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Simulated Nitrogen Deposition Decreases the Ratios of Soil C to P and N to P, Changes Soil Enzyme Activity, and Reduces Soil Microbial Biomass in Paddy Soil in Southern China

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Abstract: There have been few studies on the impact of nitrogen deposition on paddy field ecosystem; therefore, we evaluated the effects of different N deposition levels (0, 40, and 120 kg N·ha^{-1}) with the conventional nitrogen rate (180 kg N·ha^{-1}) on rice field ecosystem through two-season experiments. The results showed that 40 and 120 kg·ha^{-1} nitrogen deposition had no significant effect on rice yield, although the rice grains per panicle and the 1000-grain weight increased. The 40 and 120 kg·ha^{-1} nitrogen deposition levels had no significant effect on rice and soil total carbon/total nitrogen (TC/TN) in the two-season experiment; however, 40 and 120 kg·ha^{-1} nitrogen deposition significantly increased TP content of the rice root and soil in a short time, and continuous 120 kg·ha^{-1} nitrogen deposition significantly decreased TP content of the rice root and significantly increased TP content of the rice stem. In addition, nitrogen deposition significantly reduced total carbon/total phosphorus (TC/TP) and total nitrogen/total phosphorus (TN/TP) in the soil. The activities of soil acid phosphatase (S-ACP), β-glucosidase (S-β-GC), and N-acetyl-β-D-glucosidase (S-NAG) increased under 40 kg·ha^{-1} nitrogen deposition, while the activities of S-β-GC and S-NAG decreased under 120 kg·ha^{-1} nitrogen deposition compared with 40 kg·ha^{-1}. The microbial carbon, microbial nitrogen, microbial phosphorus, and fungal microbial biomass reduced under 40 and 120 kg·ha^{-1} nitrogen deposition. These findings suggest that, under short-term N deposition, rice and soil can adjust the C, N, P, and even the nutrient balance by themselves; however, continuous nitrogen deposition may have adverse reactions to microorganisms, thereby disrupting this balance and ultimately leading to the deterioration of paddy soil environment and a reduction in rice yield in the long term.

Keywords: nitrogen deposition; nutrient balance; soil enzyme; microbial biomass; microbial community

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1. Introduction

With the rapid development of the world, environmental problems are becoming increasingly serious. The combustion of fossil fuels, such as coal, oil, and natural gas, the emission of vehicle exhausts, and the excessive application of fertilizers release a large amount of greenhouse gases, such as carbon dioxide (CO_2), sulfur dioxide (SO_2), nitrous oxide (N_2O), methane (CH_4), etc., into the atmosphere [1]. These gases undergo a series of chemical reactions in the atmosphere, eventually forming nitrates and sulfates, and return to the ground in the forms of dry and wet depositions [2]. A dry deposition refers to the

phenomenon in which acidic substances, such as nitrates and sulfates, in the atmosphere are adsorbed by plants or are deposited on the surface by gravity, while a wet deposition refers to the carbon dioxide, methane, and acidic substances, such as nitrates and sulfates, rained from the atmosphere after a reaction with water vapor [3]. Nitrogen (N) deposition, as one of the forms of acid deposition, seriously affects the balance of the ecosystem, resulting in aquatic eutrophication, which reduces the quality of the water, the photosynthesis of water plants, and the biodiversity in the water [4]. Moreover, large N depositions in the soil acidify the soil and reduce its quality and microbial diversity, thereby declining the crop yields [5].

China is one of the fastest developing countries in recent years and one of the countries most seriously affected by N deposition [6]. Rice is the main food crop for nearly 50% of the world's population of which 90% is produced in Asia. China is one of the major rice-producing countries in the world, with a planting area of about 30 million hectares and a total output of more than 212 million tons [7]. At present, the research on N deposition mainly focuses on the field of forest, grassland, and desert ecosystems (Supplementary File). Some studies have found that short-term N deposition can promote the growth of grassland aboveground parts and improve productivity and that it promotes soil respiration [8,9]. However, N deposition decreases the forest soil pH, organic carbon content (SOC), and total phosphorus content, while it increases the nitrate-N content [5]. By changing these environmental factors, affecting the activities of hydrolase and oxidase in the soil, N deposition impacts the SOC pool and nutrient cycle [5]. Additionally, N deposition decreases the inorganic N content of desert soil, alters the soil microbial biomass and activity, and changes the soil microbial structure [10]. In contrast, some researches have shown that N deposition increases the microbial biomass and the enzymatic activities of urease, protease, and catalase in wetland soil [8]. In addition, a long-term experiment demonstrated that the N input affects the availability of soil nutrients and causes changes in the ability of microorganisms to utilize nutrients, thereby changing the community structure of the soil microorganisms and the function [11,12]. However, there are few reports on the responses of farmland ecosystems to N deposition.

Therefore, the current study tried to contribute substantially to fill the knowledge gap on the impact of N deposition on farmland ecosystems and whether or not it reduces the rice yield and changes the nutrient status and community structure of paddy soil microorganisms. Accordingly, this study hypothesized the following objectives: (1) Low N deposition will promote rice growth, while a high deposition rate will inhibit rice growth. (2) When N deposition reaches a relatively high level, the soil microbial biomass and community diversity will decrease, resulting in a yield decline. This study aimed to explore the effects of different N deposition levels on rice paddy systems through two-season rice field experiments.

2. Materials and Methods

2.1. Experimental Site

Field site was located at Zengcheng Teaching and Research Farm Base (23°14′ N, 113°38′ E), South China Agricultural University, Guangzhou, Guangdong Province, China. The trial site belongs to a subtropical monsoon climate with warm winters and hot summers.

The soil in the experimental site was sandy loam with pH of 6.01, 15.80 $g \cdot kg^{-1}$ of organic matter, 18.70 $g \cdot kg^{-1}$ of total carbon (TC), 1.93 $g \cdot kg^{-1}$ of total nitrogen (TN), 0.51 $g \cdot kg^{-1}$ of total phosphorous (TP), 10.91 $g \cdot kg^{-1}$ of total potassium, 9.06 $mg \cdot kg^{-1}$ of ammonium N (NH_4^+-N), 4.69 $mg \cdot kg^{-1}$ of nitrate N (NO_3^-N), 43.82 $mg \cdot kg^{-1}$ of available phosphorus, and 47.56 $mg \cdot kg^{-1}$ of available potassium.

2.2. Experimental Design

A field trial was conducted from April 2021 to November 2021, including two rice (*Oryza sativa* L., Huanghuazhan) growing seasons (early season from April 2021 to July 2021; late season from August to November 2021). There were three treatments with three replicates: conventional rice monocropping with 180 kg ha^{-1} nitrogen (N) fertilizer with 0, 40, and 120 kg ha^{-1} N deposition. The optimum application level of N fertilizer in rice

conventional monocropping was based on the previous studies [13,14]. Hereafter, the three treatments are labeled as zero nitrogen deposition (ZN, 180 kg ha^{-1} N), low nitrogen deposition (LN, 220 kg ha^{-1} N), and high nitrogen deposition (HN, 300 kg ha^{-1} N). The above N deposition levels were based on previous findings of Zhang et al. [15], who reported 42 kg·ha^{-1} to be the average precipitation N deposition level in the farmland ecosystem of the Pearl River Delta over the recent years. Treatments were arranged in a randomized complete block design. The field plot area of each replicate was 25 m^2 (5 m × 5 m), the row spacing of rice was 0.2 m, and each plot was irrigated and drained independently. Urea (N content: 46%) was the N fertilizer source representing conventional rice monocropping and was applied directly in the pretransplantation, tillering, heading, and mature stages of rice growth at ratios of 40%, 20%, 30%, and 10%, respectively. Ammonium nitrate was sprayed after dissolving in water as deposition source in three equal doses in the regreening, booting, and grain-filling stages of rice growth. Germinated seeds were sown on March 10 (2021 early season) and on July 15 (2021 late season) for nurseries, rising with 15 kg ha^{-1} urea fertilizer application to rice seedlings. Rice seedlings (three-leaf and one-leaflet stage) were then simultaneously transplanted into the experimental field. The management methods for fertilization were the same for both seasons. No pesticides, herbicides, or other weed control practices were applied for any treatments.

