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Postponed Nitrogen Enhanced Waterlogged Summer Maize Soil Nutrients by Regulating Microbial Diversity and Network Complexity

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Abstract: Waterlogging stress and low nitrogen use efficiency are important factors constraining summer maize production in the Huang-Huai-Hai region. However, how postponed nitrogen application modulates soil micro-ecological properties and mitigates yield loss in waterlogged summer maize fields by regulating soil microbial community diversity, co-occurrence networks, and community assembly processes remained rarely explored. In this study, summer maize at the three-leaf-one-heart stage was subjected to waterlogging for 3 (WL3), 6 (WL6), and 9 (WL9) days, and the treatment without artificial flooding was set as the control (CK). combined with three postponed nitrogen application ratios: N1 (70% basal fertilizer + 30% topdressing after stress relief), N2 (50% basal fertilizer + 30% topdressing after stress relief + 20% topdressing at the V12 stage), and N3 (30% basal fertilizer + 30% topdressing after stress relief + 40% topdressing at the V12 stage). Soil nutrients, microbial community diversity, network stability, and assembly processes were systematically analyzed. Results showed that N3 treatment increased the contents of soil total nitrogen, inorganic nitrogen, available phosphorus, alkali-hydrolyzable nitrogen and available potassium after waterlogging stress; prolonging the waterlogging duration increased the alpha diversity of soil bacteria at the flowering stage of summer maize. Under WL9 treatment, compared with N1 treatment, N2 treatment significantly increased the alpha diversity of farmland soil at the maturity stage. Linear discriminant analysis Effect Size (LEfSe) analysis revealed that under WL9 treatment, N3 treatment enriched putative nitrogen-cycling functional bacterial taxa such as *Nitrolancea* and *Defluviicoccus*, as well as saprophytic fungi including *Blastocladiiales*, *Acrophialophora*, and *Epicoccum*. Nitrate nitrogen and ammonium nitrogen were identified as key environmental factors driving bacterial and fungal community diversity and structure at the maturity stage. Under the WL9 treatment, compared with N1, the N2 and N3 treatments increased network complexity by 30.61% and 46.94%, respectively. The migration rate and migration-drift parameter of bacterial and fungal communities increased with the increase of postponed nitrogen application ratio under CK and WL3 treatments. Under waterlogging conditions, compared with N1 treatment, N3 treatment increased summer maize yield by 27.29%–33.75%. Overall, the postponed nitrogen application improved micro-ecology of summer maize under waterlogging conditions by enhancing soil nutrient status, enriching microorganisms, and increasing network stability. This provides a theoretical basis for waterlogging prevention and optimized nitrogen management in summer maize production in the Huang-Huai-Hai region.

Keywords: waterlogging stress; postponed nitrogen application; summer maize; soil microbial diversity; network complexity

1. Introduction

The Huang-Huai-Hai Region is one of China's major maize-producing areas, accounting for approximately one-third of the national corn planting area and playing a crucial role in China's food security [1–3]. In recent years, with the increasing frequency of extreme precipitation events, waterlogging has become the second-largest agricultural natural disaster in China, accounting for about 28% of natural disasters with a growing trend [4,5]. Meteorological data showed that from mid-June to early-July each year, the region experiences concentrated rainfall, during which summer maize is in the seedling stage and exhibits the most obvious response to waterlogging stress [6,7]. Our previous studies have confirmed that maize plants during this stage were particularly vulnerable and highly susceptible to environmental stressors, making them prone to waterlogging damage [8]. When subjected to waterlogging, summer maize at the seedling stage often exhibited severely retarded growth or even complete stagnation, which adversely affected subsequent development and ultimately led to significant yield losses [9,10]. Therefore, waterlogging disaster has become a key limiting factor for achieving stable and high yields of summer maize in this region, and urgent solutions are needed through scientific and effective means.

