumetric method [25]. The content of TN was determined by the Kjeldahl method [26], and the content of TP was measured using the method described by Bao [27].

2.5. Microbial DNA Extraction and 16S rDNA Sequencing

Bio-Base-XPure Soil DNA Extraction Kit (Guangzhou, China) was used to extract DNA from the soil samples, and the extraction method was performed according to the instructions provided by the manufacturer. The quality of the extracted DNA samples was examined by 1% agarose gel electrophoresis and stored at −20 °C until final use. Beijing Biomarker Technologies Ltd. (Guangzhou, China). was commissioned to perform 16S rDNA sequencing. We used Trimmomatic (version 0.33) to perform quality filtering on the raw data, and then used Cutadapt (version 1.9.1) to identify and remove primer sequences, followed by splicing of paired-end reads using USEARCH (version 8.1) and removing chimaeras using UCHIME (version 8.1), resulting in high-quality sequences for subsequent analysis. Finally, the sequences were clustered at a similarity level of 97% using USEARCH (version 10.0), with OTUs filtered by default at a threshold of 0.005% of the number of all sequences sequenced.

2.6. Statistical Analysis

The obtained data were processed using Excel 2019 (Microsoft Corporation, State of Washington, DC, USA) and analyzed by repeated measures ANOVA using SPSS 17.0 (IBM, Chicago, IL, USA), with the significance level set at $p < 0.05$, and then graphically plotted using Origin 8.0 (OriginLab, MA, USA). SPSS 17.0 was also used to calculate Spearman correlation coefficients between soil chemical properties and microbial diversity and community structure. In addition, the specific composition of each sample at the phylum level was obtained using QIIME 2 based on the identification of OTU divisions and taxonomic levels. QIIME 2 was also used to calculate α-diversity indices (Chao 1 index and Shannon index). Canonical Correspondence Analysis (CCA) of dominant phyla and environmental factors was performed using the "Vegan" package in R software (Version 4.0.5).

3. Results

3.1. Chemical Characteristics of Soil

The effects of acid rain on pH, SOC, TN, and TP contents showed consistency between the greenhouse experiment and the laboratory incubation experiment (Figures 1 and 2). Specifically, soil pH decreased with increasing acidity of NAR and increasing incubation time, and reached a minimum at 90 days in T_{SA}, which was 3.42 and 2.13% lower than CK in the laboratory incubation experiment and greenhouse experiment, respectively, while the SOC content showed the opposite trend and reached a maximum at 90 days in T_{SA}, which was 3.53 and 3.04% higher than CK in the laboratory incubation experiment and greenhouse experiment, respectively. Although the TN content increased with increasing acidity of NAR during the same incubation time, it showed a trend of increasing and then decreasing with increasing time. In addition, there was no significant difference between the treatments for the TP content ($p > 0.05$).