2.3. Sampling

2.3.1. Plant Samplings

In the rice maturity stage, three plots of 1 × 1 m^2 were selected in each replicate; the rice plants were harvested, threshed, and sun-dried (water content less than 14%), and the rice yield, number of grains per panicle, and seed-setting rate were measured. A plot of 1 m^2, including 25 rice plants, was randomly selected in each replicate, and the plant height, tiller number, and effective panicle number were measured through counting method; then, the rice plants were taken to laboratory, and each plant was separated into four parts, including rice, leaves, stems, and roots, in 2021 late season and two parts, including shoot and root, in 2021 early season. After the roots, stems, and leaves were cleaned with deionized water, they were dried to constant weight at 65 °C after being greened at 105 °C for two hours and their dryweights were measured [16]. Samples were then ground into a fine powder in a pulverizer to determine the plant physicochemical properties, i.e., total carbon, total nitrogen, and total phosphorus content.

2.3.2. Soil Samplings

In the maturity stage of rice, the rhizosphere soil of five rice plants in each plot was mixed into a soil sample and divided into three subsamples. One subsample was air-dried and sieved to determine soil physicochemical properties, i.e., soil pH value; TC; TN; TP content; and enzyme activity, including soil cellobiosidase (S-C1), β-glucosidase (S-β-GC), N-acetyl-β-D-glucosidase (S-NAG), and acid phosphatase (S-ACP). Another subsample was kept fresh to measure the microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial biomass phosphorus (MBP), and bacterial abundance, while the third subsample was freeze-dried to extract the phospholipid fatty acids (PLFAs).

2.4. Measurements and Determinations

The TC and TN contents in soil and plant samples were measured with Vario TOC element analyzer (Elementar, Hanau, Germany) through combustion method. The TP content in soil and plant samples was determined through UV spectrophotometry (UV-1750, Shimadzu International Trading (Shanghai) Co., Ltd., Shanghai, China) after digestion with H_2SO_4-$HClO_4$ [17].

According to previous research, S-C1, S-β-GC, S-NAG, and S-ACP activities are closely related to the C, N, and P transfer [18]. The activity of these four soil enzymes was measured to indicate the characteristics of soil processes, and determined with microplate reader through the method of multiwell-plate fluorescence spectrometry.

2.5. Soil Microbial Biomass

The chloroform fumigation and PLFA methods were used to determine the soil microbial biomass. In brief, two samples (10 g of fresh soil) were extracted using 0.5 mol L^{-1} K_2SO_4 and $NaHCO_3$ solutions with 24 h chloroform fumigation under vacuum conditions and without fumigation, respectively. Then, the extracts were filtered through filter paper, and the filtrates were used to quantify the dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) content using the Vario TOC element analyzer (K_2SO_4 solutions) as well as the dissolved organic phosphorus (DOP) content through UV spectrophotometry ($NaHCO_3$ solutions) [19]. The microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorus (MBP) were calculated as the difference in DOC, DON, and DOP contents between fumigated and nonfumigated soil extracts with correction factor of 0.45, 0.54, and 0.40 [20].

$$MBC = (DOC_{fumigated} - DOC_{nonfumigated})/0.45$$

$$MBN = (DON_{fumigated} - DON_{nonfumigated})/0.54$$

$$MBP = (DOP_{fumigated} - DOP_{nonfumigated})/0.40$$

The PLFA analysis was conducted based on previous studies [21]. Briefly, the PLFAs were extracted from 8 g of freeze-dried soil using phosphate-buffer–chloroform–methanol solutions and were then eluted with chloroform, acetone, and methanol. The collected PLFAs were identified using the Sherlock Microbial Identification System (Version 6.2, MIDI Inc., Newark, DE, USA) in Agilent 7890A GC and were quantified on the basis of 19:0 (internal standard). Finally, several microbial groups (bacteria, Gram-positive bacteria (G+), Gram-negative bacteria (G−), Gram-positive bacteria/Gram-negative bacteria ratio (G+/G−), actinobacteria, fungi, fungi/bacteria ratio (F/B), arbuscular mycorrhizal fungi (AMF), sulfate-reducing bacteria (SRB), and methane-oxidizing bacteria (MOB)) were classified by summing the specific PLFAs.

2.6. Soil Bacterial Community Structure by Determining 16S RNA Genes

Soil bacterial community structure was determined using alpha diversity and abundance of each genus, which were amplified 16S rRNA genes with universal primers F338 (ACTCCTACGGGAGGCAGCAG) and R806 (GGACTACHVGGGTWTCTAAT), and using the Majorbio Cloud Platform for interactive analysis. Alpha diversity analysis results were expressed by Shannon, Simpson, Ace, and Chao indices. The abundance of each genus was used to analyze the significance among different treatments through multivariate variance analysis, and the results were expressed by R^2 and p value.

2.7. Data Analysis

Statistical analyses were performed using SPSS 26.0. Data are presented as the mean value and standard error. The significance level among the three treatments was statistically analyzed through Duncan multiple comparison tests and one-way ANOVA. The MANOVA was used to analyze the significance between control and N deposition treatments ($p < 0.05$).

3. Results

3.1. Rice Yield and Biomass

In the early season, the HN treatment significantly increased the 1000-grain weight of the rice by 20.3% compared with ZN. In the late season, the HN treatment significantly increased the grains per panicle by 88.36% relative to ZN and significantly increased the 1000-grain weight by 11.2% compared with LN. However, N deposition did not have a significant effect on the rice yield, number of tillers, number of effective panicles, dry weight, or root–shoot ratio (Table 1).

Table 1. Rice yield and biomass under nitrogen deposition treatments. ES and LS refer to 2021 early and late seasons, respectively. ZN, LN, and HN refer to zero nitrogen deposition, low nitrogen deposition, and high nitrogen deposition, respectively. The data are expressed as mean ± standard error. Different lowercase letters denote a significant difference ($p < 0.05$).

		Yield (t·ha^{-1})	Number of Tillers	Number of Effective Panicles	Grains per Panicle	1000-Grain Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Root–Shoot Ratio
ES	ZN	6.72 ± 0.05 a	13.73 ± 1.07 a	13.13 ± 0.97 a	76.75 ± 2.52 a	24.55 ± 0.31 b	44.52 ± 4.78 a	2.20 ± 0.49 a	0.049 ± 0.008 a
	LN	5.72 ± 0.20 a	12.73 ± 0.37 a	11.60 ± 0.64 a	84.94 ± 13.10 a	23.41 ± 1.09 b	37.57 ± 2.06 a	1.73 ± 0.32 a	0.046 ± 0.007 a
	HN	6.72 ± 0.55 a	13.13 ± 0.13 a	12.67 ± 0.13 a	78.50 ± 7.49 a	29.53 ± 1.18 a	45.25 ± 2.32 a	2.37 ± 0.44 a	0.054 ± 0.013 a
LS	ZN	7.17 ± 1.11 a	11.06 ± 0.43 a	10.17 ± 0.73 a	50.84 ± 3.53 b	25.45 ± 0.77 ab	27.94 ± 0.92 a	1.73 ± 0.05 a	0.062 ± 0.002 a
	LN	8.28 ± 0.87 a	11.78 ± 1.20 a	10.78 ± 0.78 a	59.60 ± 7.94 ab	24.06 ± 0.20 b	26.34 ± 2.25 a	1.77 ± 0.18 a	0.068 ± 0.007 a
	HN	7.89 ± 1.36 a	9.78 ± 0.31 a	9.39 ± 0.29 a	95.76 ± 17.07 a	26.76 ± 1.01 a	27.51 ± 2.84 a	1.64 ± 0.09 a	0.060 ± 0.003 a

3.2. Rice Plant TC, TN, and TP Contents and TC/TN, TC/TP, and TN/TP Ratios

In the early season, LN significantly increased the TP content of the rice root by 112.5%, and HN significantly increased the TP content of the rice root by 127.5% compared with ZN. In the late season of 2021, HN significantly increased the TP content of the rice stem by 51.14% and reduced the TC/TP ratio by 35.20%, but HN significantly reduced the TP content of the rice root by 25% relative to ZN. N deposition did not have a significant effect on the rice TC or TN contents or on the TC/TN or TN/TP ratios (Table 2).