Nitrogen fertilizer, as a crucial input in agricultural production, plays an irreplaceable role in ensuring global food security. However, long-term issues such as low utilization efficiency and severe nutrient loss have persisted in nitrogen fertilizer application in China. Statistics show that the nitrogen use efficiency in China is only 30%–35%, far lower than the 60% level in developed countries [11,12]. Therefore, improving nitrogen fertilizer management practices and enhancing nitrogen use efficiency are of vital significance for increasing crop yield, reducing fertilizer losses, and protecting the ecological environment. Several studies have illustrated that conventional nitrogen application resulted in approximately 40%–50% nitrogen loss via volatilization and leaching [13,14]. Addressing this, the concept of “precision fertilization based on crop demand” was proposed, which has led to the emergence of nitrogen fertilizer postponement techniques. Several studies have shown that delaying the application of 30%–50% of nitrogen fertilizer until the V12 stage of maize can increase the nitrogen utilization rate by 15%–20% and reduce nitrogen loss by more than 25% [15–17]. Waterlogging stress induces soil nutrient leaching and denitrification losses, thereby impairing root nutrient uptake capacity, hindering dry matter accumulation, and ultimately suppressing crop growth and development [14,18]. The implementation of a nitrogen postponement strategy can reallocate part of the fixed N dose to post-stress topdressing to offset the nitrogen loss caused by waterlogging, promote the recovery of root vitality, enhance leaf chlorophyll content and photosynthetic enzyme activity, thereby alleviating the inhibition of waterlogging stress on crop growth and reducing yield loss [19,20].

Soil microbial community diversity and structure are pivotal for sustaining agroecosystem balance by regulating the efficiency of soil nutrient cycling, driving organic matter transformation, and shaping crop stress resilience [21,22]. Under waterlogging stress, soil physicochemical properties, such as porosity, structure, and pH, suffered significant deterioration, which in turn exerted adverse effects on

microbial diversity and community structure [23,24]. Previous studies confirmed that waterlogging stress led to a sharp decline in the abundance of microorganisms involved in nitrification, thereby suppressing the metabolic activity of nitrifying communities, while the enrichment of anaerobic diazotrophic bacteria such as *Geobacter* enhances nitrogen fixation to help sustain plant growth under such conditions [25,26]. Numerous studies revealed that nitrogen postponement application enhanced the soil available nitrogen content, improved the diversity and structure of bacterial communities, and also contributed to facilitating the recovery of key functional microbial taxa such as ammonia-oxidizing bacteria and nitrifying bacteria, and strengthening nitrogen transformation and fixation processes [27–29]. However, at present, there is still a lack of systematic and in-depth explanations for the underlying mechanism of how the nitrogen postponement application drives the recovery of crop growth and yield improvement by regulating the soil microbial community structure under waterlogging stress.

This study investigated the effects of postponed nitrogen application on soil nutrients, microbial community diversity, and network stability in summer maize under varying waterlogging durations. The objectives were to [1] analyze the compensatory effects of postponed nitrogen application on soil nutrients and crop yields under waterlogging conditions; [2] examine the response of soil fungal and bacterial community composition and diversity to postponed nitrogen application under waterlogging stress; and [3] explore whether changes in nutrients can regulate the relationships among soil microorganisms to mitigate the adverse effects of waterlogging stress. This study aimed to provide novel insights and practical field management strategies for summer maize cultivation in the Huang-Huai-Hai region of China.

2. Materials and Methods

2.1. Experimental Site

This field experiment was conducted at the Shuangshou Seed Industry Crop Research Institute in Suixi County, Huaibei City, Anhui Province (33°46'N, 116°45'E) in 2024. The region has a warm temperate semi-humid monsoon climate with a mean annual temperature of 14.5 °C, mean annual precipitation of 862.5 mm, mean annual evaporation of 1743.1 mm, and mean annual frost-free period of 203 days. The soil used in this experiment was a lime concretion black soil, classified as a Vertisol in the USDA Soil Taxonomy, with the following physicochemical properties in the surface layer (0–20 cm): pH 8.00, organic matter content 23.40 g·kg⁻¹, total nitrogen 1.51 g·kg⁻¹, alkali-hydrolyzable nitrogen 120.12 mg·kg⁻¹, available phosphorus 46.67 mg·kg⁻¹, and available potassium 492.44 mg·kg⁻¹.

2.2. Experimental Design

A split-plot design was adopted in this field experiment, with waterlogging duration as the main plot and postponed nitrogen application as the subplot, and was randomly assigned to main plots within each block. Each treatment included three blocks, and the area of each plot was 20 m² (4 m × 5 m). Based on the previous