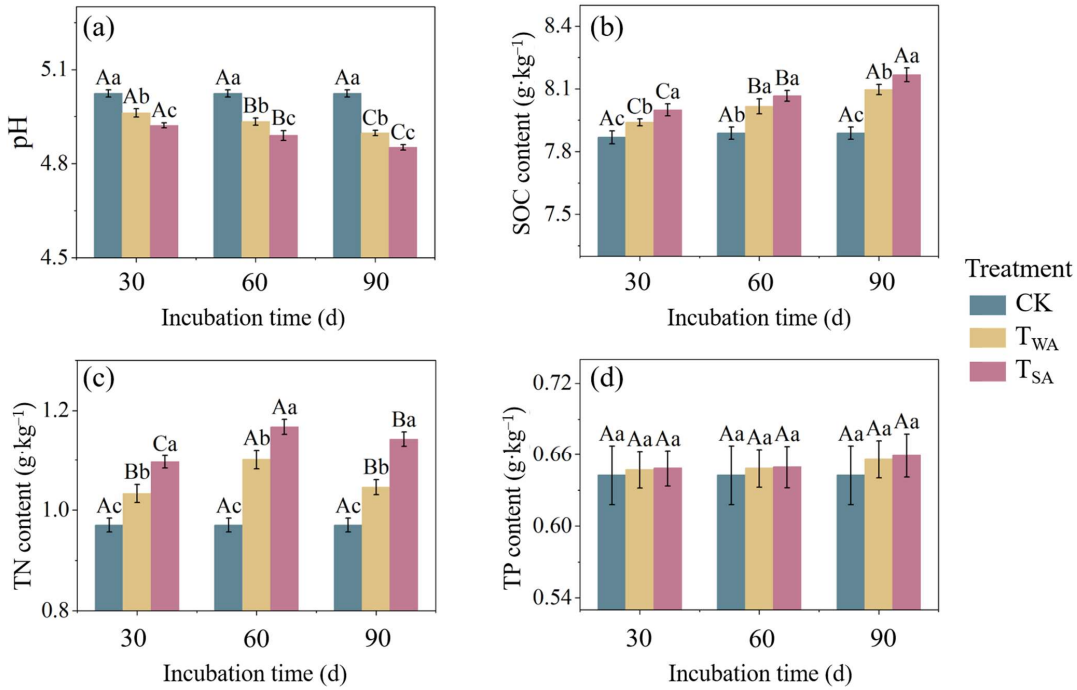

Figure 1. Effects of acid rain on soil chemical properties in laboratory incubation experiment. Different lowercase letters above the columns indicate significant differences between treatments under the same incubation time ($p \leq 0.05$), and different uppercase letters above the columns indicate significant differences between incubation times under the same treatment ($p \leq 0.05$). CK: blank control; T_{WA}: weak acid rain; T_{SA}: strong acid rain. (**a**–**d**) represent different chemical properties as pH, SOC, TN, and TP, respectively. SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus.

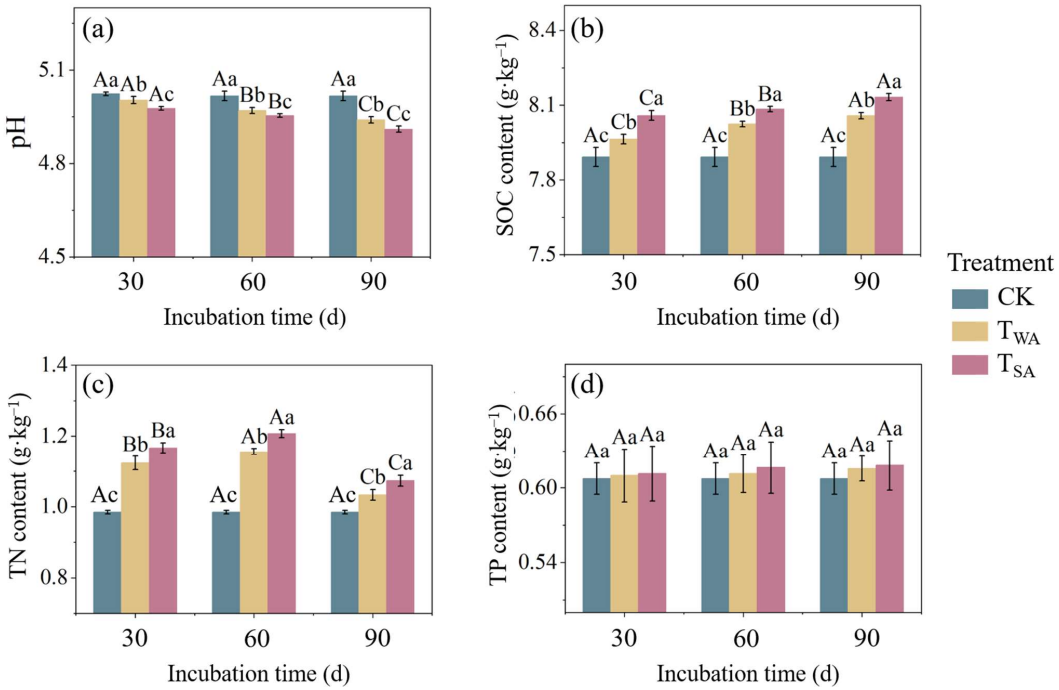

Figure 2. Effects of acid rain on soil chemical properties in greenhouse experiment. Different lowercase letters above the columns indicate significant differences between treatments under the same incubation time ($p \leq 0.05$), and different uppercase above the columns indicate significant differences between incubation times under the same treatment ($p \leq 0.05$). CK: blank control; T_{WA}: weak acid rain; T_{SA}: strong acid rain. (**a**–**d**) represent different chemical properties as pH, SOC, TN, and TP, respectively. SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus.

3.2. Change in Soil Bacterial Diversity

As can be seen from Tables 1 and 2, the Chao1 richness and Shannon index of soil bacteria varied significantly between treatments during the same incubation time ($p < 0.05$). Compared with CK, NAR treatments significantly decreased Chao1 richness and Shannon index ($p < 0.05$). In the same NAR treatment, Chao1 richness and Shannon index also tended to decrease with increasing time. To be specific, compared with the 30-day T_{SA}, the Chao1 richness and Shannon index in the 90-day T_{SA} significantly reduced by 38.71 and 46.72%, respectively, in the laboratory incubation experiment. Similarly, in the greenhouse experiment, both significantly reduced by 24.94 and 42.06%, respectively, in the 90-day T_{SA}.