3.3. Soil Physicochemical Properties

The results indicated a higher effect from LN than HN on the soil TP and on the TC/TP and TN/TP ratios (Figure 1). In the early season, LN significantly raised the TP content by 149%, while it decreased the soil TC/TP and TN/TP ratios by 63% and 61% compared with ZN. On the other hand, the HN treatment significantly augmented the TP content by 125%, while it reduced the soil TC/TP and TN/TP ratios by 55% and 52% relative to ZN (Figure 1C,E,F). N deposition had no significant effect on the TC or TN content, TC/TN ratio, or pH value of the soil (Figure 1A,B,D,G).

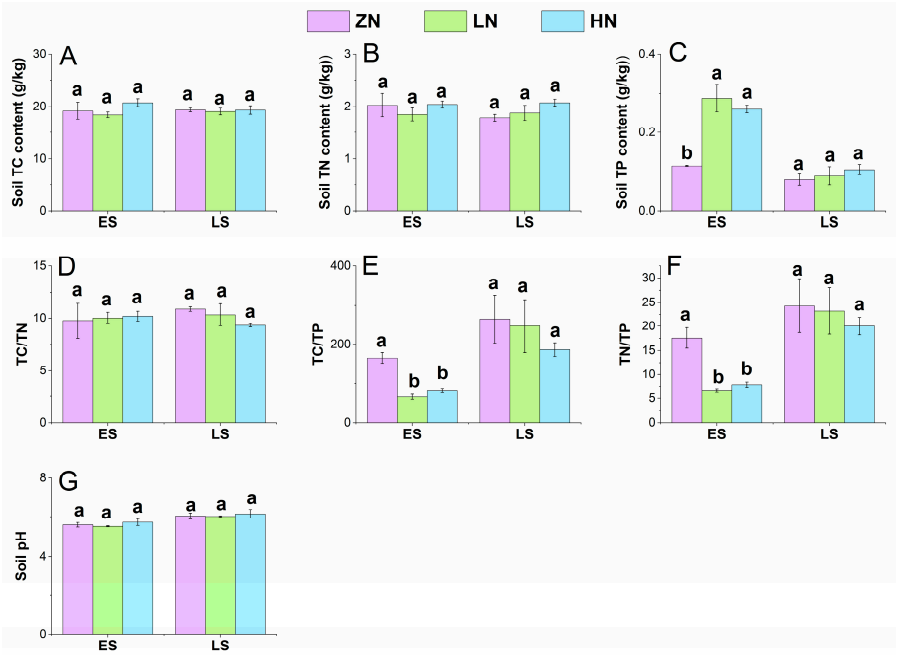

Figure 1. Total carbon (TC, **A**), total nitrogen (TN, **B**), and total phosphorus (TP, **C**) contents; TC/TN (**D**), TC/TP (**E**), and TN/TP (**F**) ratios; and pH (**G**) of soil under nitrogen (N) deposition treatments. ES and LS refer to early and late seasons, respectively; ZN, LN, and HN refer to zero nitrogen deposition, low nitrogen deposition, and high nitrogen deposition, respectively. The data are expressed as mean ± standard error. Different lowercase letters denote a significant difference ($p < 0.05$).

Table 2. TC, TN, and TP contents and TC/TN, TC/TP, and TN/TP ratios of rice plant under N deposition treatments. ES and LS refer to 2021 early and late seasons, respectively; ZN, LN, and HN refer to zero nitrogen deposition, low nitrogen deposition, and high nitrogen deposition, respectively. The data are expressed as mean ± standard error. Different lowercase letters denote a significant difference ($p < 0.05$).

		TC Content (g/kg)			TN Content (g/kg)			TP Content (g/kg)			TC/TN Ratio			TC/TP Ratio			TN/TP ratio		
		Shoot		Root	Shoot		Root	Shoot		Root	Shoot		Root	Shoot		Root	Shoot		Root
ES	ZN	196.13 ± 41.15 a		311.83 ± 33.76 a	4.88 ± 0.87 a		7.43 ± 0.79 a	0.52 ± 0.18 a		0.40 ± 0.19 b	39.73 ± 1.94 a		41.93 ± 0.40 a	1591.22 ± 983.98 a		1182.03 ± 558.35 a	0.64 ± 0.35 a		32.05 ± 16.73 a
	LN	229.11 ± 12.51 a		310.41 ± 24.89 a	5.73 ± 0.50 a		8.12 ± 1.16 a	0.40 ± 0.02 a		0.85 ± 0.04 a	40.25 ± 1.88 a		38.89 ± 2.34 a	1790.68 ± 763.27 a		2505.75 ± 23.97 a	0.54 ± 0.06 a		9.78 ± 1.95 a
	HN	159.30 ± 19.26 a		367.86 ± 63.55 a	4.33 ± 0.23 a		8.56 ± 1.13 a	0.58 ± 0.24 a		0.91 ± 0.03 a	36.51 ± 2.68 a		42.55 ± 1.84 a	438.44 ± 225.43 a		2391.68 ± 346.47 a	0.88 ± 0.64 a		9.46 ± 1.44 a
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
LS	ZN	355.14 ± 99.65 a	346.53 ± 4.63 a	307.68 ± 7.05 a	10.90 ± 3.47 a	8.02 ± 0.30 a	7.22 ± 0.55 a	0.99 ± 0.13 a	0.88 ± 0.12 b	1.76 ± 0.45 a	33.22 ± 1.62 a	43.35 ± 2.18 a	43.02 ± 2.80 a	392.77 ± 160.65 a	410.06 ± 56.66 a	177.46 ± 17.34 a	12.21 ± 5.45 a	9.58 ± 1.64 a	4.20 ± 0.64 a
	LN	260.94 ± 16.50 a	349.11 ± 6.75 a	252.48 ± 32.44 a	8.08 ± 0.40 a	9.17 ± 0.63 a	6.83 ± 0.77 a	0.85 ± 0.04 a	0.89 ± 0.02 b	1.55 ± 0.08 ab	32.28 ± 1.04 a	38.37 ± 2.37 a	36.73 ± 1.86 a	309.79 ± 31.57 a	392.99 ± 16.26 a	161.70 ± 13.63 a	9.56 ± 0.71 a	10.34 ± 0.88 a	4.38 ± 0.30 a
	HN	371.11 ± 92.51 a	352.00 ± 10.91 a	297.06 ± 24.01 a	12.67 ± 1.49 a	9.18 ± 0.48 a	7.38 ± 0.38 a	1.02 ± 0.06 a	1.33 ± 0.07 a	1.32 ± 0.19 b	28.35 ± 4.31 a	38.62 ± 2.77 a	40.39 ± 3.55 a	359.85 ± 80.41 a	265.70 ± 6.05 b	234.61 ± 39.31 a	12.41 ± 0.93 a	6.95 ± 0.52 a	5.86 ± 1.02 a

3.4. Soil Enzyme Activities

In the early season, LN significantly increased the S-NAG and S-β-GC activity by 77% and 56% compared with ZN, while HN did not have a significant difference with ZN (Figure 2B,D). In the late season, LN significantly amplified the S-ACP and S-NAG activity by 11% and 250% relative to ZN. On the other hand, HN significantly augmented the S-ACP and S-NAG activity by 9% and 113% compared with ZN (Figure 2A,B). N deposition did not have a significant effect on the S-C1 activity of the soil (Figure 2C).

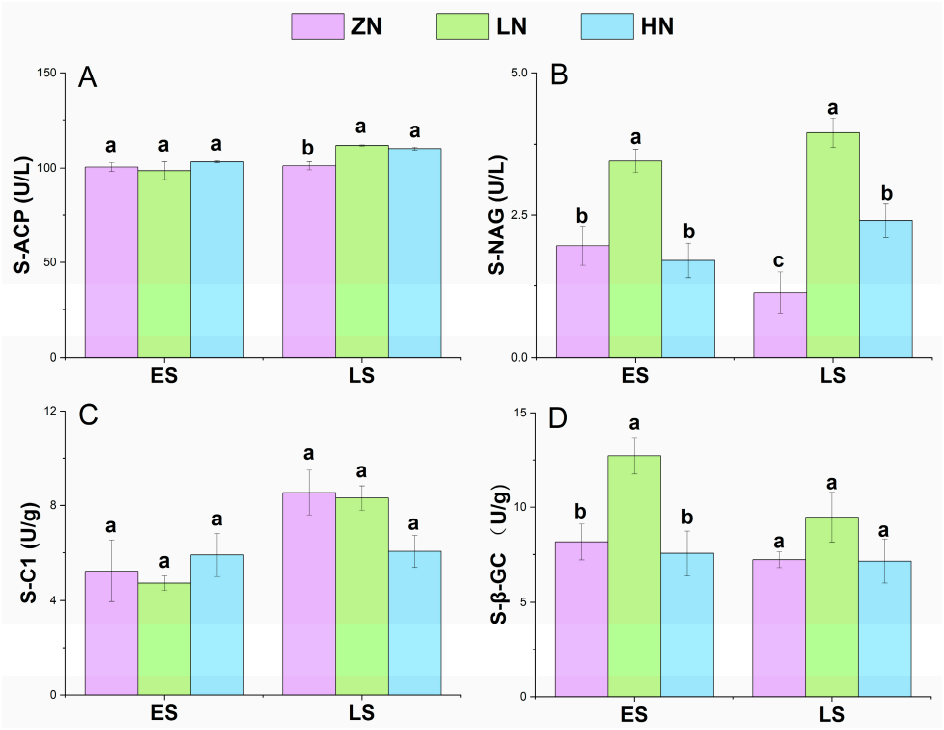

Figure 2. The activities of soil acid phosphatase (S-ACP, **A**), soil N-acetyl-β-D-glucosidase (S-NAG, **B**), soil cellobiosidase (S-C1, **C**), and soil β-glucosidase (S-β-GC, **D**) under N deposition treatments. ES and LS refer to early and late seasons, respectively; ZN, LN, and HN refer to zero nitrogen deposition, low nitrogen deposition, and high nitrogen deposition, respectively. The data are expressed as mean ± standard error. Different lowercase letters denote a significant difference ($p < 0.05$).

3.5. Soil Microbial Biomass

In the early season, the HN treatment significantly dropped the MBP content by 88% compared with ZN (Figure 3C). In the late season, LN significantly reduced the MBN content by 34% relative to the ZN treatment. The HN treatment significantly decreased the MBC and MBN contents by 28% and 38% compared with ZN, respectively (Figure 3A,B).

In addition, the soil microbial biomass generally showed a downward trend under the N deposition treatments in the late season of 2021 (Figure 3). In the late season, LN and HN significantly reduced the microbial biomass of the fungal flora by 41% and 45% and reduced the fungi/bacteria ratio by 32% and 27% compared with ZN (Figure 3J,K). N deposition did not have a significant effect on the biomass of other microorganisms.

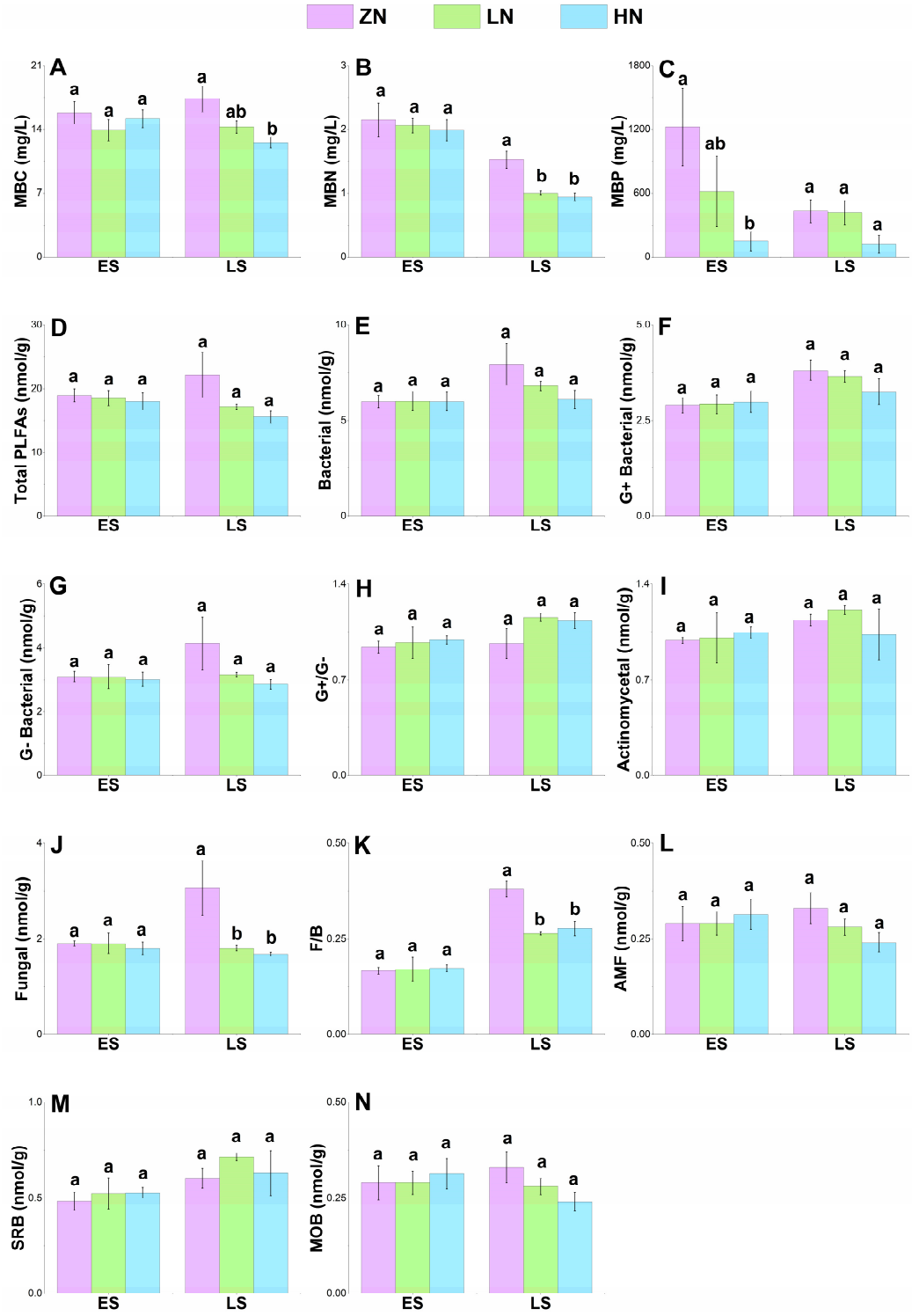

Figure 3. Microbial biomass carbon (MBC, **A**), microbial biomass nitrogen (MBN, **B**), microbial biomass phosphorus (MBP, **C**), and PLFA content (total PLFA, **D**; bacterial, **E**; G+ bacterial, **F**; G− bacterial, **G**; G+/G− ratio, **H**; actinomycetal, **I**; fungal, **J**; F/B ratio, **K**; AMF, **L**; SRB, **M**; and MOB, **N**) of soil under N deposition treatments. ES and LS refer to early and late seasons, respectively; ZN, LN, and HN refer to zero nitrogen deposition, low nitrogen deposition, and high nitrogen deposition, respectively. The data are expressed as mean ± standard error. Different lowercase letters denote a significant difference ($p < 0.05$).