Table 1. Bacterial Chao1 richness under acid rain.

Experiment Type	Incubation Time (Days)	CK	T_{WA}	T_{SA}
Laboratory incubation	30	750 ± 51 Aa	689 ± 66 Ab	589 ± 33 Ac
	60	748 ± 46 Aa	730 ± 11 Aa	491 ± 57 Bb
	90	752 ± 21 Aa	560 ± 30 Bb	361 ± 32 Cc
Greenhouse	30	1487 ± 33 Aa	1117 ± 94 Ab	850 ± 54 Ac
	60	1483 ± 31 Aa	1047 ± 47 ABb	732 ± 17 Bc
	90	1484 ± 35 Aa	958 ± 36 Bb	638 ± 22 Cc

CK: blank control; T_{WA}: weak acid rain; T_{SA}: strong acid rain. Different lowercase letters in the same row indicate significant differences between treatments under the same incubation time ($p < 0.05$); different uppercase letters in the same column indicate significant differences between incubation times under the same treatment ($p < 0.05$).

Table 2. Bacterial Shannon diversity index under acid rain.

Experiment Type	Incubation Time (Days)	CK	T_{WA}	T_{SA}
Laboratory incubation	30	7.04 ± 0.07 Aa	6.56 ± 0.07 Ab	6.40 ± 0.16 Ab
	60	7.03 ± 0.06 Aa	6.09 ± 0.05 Bb	5.19 ± 0.05 Bc
	90	7.03 ± 0.05 Aa	4.39 ± 0.04 Cb	3.41 ± 0.03 Cc
Greenhouse	30	8.72 ± 0.09 Aa	7.75 ± 0.06 Ab	6.80 ± 0.09 Ac
	60	8.73 ± 0.19 Aa	7.63 ± 0.47 Ab	5.81 ± 0.15 Bc
	90	8.74 ± 0.16 Aa	5.93 ± 0.04 Bb	3.94 ± 0.02 Cc

CK: blank control; T_{WA}: weak acid rain; T_{SA}: strong acid rain. Different lowercase letters in the same row indicate significant differences between treatments under the same incubation time ($p < 0.05$); different uppercase letters in the same column indicate significant differences between incubation times under the same treatment ($p < 0.05$).

3.3. Change in Soil Bacterial Community Structure

The results of the soil bacterial community structure analysis are shown in Figures 3 and 4. Nine known phyla that ranked in the top ten in relative abundance in all soil samples throughout the incubation period, and there were four dominant phyla that were common to both the laboratory incubation experiment and the greenhouse experiment, namely Proteobacteria, Actinobacteriota, Acidobacteriota, and Chloroflexi. Among them, the relative abundance of Proteobacteria and Actinobacteriota was higher in CK than in NAR treatments, while Acidobacteriota and Chloroflexi abundance was higher in NAR treatments, especially in T_{SA} than in CK.

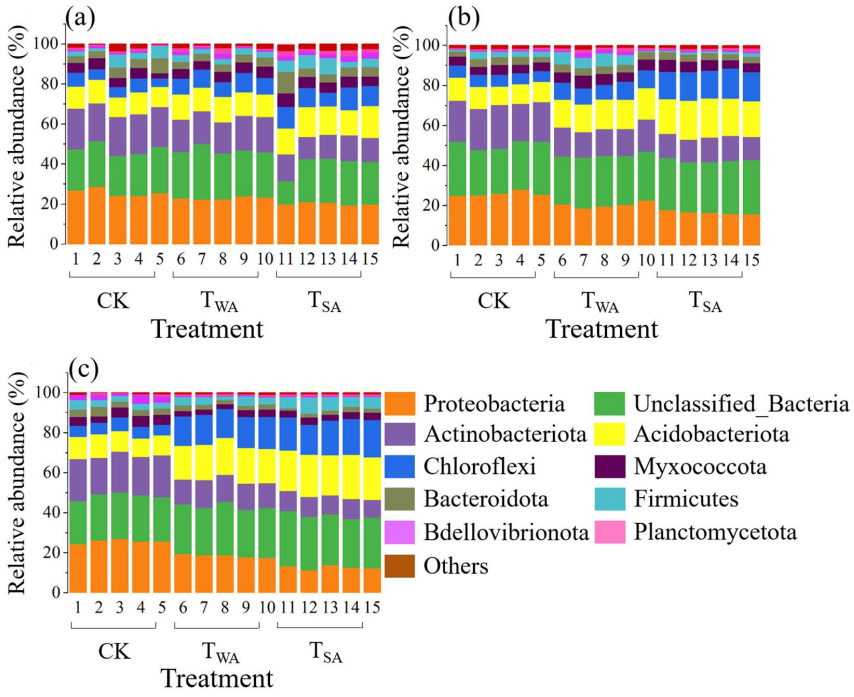

Figure 3. Relative abundance of soil bacteria at phylum level under acid rain in laboratory incubation experiment. (**a**–**c**) represent 30 days, 60 days, and 90 days, respectively. Numbers 1 to 5 represent CK repeats; numbers 6 to 10 represent T_{WA} repeats; numbers 11 to 15 represent T_{SA} repeats. CK: blank control; T_{WA}: weak acid rain; T_{SA}: strong acid rain.