3.6. Soil Microbial Community Structure

N deposition did not have a significant effect on the four indices of alpha diversity of bacterial community, including the Ace, Chao, Shannon, and Simpson indices (Figure 4).

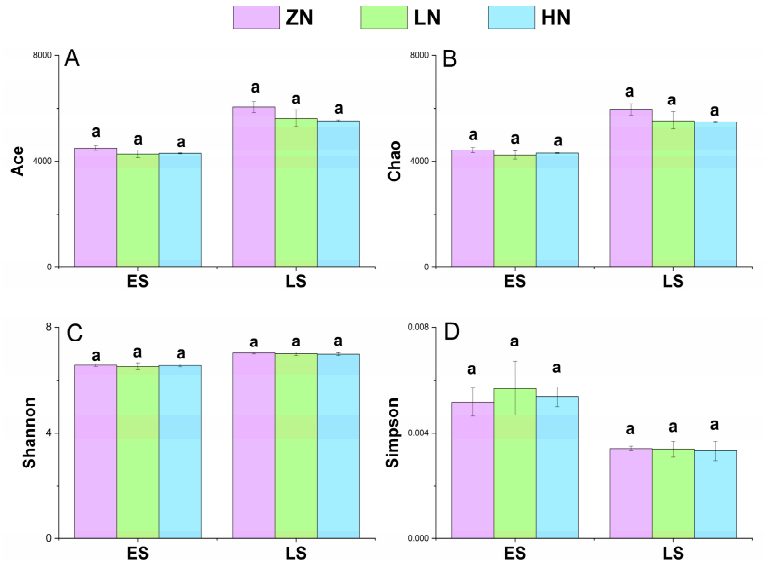

Figure 4. Ace (**A**), Chao (**B**), Shannon (**C**), and Simpson (**D**) indices of soil bacterial community under N deposition treatments. ES and LS refer to early and late seasons, respectively; ZN, LN, and HN refer to zero nitrogen deposition, low nitrogen deposition, and high nitrogen deposition, respectively. The data are expressed as mean ± standard error. Different lowercase letters denote a significant difference ($p < 0.05$).

The N deposition treatments had no significant effect on the soil bacterial community structure in the short term (Figure 5).

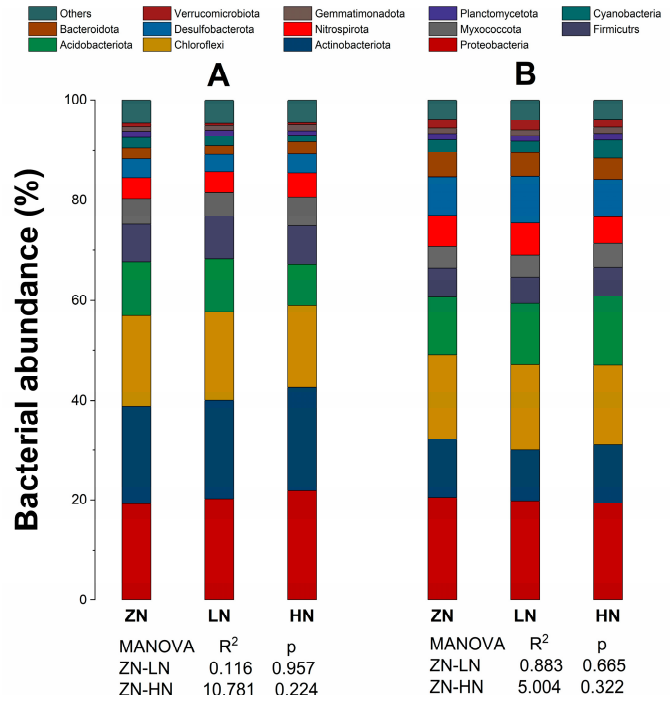

Figure 5. Bacterial abundance under N deposition treatments in 2021 early season (**A**) and late season (**B**). ZN, LN, and HN refer to zero nitrogen deposition, low nitrogen deposition, and high nitrogen deposition, respectively. The R^2 and p values were obtained from the MANOVA.

4. Discussion

4.1. Effects of Nitrogen Deposition on Rice Yield and Biomass

Nitrogen is one of the important nutrient elements affecting rice growth; it can promote rice tillering, speed up rice growth, enhance rice stress resistance and dry matter accumulation, and ultimately increase the rice yield [22].

In this study, the N application rate of 180 kg·ha^{-1} was set for the ZN treatment combined with two N deposition levels of 40 kg·ha^{-1} and 120 kg·ha^{-1}. The results showed that the N deposition treatment significantly increased some components of the rice biomass (i.e., the grain number per panicle and the 1000-grain weight) but had no significant effect on the yield, which is consistent with the previous studies [22,23]. This may be because the rice leaves absorbed a small amount of the N sprayed on the rice leaves, which promoted the photosynthesis of the rice leaves and the accumulation of the grain dry matter and finally increased the number of grains per panicle and the 1000-grain weight of the rice [24]. Because the N application rate of the ZN treatment reached the optimum N rate for rice planting, it had a high yield benefit, and N deposition had no significant impact on the rice yield [25]. Vicensi et al. [26] reported that, with a continuous increase in the N deposition rate, the yield and biomass of rice will grow to a certain extent in a short period of time but that, with the continuous accumulation of N in the soil, the effect of N on rice growth will gradually decrease until the yield and biomass reach the threshold of the N effect. In the present study, there were no significant differences in the number of rice tiller and effective panicle, rice dry weight and root–shoot ratio among the treatments. Therefore, we speculate that the 180 kg N·ha^{-1} applied to the soil through urea had already maximized rice tillering, the panicle formation rate, and rice plant growth, whilst excess N deposition had no significant effect on the growth conditions of rice tillering and panicle formation.

4.2. Effects of Nitrogen Deposition on Rice TC, TN, and TP Contents

The nutrient content in rice plants is mainly related to the ability of the roots to absorb nutrients and the soil nutrient content. In the early season, N deposition did not have a significant effect on the TC and TN contents in the rice shoots and roots, which is consistent with the findings of Finnan et al. [27]. This may be because the application of 180 kg N·ha^{-1} as urea added a large amount of C and N elements to the soil, which provided enough nutrients for rice growth, while the N deposition as NH_4NO_3 was preserved in the soil. Therefore there were no significant changes in the TC or TN content or in the C/N ratio in the rice plants. Inconsistent with the previous study [22], the TP content of the rice roots significantly increased under N deposition in this study due to the effect of N deposition in stimulating an increase in the soil S-ACP activity (Figure 2A). That caused soil decomposition to release phosphorus for the rice roots absorbing to maintain the TC/TP and TN/TP ratios of the rice.

In the late season, we further analyzed the aboveground parts of the rice in two parts: stem and leaf. Inconsistent with the previous studies [27–29], N deposition did not have a significant effect on the TC or TN content in the stem or leaf as well as in the whole rice plants (Table 2). The TP content of the rice stem increased, while it reduced in the roots under the HN treatment due to the change in the rhizosphere environment with continuous N deposition, which caused the rice roots to reduce their absorption of soil phosphorus, to even the nutrients for their nutrient balance, and to transfer the P element absorbed from the soil to the aboveground parts due to an increase in the soil S-ACP activity, maintaining the rice C/P and N/P ratios in the aboveground parts [30]. N deposition did not have a significant effect on the TC/TN, TC/TP, or TN/TP ratios in the stems, leaves, or roots of the rice in the early or late season, further verifying that there is a mechanism in rice to maintain its own C, N, and P and even all the nutrient balances (Table 2).

4.3. Effects of Nitrogen Deposition on Soil Physicochemical Properties

Inconsistent with the previous study [31], N deposition did not have a significant effect on the TC or TN contents of the soil due to the enough amount of C and N that

provided from the application of basic N fertilizer(180 kg·N ha^{-1} as urea). During the deposition of nitrogen that was absorbed by the rice leaves and plants under the influence of unfavorable factors, such as evaporation and a loss in the paddy environment, the TC and TN content of the soil did not increase significantly. In addition, in the early season, the soil TP content significantly increased under N deposition, agreeing well with the previous study [32]. Perhaps it was because N deposition stimulated an increase in the soil S-ACP activity (Figure 2A), which decomposed the soil organic matter (SOM) and released the P element. That resulted in significant increases in the soil TP content, thereby declining in TC/TP and TN/TP; and temporarily unbalancing C, N, and P in the soil within a short time. However, in the late season, N deposition did not have a significant effect on the soil TC, TN, or TP contents or on the TC/TN, TC/TP, or TN/TP ratios. This is also consistent with previous studies [33]. This may be because the soil also had the ability to maintain the C, N, and P nutrient balances. Meanwhile, the soil pH of the two N deposition treatments did not change significantly but showed an upward trend, which is consistent with previous studies [31,33]. This indicates that the soil N accumulation in the short term was insufficient and did not cause soil acidification in this study.

4.4. Effects of Nitrogen Deposition on Soil Enzyme Activities

The four enzymes S-C1, S-β-GC, S-NAG, and S-ACP participate in and promote the transformation and decomposition of C, N, and P sources, which reflects the characterization and activity of the soil microorganisms that play an important role in the soil nutrient balance [18,34].

In the late season, the S-ACP activity of the two N addition rates increased significantly, indicating that N deposition increased the soil N content, stimulated soil decomposition and released P, and stabilized the N/P ratio in the soil [24,35]. In the early season, the activity of S-β-GC of the LN treatment increased significantly, indicating that N deposition also stimulated soil decomposition and the release of C so that the C/N ratio in the soil was in a stable state [36,37]. That is because the N content in the soil may be increased under N deposition, so, with the release of C, the ratio of C/N was maintained. In addition, with an increase in N deposition, the S-NAG activity of the LN treatment increased to be higher than that of HN. The low N deposition level may increase the enzyme activity of soil N cycling. Meanwhile, high N deposition may change the environment of the paddy soil and reduce the microbial biomass, resulting in a decrease in the enzyme activities of soil N cycling [38].

4.5. Effects of Nitrogen Deposition on Soil Microbial Biomass

There is currently a great deal of uncertainty about the effect of N deposition on the soil microbial biomass, which is related to the biome, N source, duration of the experiments, and climatic factors [39,40].

In the present study, the MBC, MBN, and MBP, as indicators of the soil microbial community activity, decreased significantly with an increasing N deposition rate. Previous studies have shown that this may be caused by acidification due to an increase in the available soil N content [41]. As mentioned above, the soil N accumulation with the two N deposition rates was insufficient and did not cause soil acidification. Therefore, the significant decrease in the soil microbial biomass may be caused by the improvement of the soil N availability. Moreover, most studies have a common view that N deposition beyond the tolerance range of the microorganisms will inevitably lead to a decline in the soil microbial biomass [30,38,42].

Meanwhile, the microbial biomass of the fungal flora and the F/B ratio in the soils treated with the two N addition levels in this study significantly reduced, which is consistent with previous studies [43–45]. These studies showed that the microbial biomass of soil fungal flora is negatively correlated with pH and ammonium N [40]. In the present study, the soil pH values of the two N deposition treatments were not significantly different from those of the ZN treatments. However, ammonium nitrate using for N deposition

will increase the content of soil ammonium nitrogen, so we speculate that fungi are more sensitive to changes in the soil ammonium nitrogen content. Fungi play an important role in promoting plant growth among which rhizobacteria fungi can promote the absorption of the soil nutrients by the rice roots and provide more nutrients directly to the rice roots [30]. Once the microbial biomass of the fungal flora decreases, the ability of rice to absorb nutrients will also decrease. This will weaken the beneficial interaction between rice roots and microorganisms, resulting in a decrease in soil MBC, MBN, MBP, and total biomass [46].

4.6. Effects of Nitrogen Deposition on Soil Microbial Community Structure

Nitrogen deposition has been investigated to cause significant changes in the composition of the soil microbial community [47–49]. In the current study, N deposition did not significantly affect bacteria diversity and abundance, but there were significant differences in the biomass of fungi and ratio of fungi to bacteria (Figure 3), which indicated that N deposition altered the composition of fungi and bacteria. This result supported the previous studies which reported that N deposition had greater negative effect on fungi than bacteria [50,51]. There may be competition between the plants and microorganisms for other limited resources, resulting in the inability of the mycorrhizal fungi to obtain other nutrients from the plants, which in turn reduced the biomass of the fungi and changed the soil microbial community structure (Figure 3I) [52–54]. Therefore, we may consider that N deposition had a significant effect on soil microbial community structure by altering the composition of fungi and bacteria, but how fungal community changes with increasing N deposition needs to be confirmed through long-term experiments in the future.

5. Conclusions

In general, short-term nitrogen deposition had no significant effect on the rice yield but significantly increased the rice partial yield components (i.e., the grains per panicle and the 1000-grain weight). With an increase in soil N deposition, the rice regulated its carbon, nitrogen, and phosphorus by reducing the nutrient uptake by the roots, while microorganism regulated soil carbon, nitrogen, and phosphorus by changing the enzyme activities related to soil C, N, and P cycling. Moreover, nitrogen deposition significantly reduced soil MBC, MBN, and MBP contents, decreased the fungal microbial biomass and altered the soil microbial community structure in the paddy fields. These findings provide a new perspective on the responses of paddy ecosystem to nitrogen deposition, but there are great uncertainties in the short-term paddy field experiments. Therefore, long-term investigations are needed to further verify the more real effects, processes and the related mechanisms.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13092249/s1, Figure S1: Biblionmetric analysis on cnki.net (Accessed on 27 September 2022). Figure S2: Average temperature and rainfall in 2021.