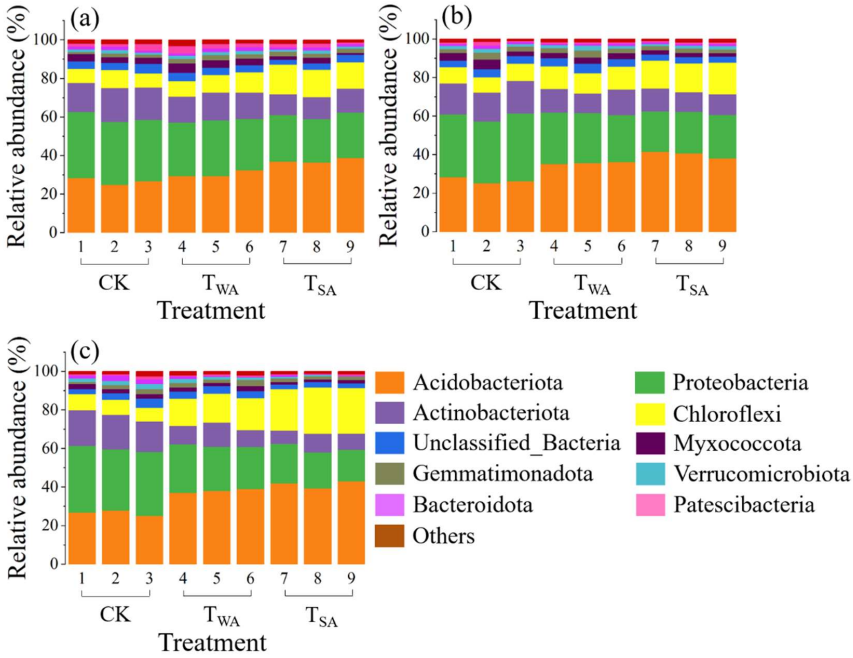

Figure 4. Relative abundance of soil bacteria at phylum level under acid rain in greenhouse experiment. (**a**–**c**) represent 30 days, 60 days, and 90 days, respectively. Numbers 1 to 3 represent CK repeats; numbers 4 to 6 represent T_{WA} repeats; numbers 7 to 9 represent T_{SA} repeats. CK: blank control; T_{WA}: weak acid rain; T_{SA}: strong acid rain.

The relative abundance of the shared dominant phyla was tested for significance of differences and the results are shown in Figures 5 and 6. Compared with CK, NAR treatments (T_{WA} and T_{SA}) significantly reduced the relative abundance of Proteobacteria and Actinobacteria ($p < 0.05$), but significantly increased the relative abundance of Acidobacteria and Chloroflexi ($p < 0.05$).

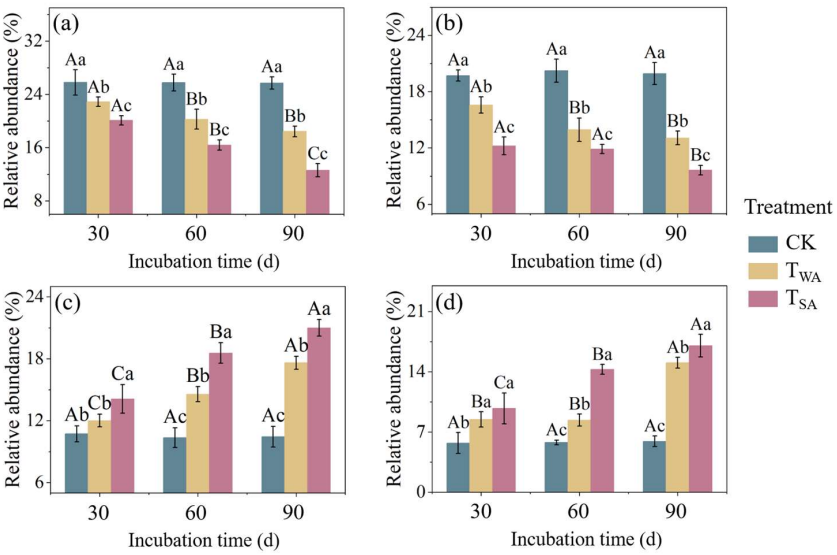

Figure 5. Effects of acid rain on the dominant bacterial phylum in laboratory incubation experiment. (**a**–**d**) represent the dominant phyla, which are Proteobacteria, Actinobacteriota, Acidobacteriota, and Chloroflexi. Different lowercase letters above the columns indicate significant differences between treatments under the same incubation time ($p \leq 0.05$), and different uppercase letters above the columns indicate significant differences between incubation times under the same treatment ($p \leq 0.05$). CK: blank control; T_{WA}: weak acid rain; T_{SA}: strong acid rain.

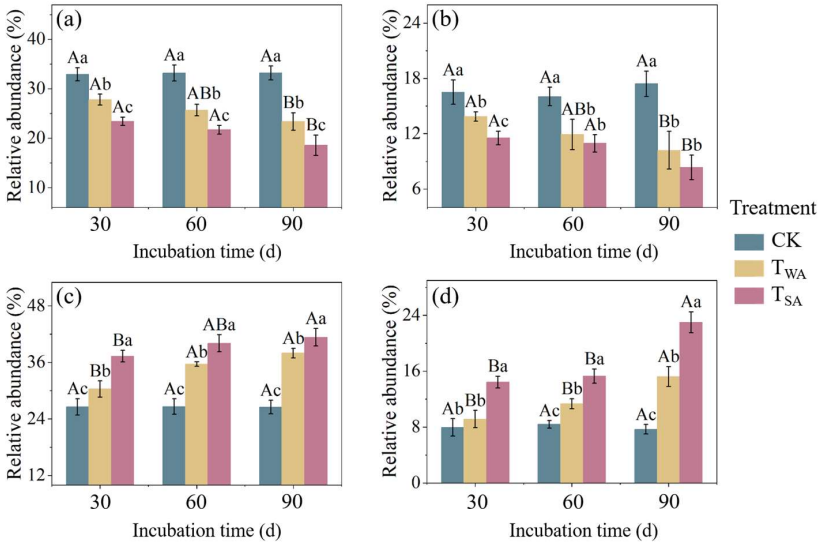

Figure 6. Effects of acid rain on the dominant bacterial phylum in greenhouse experiment. (**a**–**d**) represent the dominant phyla, which are Proteobacteria, Actinobacteriota, Acidobacteriota, and Chloroflexi. Different lowercase letters above the columns indicate significant differences between treatments under the same incubation time ($p \leq 0.05$), and different uppercase letters above the columns indicate significant differences between incubation times under the same treatment ($p \leq 0.05$). CK: blank control; T_{WA}: weak acid rain; T_{SA}: strong acid rain.

A comparison of the differences between T_{WA} and T_{SA} revealed that the relative abundance of the Chloroflexi was not significantly different between the two in the laboratory incubation experiment at 30 days ($p > 0.05$) (Figure 5d), whereas in the greenhouse experiment—except for the relative abundance of the Actinobacteria, which was not significantly different between T_{WA} and T_{SA} at 60 and 90 days ($p > 0.05$) (Figure 6b)—the relative abundance of all the other dominant phyla differed significantly between the two treatments ($p < 0.05$).

A comparison of the differences between different incubation times under the same treatment revealed that the relative abundance of Proteobacteria and Actinobacteria significantly decreased at 90 days compared with 30 days ($p < 0.05$). The opposite was true for Acidobacteria and Chloroflexi.

Overall, the relative abundance of Proteobacteria and Actinobacteria tended to decrease with increasing acid rain acidity and time throughout the incubation period, while the relative abundance of Acidobacteria and Chloroflexi tended to increase with increasing acid rain acidity and time.

3.4. Soil Chemical Properties in Relation to Microbial Diversity and Community Composition

3.4.1. Relationship between Soil Chemical Properties and Microbial Diversity

A Spearman correlation analysis of soil bacterial diversity with soil chemical properties showed that Shannon and Chao1 indices were significantly and positively correlated with soil pH ($p < 0.01$), whereas they were significantly and negatively correlated with SOC and TN contents ($p < 0.01$). In addition, Shannon and Chao1 indices were not significantly correlated with TP content ($p > 0.05$) (Table 3).

Table 3. Spearman coefficients of correlation between soil bacterial community α-diversity indices and soil chemical properties.

Experiment Type	α-Diversity	pH	SOC	TN	TP
Laboratory incubation	Shannon	0.858 **	−0.880 **	−0.722 **	−0.234
	Chao1	0.865 **	−0.800 **	−0.742 **	−0.161
Greenhouse	Shannon	0.865 **	−0.946 **	−0.618 **	−0.223
	Chao1	0.889 **	−0.932 **	−0.694 **	−0.261

** indicates significant correlation at the 0.01 level (two-tailed). SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus.