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References

1. Dentener, F.; Drevet, J.; Lamarque, J.F.; Bey, I.; Eickhout, B.; Flore, A.M.; Hauglustaine, D.; Horowitz, L.W.; Krol, M.; Kulshrestha, U.C.; et al. Nitrogen and sulfur deposition on regional and global scales: A multimodel evaluation. *Glob. Biogeochem. Cycles* **2006**, *20*, 1–21.
2. Liu, X.J.; Zhang, Y.; Han, W.X.; Tang, W.X.; Tang, A.H.; Shen, J.L.; Cui, Z.L.; Vitousek, P.; Erisman, J.W.; Goulding, K.; et al. Enhanced nitrogen deposition over China. *Nature* **2013**, *494*, 459–462.
3. Fowler, D.; Pyle, J.A.; Raven, J.A.; Sutton, M.A. The global nitrogen cycle in the twenty-first century: Introduction. *Biol. Sci.* **2013**, *368*, 1–2.
4. Xu, F.D.; Gao, Y.; Dong, W.Y.; Hao, Z.; Xu, Y.J. Impact of atmospheric nitrogen and phosphorus wet deposition on nitrogen and phosphorus export and associated water quality: A case study of forest watershed in the red soil area, southern China. *Acta Ecol. Sin.* **2016**, *36*, 6409–6419. (In Chinese)
5. Mao, Q.G.; Lu, X.K.; Zhou, K.J.; Chen, H.; Zhu, X.M.; Mori, T.K.; Mo, J.M. Effects of long- term nitrogen and phosphorus depositions on soil acidification in an N-rich tropical forest. *Geoderma* **2017**, *285*, 57–63.
6. Lu, X.K.; Mo, J.M.; Zhang, W.; Mao, Q.G.; Liu, R.Z.; Wang, C.; Wang, S.H.; Zheng, M.H.; Mori, T.; Mao, J.H.; et al. Effects of simulated atmospheric nitrogen deposition on forest ecosystems in China: An overview. *J. Trop. Subtrop. Bot.* **2019**, *27*, 500–522. (In Chinese)
7. The State of National Grain Production in China. 2020. Available online: http://www.stats.gov.cn/xxgk/jd/sjjd2020/202012/t20201211_1808749.html (accessed on 16 November 2021). (In Chinese)
8. Yan, Z.Q.; Qi, Y.C.; Li, S.J.; Dong, Y.S.; Peng, Q.; He, Y.L.; Li, Z.L. Soil microorganisms and enzyme activity of grassland ecosystem affected by changes in precipitation pattern and increase in nitrogen deposition-a review. *Microbiol. China* **2017**, *44*, 1481–1490. (In Chinese)
9. Tu, L.H.; Dai, H.Z.; Hu, T.X.; Zhang, J.; Luo, S.H. Effects of simulated nitrogen deposition on soil respiration in a *Bambusa pervariabilis* × *Dendrocala mopsi* Plantation in Rainy Area of West China. *Chin. J. Appl. Ecol.* **2011**, *22*, 829–836. (In Chinese)
10. Midolo, G.; Alkemade, R.; Schipper, A.M.; Benitez-Lopez, A.; Perring, M.P.; De Vries, W. Impacts of nitrogen deposition on plant species richness and abundance: A global meta-analysis. *Glob. Ecol. Biogeogr.* **2019**, *28*, 398–413.
11. Rousk, J.; Philip, C.B.; Brookes, P.C.; Baath, E. Fungal and bacterial growth response to N fertilization and pH in the 150-year 'Park Grass' UK grassland experiment. *FEMS Microbiol. Ecol.* **2011**, *76*, 89–99.
12. Lin, B.L.; Kumon, Y.; Inoue, K.; Tobari, N.; Xue, M.Q.; Tsunemi, K.; Terada, A. Increased nitrogen deposition contributes to plant biodiversity loss in Japan: Insights from long-term historical monitoring data. *Environ. Pollut.* **2021**, *290*, 118033. [CrossRef]
13. Hou, X.D.; Liu, S.T.; Wang, S.J.; Jia, S.G.; Hou, Y.L.; Lu, L. Correlation analysis of fertilization parameters in rice. *J. Agric. Resour. Environ.* **2019**, *36*, 614–619. (In Chinese)
14. Hou, Y.P.; Yang, J.; Li, Q.; Qin, Y.B.; Kong, L.L.; Yin, C.X.; Wang, L.C.; Xie, J.G. Effects of nitrogen application on yield, nitrogen utilization and accumulation of soil inorganic nitrogen of rice. *Chin. J. Soil Sci.* **2016**, *47*, 118–124. (In Chinese)
15. Zhang, Q.; Chang, M.; Wang, X.M. Advances in nitrogen deposition observation in China and its application in the Pearl River Delta. *China Environ. Sci.* **2017**, *37*, 4401–4416. (In Chinese)
16. Hei, Z.W.; Xiang, H.M.; Zhang, J.E.; Liang, K.M.; Ren, X.Q.; Sun, Y.R.; Wu, R.Q. Water mimosa (*Neptunia oleracea* Lour.) can fix and transfer nitrogen to rice in their intercropping system. *J. Sci. Food Agric.* **2021**, *102*, 156–166. [PubMed]
17. Lu, R.K. *Soil Argro-Chemical Analysis*; Agricultural Technical Press of China: Beijing, China, 2000. (In Chinese)
18. Mecino, C.; Godoy, R.; Matus, F. Soil enzymes and biological activity at different levels of organic matter stability. *J. Soil Sci. Plant Nutr.* **2016**, *16*, 14–30.
19. Li, Y.; Zhao, X.R.; Li, G.T.; Lin, Q.M. Effect of grazing on soil microbial biomass phosphorus and phosphatase activities on different topographic unit of a typical steppe. *Acta Ecol. Sin.* **2022**, *42*, 4137–4149. (In Chinese)
20. Yang, X.Y.; Zhou, S.S.; Sun, B.H.; Wang, B.Q.; Zhang, S.L.; Gu, Q.Z. Microbial properties of a loess soil as affected by various nutrient management practices in the loess plaseau of China. *Commun. Soil Sci. Plant* **2010**, *41*, 956–967.
21. Liu, Z.Q.; Liu, Z.X.; Wu, L.Z.; Li, Y.Z.; Wang, J.; Wei, H.; Zhang, J.E. Effect of polyethylene mircroplastics and acid rain on the agricultural soil ecosystem in Southern China. *Environ. Pollut.* **2022**, *303*, 119094.
22. Zhang, J.F.; Feng, Y.H.; Xu, G.L.; Guan, Z.C.; Su, W.; Ou, D.; Wang, L.L.; Chen, Y. Effects of different nitrogen rates on growth and yield of Nei 5 You 5399 of hybrid rice. *Hybrid Rice* **2019**, *34*, 61–66. (In Chinese)
23. Feng, S.Z.; Ding, X.D.; Wang, S.W.; Li, C.Y.; Cheng, D.D.; Zhang, S.R.; Zhou, W. Effects of different nitrogen application rates on photosynthetic rate, nitrogen fertilizer conversion efficiency and yield of rice in Linyi region. *Acta Agric. Shanghai* **2021**, *37*, 88–91. (In Chinese)
24. Yan, K.; Jiang, Y.L.; Xiao, T.J.; Dai, Q.G. Effects of nitrogen amount on grain yield and quality of rice on saline-alkaline land. *China Rice* **2017**, *23*, 35–39. (In Chinese)
25. Lewandowski, I.; Kauter, D. The influence of nitrogen fertilizer on the yield and combustion quality of whole grain crops for solid fuel use. *Ind. Crops Prod.* **2003**, *17*, 103–117.

26. Vicensi, M.; Lopes, C.; Koszalka, V.; Umburanas, R.C.; Vidigal, J.C.B.; Avila, F.W.; Muller, M.M.L. Soil fertility, root and aboveground growth of black oat under gypsum and urea rates in no till. *J. Soil Sci. Plant Nutr.* **2020**, *20*, 1271–1286.
27. Finnan, J.; Burke, B. Nitrogen fertilization of *Miscanthus* × *giganteus*: Effects on nitrogen uptake, growth, yield and emissions from biomass combustion. *Nutr. Cycl. Agroecosyst.* **2016**, *106*, 249–256.
28. Yan, Z.D.; Guo, P.Y.; Yuan, X.Y.; Xiao, X.L.; Wang, D.M. Effect of foliar application of nitrogen fertilizer on photosynthetic characteristics and yield of foxtail millet cultivar 'Zhangzagu 10' *Setaria italic* (L.) Beauv. *J. Shanxi Agric. Univ. (Nat. Sci. Ed.)* **2018**, *39*, 37–41. (In Chinese)
29. Tian, T.; Wang, J.G.; Wang, H.J.; Cui, J.; Shi, X.Y.; Song, J.H.; Li, W.D.; Zhong, M.T.; Qiu, Y.; Xu, T. Nitrogen application alleviates salt stress by enhancing osmotic balance, ROS scavenging, and photosynthesis of rapeseed seedlings (*Brassica napus*). *Plant Signal. Behav.* **2022**, *17*, 2081419.
30. Lu, X.K.; Mo, J.M.; Gilliam, F.S.; Zhou, G.Y.; Fang, Y.T. Effects of experimental nitrogen depositions on plant diversity in an old-growth tropical forest. *Glob. Chang. Biol.* **2010**, *16*, 2688–2700.
31. Anning, D.K.; Qiu, H.Z.; Zhang, C.H.; Ghanney, P.; Zhang, Y.J.; Guo, Y.J. Maize straw return and nitrogen rate effects on patato (*Solanum tuberosum* L.) performance and soil physicochemical characteristics in northwest China. *Sustainability* **2021**, *13*, 5508.
32. Ramteke, P.R.; Vashisht, B.B.; Sharma, S.; Jalota, S.K. Assessing and ranking influence of rates of rice (*Oryza sativa* L.) straw incorporation and N fertilizer on soil physicochemical properties and wheat (*Triticum aestivum* L.) yield in rice-wheat system. *J. Soil Sci. Plant Nutr.* **2022**, *22*, 515–526.
33. Behnke, G.D.; David, M.B.; Voigt, T.B. Greenhouse gas emissions, nitrate leaching, and biomass yields from production of miscanthus×giganteus in Illinois, USA. *Bioenergy Res.* **2012**, *5*, 801–813. [CrossRef]
34. Xiang, Z.Y.; Wang, C.T.; Song, W.B.; Silang, S.; Garong, R.; Dawa, Z.; Zhaxi, L. Advances on soil enzymatic activities in grassland ecosystem. *Pratacult. Sci.* **2011**, *28*, 1801–1806.
35. Tian, M.Y.; Yu, C.J.; Wang, J.K.; Ding, F.; Chen, Z.H.; Jiang, N.; Jiang, H.; Jiang, L.J. Effect of nitrogen depositions on soil pH, phosphorus contents and phosphatase activities in grassland. *Chin. J. Appl. Ecol.* **2020**, *31*, 2985–2992. (In Chinese)
36. Sekaran, U.; Mccoy, C.; Kumar, S.; Subramanian, S. Soil microbial community structure and enzymatic activity responses to nitrogen management and landscape positions in switchgrass (*Panicum virgatum* L.). *Glob. Chang. Biol. Bioenergy* **2019**, *11*, 836–851. [CrossRef]
37. Zhang, Y.; Wang, C.M.; Xu, K.; Yang, X.T. Effect of simulated nitrogen deposition on soil enzyme activities in a temperate forest. *Acta Ecol. Sin.* **2017**, *37*, 1956–1965. (In Chinese)
38. Zhang, N.L.; Wan, S.Q.; Li, L.H.; Bi, J.; Zhao, M.M.; Ma, K.P. Impacts of urea N deposition on soil microbial community in a semi-arid temperate steppe in northern China. *Plant Soil* **2008**, *311*, 19–28. [CrossRef]
39. Yan, G.Y.; Xing, Y.J.; Han, S.J.; Zhang, J.H.; Wang, Q.G.; Mu, C.C. Long-time precipitation and nitrogen deposition increase alter soil nitrogen dynamic by influencing soil bacterial communities and functional groups. *Pedosphere* **2020**, *30*, 363–377. [CrossRef]
40. Wang, Y.S.; Cheng, S.L.; Fang, H.J.; Yu, G.R.; Xu, X.F.; Xu, M.J.; Wang, L.; Li, X.Y.; Si, G.Y.; Geng, J.; et al. Contrasting effects of ammonium and nitrate inputs on soil CO_2 emission in a subtropical coniferous plantation of Southern China. *Biol. Fertil. Soils* **2015**, *51*, 815–825. [CrossRef]
41. Zhou, Z.H.; Wang, C.K.; Zheng, M.H.; Jiang, L.F.; Luo, Y.Q. Patterns and mechanisms of responses by soil microbial communities to nitrogen deposition. *Soil Biol. Biochem.* **2017**, *115*, 433–441. [CrossRef]
42. Janssens, I.A.; Dieleman, W.; Luyssaert, S.; Subke, J.A.; Reichstein, M.; Ceulemans, R.; Ciais, P.; Dolman, A.J.; Grace, J.; Matteucci, G.; et al. Reduction of forest soil respiration in response to nitrogen deposition. *Nat. Geosci.* **2010**, *3*, 315–322. [CrossRef]
43. Van Diepen, L.T.A.; Lilleskov, E.A.; Pregitzer, K.S. Simulated nitrogen deposition affects community structure of arbuscular mycorrhizal fungi in northern hardwood forests. *Mol. Ecol.* **2011**, *20*, 799–811. [CrossRef] [PubMed]
44. Averill, C.; Dietze, M.C.; Bhatnagar, J.M. Continental-scale nitrogen pollution is shifting forest mycorrhizal associations and soil carbon stocks. *Glob. Chang. Biol.* **2018**, *24*, 4544–4553. [CrossRef] [PubMed]
45. Jo, I.; Fei, S.L.; Oswalt, C.M.; Domke, G.M.; Phillips, R.P. Shifts in dominant tree mycorrhizal associations in response to anthropogenic impacts. *Sci. Adv.* **2019**, *5*, eaav6358. [CrossRef]
46. Feng, G.; Zhang, Y.F.; Li, X.L. Effect of external hyphae of arbuscular mycorrhizal plant on water-stable aggregates in sandy soil. *J. Soil Water Conserv.* **2001**, *15*, 99–102. (In Chinese)
47. Tian, D.S.; Niu, S.L. A global analysis of soil acidification caused by nitrogen deposition. *Environ. Res. Lett.* **2015**, *10*, 024019. [CrossRef]
48. Wang, C.; Liu, D.W.; Bai, E. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen deposition. *Soil Biol. Biochem.* **2018**, *120*, 126–133. [CrossRef]
49. Kaspari, M.; Bujan, J.; Weiser, M.D.; Ning, D.L.; Michaletz, S.T.; He, Z.L.; Enquist, B.J.; Waide, R.B.; Zhou, J.Z.; Turner, B.L. Biogeochemistry drives diversity in the prokaryotes, fungi, and invertebrates of a Panama forest. *Ecology* **2017**, *98*, 2019–2028. [CrossRef] [PubMed]
50. Wang, H.; Liu, S.R.; Zhang, X.; Mao, Q.G.; Li, X.Z.; You, Y.M.; Wang, J.X.; Zheng, M.H.; Zhang, W.; Lu, X.K.; et al. Nitrogen deposition reduces soil bacterial richness, while phosphorus deposition alters community composition in an old-growth N-rich tropical forest in southern China. *Soil Biol. Biochem.* **2018**, *127*, 22–30. [CrossRef]

51. Fierer, N.; Lauber, C.L.; Ramirez, K.S.; Zaneveld, J.; Bradford, M.A.; Knight, R. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J.* **2012**, *6*, 1007–1017. [CrossRef]
52. Yan, G.Y.; Han, S.J.; Wang, Q.G.; Wang, X.C.; Hu, C.Y.; Xing, Y.J. Variations of the effects of reduced precipitation and N deposition on the microbial diversity among different seasons in a temperate forest. *Appl. Soil Ecol.* **2021**, *166*, 103995. [CrossRef]
53. Lauber, C.L.; Hamady, M.; Knight, R.; Fierer, N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.* **2009**, *75*, 5111–5120. [CrossRef] [PubMed]
54. Kuzyakov, Y.; Xu, X.L. Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytol.* **2013**, *198*, 656–669. [CrossRef] [PubMed]

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