3.4.2. Relationship between Soil Chemical Properties and Microbial Community Composition

In the laboratory incubation experiment, the first axis explained a variation of 79.5%, the second axis was 0.4%, and the cumulative explanatory variable for the two axes was 79.9% (Figure 7a). In particular, soil pH (R^2 = 0.57, p = 0.002) was the key factor affecting the variation in the relative abundance of dominant phyla. In the greenhouse experiment, the first axis explained a variation of 71.4%, the second axis was 2.4%, and the two-axis cumulative explanatory variable was 73.8% (Figure 7b). Similarly, soil pH (R^2 = 0.53, p = 0.001) was the key influence factor.

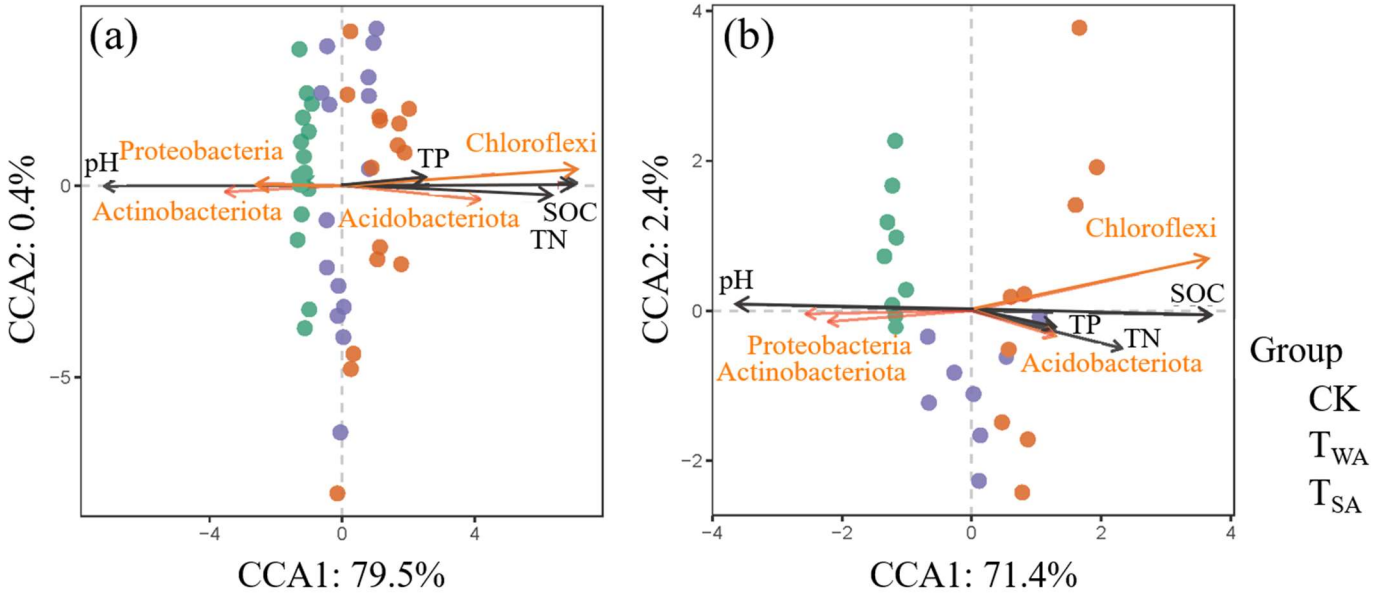

Figure 7. Canonical Correspondence Analysis (CCA) of dominant phyla and environmental factors. (**a**) represents laboratory incubation experiment; (**b**) represents greenhouse experiment. SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus.

The results of correlation analysis showed (Table 4) that soil pH value was significantly positively correlated with the relative abundance of Proteobacteria and Actinobacteria, while it was significantly negatively correlated with the relative abundance of Acidobacteria and Chloroflexi ($p < 0.01$). Conversely, the SOC and TN contents were significantly negatively correlated with the relative abundance of Proteobacteria and Actinobacteria, while they were significantly positively correlated ($p < 0.01$) with the relative abundance of Acidobacteria and Chloroflexi ($p < 0.01$). In addition, soil TP content was not significantly correlated with the relative abundance of any of the four dominant phyla ($p > 0.05$).

Table 4. Spearman coefficients of correlation between the relative abundance of dominant phyla of soil bacteria and soil chemical properties.

Experiment Type	The Relative Abundance	pH	SOC	TN	TP
Laboratory incubation	Proteobacteria	0.831 **	−0.812 **	−0.768 **	−0.246
	Actinobacteriota	0.815 **	−0.784 **	−0.762 **	−0.288
	Acidobacteriota	−0.827 **	0.819 **	0.745 **	0.198
	Chloroflexi	−0.794 **	0.789 **	0.679 **	0.287
Greenhouse	Proteobacteria	0.771 **	−0.768 **	−0.681 **	−0.185
	Actinobacteriota	0.778 **	−0.794 **	−0.587 **	−0.234
	Acidobacteriota	−0.796 **	0.735 **	0.656 **	0.232
	Chloroflexi	−0.799 **	0.748 **	0.592 **	0.065

** indicates significant correlation at the 0.01 level (two-tailed). SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus.

4. Discussion

4.1. Effects of Nitric Acid Rain on Soil Chemical Properties

The results of this study showed that NAR significantly decreased soil pH, but kept a higher SOC and TN contents and had no significant effect on TP content. AR may weaken soil acid buffering capacity by increasing the leaching of salt-based ions from the soil and decreasing the exchangeable cation contents, thereby causing soil acidification [28]. In this study, the acidification effect of strong NAR (pH 3.0) treatment was found to be significantly higher than that of weak NAR (pH 5.0) treatment, which is a result of the large input of hydrogen ions (H^+) by strong NAR. In addition, the decrease in soil pH with incubation time in this study indicated that the acidification of the soil was continuously increasing under continuous AR leaching, which is consistent with the results of previous studies [29,30]. It has been found that soil pH is significantly negatively correlated with SOC content [31], suggesting that relatively low soil pH may be beneficial for SOC accumulation. The reason for this may be that soil acidification induced by AR causes a significant decrease in soil microbial biomass carbon content [32]. Another possible reason is that AR inhibits the activity of enzymes involved in the SOC cycle [33]. The decrease in microbial biomass and related enzyme activities ultimately leads to a decrease in microbial utilization of C sources, which facilitates the accumulation of SOC. It was also found that AR converted a large amount of non-available N into available N in the soil [34], and NAR also input a large amount of nitrate nitrogen (NO_3^-), resulting in an increase in TN content. However, the amount of N loss was gradually greater than the amount of conversion with increasing time, which may explain why the TN content showed a first increase and then a decrease in this study. In addition, NAR had no significant effect on TP content in the present study, this may be that the P content in the soil is relatively low and exists in the forms of ferric phosphate and other related phosphate compounds which are not readily soluble in acidic soils.

4.2. Effects of Nitric Acid Rain on Soil Microbial Diversity

Chao1 index and Shannon index are two important indicators of bacterial diversity, which are important markers of soil health [35] and represent the complexity and stability of bacterial communities [36,37]. Among them, the Shannon index reflects the species diversity and the Chao1 index reflects the species richness. The results of this study showed that NAR significantly decreased Chao1 richness and Shannon diversity index of bacteria ($p < 0.05$), which may be due to some bacterial populations were unable to adapt or even died as a result of the decrease in soil pH [38]. The present results are consistent with previous findings. Zhang et al. [39] found that the number of soil bacteria decreased with the decrease of the pH value of AR. Sun and Quan [34] also found that AR significantly reduced the richness of the bacterial community. These researches suggest that AR can inhibit the growth and reproduction of most bacteria in the soil [40], and this inhibition increases with increasing acidity of AR.

In addition, this study found that the soil microbial diversity under NAR showed a decreasing trend with increasing treatment time. A possible reason is that species which are not adapted to the soil acidification environment are gradually eliminated with the increase of time of AR leaching [41].

4.3. Effects of Nitric Acid Rain on Soil Bacterial Composition

Bacterial communities are widely distributed in agricultural soils and play important roles in ecosystem and soil nutrient transformation [12,42,43]. Currently, microbial communities have been studied in different ecosystems [44], such as forests, grasslands, tundra, and deserts. Numerous studies have found that Proteobacteria, Acidobacteriota, Actinobacteriota, and Chloroflexi were the dominant phyla in bacterial communities [45–48], which is consistent with our results. Proteobacteria has a wide ecological niche and is highly adaptable to the soil environment [49]. Actinobacteria is Gram-positive bacteria with strong metabolic and biosynthetic capabilities, and it has been reported that large numbers of Actinobacteria exist in acidic soils [50]. Acidobacteria is also widely distributed in acidic soils due to its high sensitivity to soil pH and its acidophilic properties [36]. Chloroflexi has a strong ability to resist environmental changes and can exist in nutrient-poor soils [51]. Our results showed that Proteobacteria, Actinobacteriota, Acidobacteriota, and Chloroflexi were the dominant phyla in the present study, indicating that these four phyla were adapted to acidic environments. However, NAR increased the relative abundance of Acidobacteria and Chloroflexi, but decreased the relative abundance of Proteobacteria and Actinobacteria, indicating that there are differences in their response to acidic environments. This is consistent with the fact that eutrophic groups (Proteobacteria, Actinobacteria) decrease with AR leaching, while oligotrophic groups (Acidobacteria, Chloroflexi) exhibit the opposite pattern [52].

4.4. Drivers of Microbial Diversity and Community Composition under Nitric Acid Rain Stress

AR reduces soil pH, and changes in soil pH drive changes in bacterial diversity. Studies have found that soil chemical properties are related to soil bacterial diversity [53–55]. Further studies indicated that changes in soil bacterial community diversity are mainly dependent on soil pH values [56,57]. In this study, Spearman correlation analysis showed that soil pH was significantly and positively associated with Chao1 richness and Shannon diversity index ($p < 0.01$) under the NAR treatment. Therefore, the bacterial diversity indices were the lowest in the strong NAR treatment (pH 3.0) among all the samples. This is similar to the findings of Fierer and Jackson [58]. They collected soil samples from North and South America and found the lowest bacterial diversity in the Peruvian Amazon, where the soil was the most acidic. Bacteria generally prefer neutral conditions and are sensitive to changes in soil pH [59]. In acidic environments, bacterial growth is highly susceptible to inhibition [60], resulting in a decrease in diversity. However, there are also some studies with opposite conclusions. He et al. [61] reported that AR at pH 2.5 significantly improved Chao1 and Shannon indices of soil microorganisms, and Zhou et al. [19] also found that NAR at pH 2.5 significantly increased Chao1 and Shannon indices of bacterial communities in northern subtropical forest soil. This difference in results can be attributed to the "fertilizer effect" of AR [18]. When AR with strong acidity (e.g., pH 2.5) enters the soil, the stimulatory effect of the fertilizer effect is higher than the inhibitory effect of acidity, leading to an increase in soil bacterial diversity. However, the "fertilizer effect" of AR is likely to be short-term. This is because, with the extension of AR leaching time, there is a gradual loss of salt-based cations (e.g., Ca^{+}, Na^{+}, Mg^{2+}), leading to nutrient scarcity in the soil [62] and further acidification of the soil [63], all of which are not conducive to the growth of microorganisms in the soil. In addition, lower soil pH could affect soil microbial activity, leading to a decrease in the decomposition rate of SOC [64], thus forming a significant negative correlation between SOC and bacterial diversity indices ($p < 0.01$).

Changes in soil chemical properties are bound to affect microbial community composition as well [65]. The CCA analysis and Spearman correlation analysis of this study

indicated that soil pH was an important control factor for changes in microbial community structure, which showed a highly significant negative correlation with the relative abundance of Acidobacteria and Chloroflexi, while showing a highly significant positive correlation with the relative abundance of Proteobacteria and Actinobacteria ($p < 0.01$). Previous studies gave the same results. Gao et al. [66] confirmed that pH is the main factor affecting soil microbial communities. This phenomenon may be related to acidic environments altering competitive outcomes [67], as acidic environments are more favorable for the growth and reproduction of oligotrophic groups (Acidobacteria, Chloroflexi), while eutrophic groups (Proteobacteria, Actinobacteria) have poorer acid tolerance than oligotrophic groups [68] and are in a competitive disadvantage under acidic conditions. This study also found that SOC and TN contents were significantly correlated with the relative abundance of dominant bacterial phyla. Maestre et al. [69] obtained the same conclusion in their study. Their results showed that SOC content was strongly correlated with the relative abundance of the dominant bacterial phylum. As a limiting nutrient in soil [70], N is equally relevant to changes in soil bacterial community structure [71]. Magill and Aber [72] and Li et al. [73] found that the relative abundance of Acidobacteria gradually increased with the increase in soil TN content. This suggests that changes in soil C/N ratio also have a direct effect on bacterial community structure [74].

5. Conclusions

In this study, NAR significantly decreased the bacterial Chao1 richness and Shannon index, both of which decreased with increasing NAR acidity and treatment time, and the soil pH was the key factor driving the changes in soil bacterial community structure. Proteobacteria, Actinobacteriota, Acidobacteriota, and Chloroflexi were the dominant phyla in the present study, but the relative abundance of the dominant phyla varied significantly among treatments. Our findings suggest that soil acidification caused by NAR can alter the structure and diversity of bacterial communities in farmland soils.

In order to reduce the impact of acid rain on eco-environment, we propose the following prevention and control countermeasures: (1) to optimize and adjust the industrial structure and plan the layout of clean energy utilization; (2) to vigorously develop and promote the application of environmentally friendly, efficient, clean, and energy-saving technologies; and (3) to strengthen environmental management and global environmental governance.

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