experimental design [8], waterlogging stress was imposed at the three-leaf-one-heart stage of summer maize, with three waterlogging durations: 3 (WL3), 6 (WL6), and 9 (WL9) days, with a control group receiving normal water treatment (CK). To ensure the effectiveness of the waterlogging regime, waterproof sandbags were placed around each plot, water delivery pipelines were connected to a well, and a drainage valve was installed at the inlet of each plot. Due to soil water leakage in the experimental plots, the water flow rate was adjusted for continuous 24-hour irrigation, maintaining a 1–2 cm water layer on the soil surface to achieve the desired waterlogging intensity. To prevent mutual interference of moisture between different treatments, adjacent plots were spaced 5 meters apart, and 4 m long × 1 m high plastic plates were inserted between adjacent plots to block lateral water leakage. The three postponed nitrogen application treatments were as follows: (1) 70% basal fertilizer + 30% topdressing one day after stress relief (N1); (2) 50% basal fertilizer + 30% topdressing one day after stress relief + 20% topdressing at the V12 stage (N2); (3) 30% basal fertilizer + 30% topdressing one day after stress relief + 40% topdressing at the V12 stage (N3). The total pure nitrogen (N) application rate was $270 \text{ kg} \cdot \text{hm}^{-2}$, with urea as the N source. Basal nitrogen fertilizer was applied on 15 June. The post-stress topdressing was conducted on 2 July for WL3 and CK, 5 July for WL6, and 8 July for WL9. The topdressing at the V12 stage was applied on 25 July. The application rates of pure phosphorus (P_2O_5) and potassium (K_2O) were both $90 \text{ kg} \cdot \text{hm}^{-2}$, applied entirely as basal fertilizers. The tested summer maize cultivar was Zhengdan 958, sown on 15 June and harvested on 10 October. The row spacing was 60 cm, and the plant spacing was 24.7 cm, corresponding to a planting density of 67,500 plants hm^{-2} . All other agronomic management practices followed local conventional field management methods.

2.3. Soil Sampling

A total of 72 soil samples at a depth of 0–20 cm were collected using a clean spade following the five-point sampling method at the flowering and maturity stages. The five soil cores collected within each plot were fully pooled into one composite sample. Soil sampling at the flowering and maturity was performed in the identical plots, including 12 treatments, 2 stages, and 3 repetitions. After removing roots, stones, and debris, the samples were sieved through a 2 mm mesh and divided into two portions. One sub-sample was stored at -80°C for DNA extraction, and the other was preserved at -20°C for soil property analyses. Soil properties, including soil pH, organic matter (OM), total nitrogen (TN), Alkali-hydrolyzable nitrogen (AN), available phosphorus (AP), available potassium (AK), ammonium nitrogen ($\text{NH}_4^+\text{-N}$), and nitrate nitrogen ($\text{NO}_3^-\text{-N}$). Specific assays were described in the Supplementary Material.

2.4. DNA Extraction and Sequence Analysis

Total DNA was extracted from 500 mg of soil samples using the FastDNA[®] Spin Kit (MP Biomedicals Co., Ltd., Santa Ana, CA, USA) in strict accordance with the manufacturer's instructions. The concentration and purity of the extracted DNA were determined using a NanoDrop UV-Vis 2000 spectrophotometer (Thermo Scientific,

Wilmington, DE, USA), and DNA quality was estimated using 1% agarose gel electrophoresis. The total DNA from all samples was submitted to Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) for subsequent sequencing. The unique barcoded primer pair 338F/806R and ITS1F/ITS2R were used to amplify the V3–V4 region of bacterial 16S rRNA genes and internal transcribed spacer (ITS) region of fungal rRNA genes, respectively. Comprehensive polymerase chain reaction (PCR) reaction parameters are presented in the Supplementary Material. PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified with QuantiFluor™-ST (Promega, Madison, WI, USA) according to the manufacturer's protocols. Purified amplicons were pooled in equimolar amounts and subjected to paired-end 300 bp sequencing (2×300 bp) on an Illumina MiSeq platform at Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). PCR products were purified with the AxyPrep DNA Gel Extraction Kit from Axgen Biosciences (Union City, CA, USA) and quantified via QuantiFluor™-ST (Promega, Madison, WI, USA) in accordance with the corresponding manuals.

For the acquired 16S rRNA and ITS rRNA gene sequencing reads, demultiplexing and quality control were initially carried out utilizing Trimmomatic software, and subsequently, sequence assembly was conducted with FLASH (v1.2.11, Johns Hopkins University, Baltimore, MD, USA, <http://ccb.jhu.edu/software/FLASH/>). Detailed analytical procedures are delineated in the Supplementary Material. Sequences derived from DADA2 denoising processing are denoted as amplicon sequence variants (ASVs). Fungal and bacterial ASV sequences were aligned against the UNITE database (v8.2 University of Tartu, Tartu, Estonia, <http://unite.ut.ee/index.php>) and the Silva database (v138, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany, <https://www.arb-silva.de/>), respectively. The raw sequencing data for bacteria and fungi generated in this study have been deposited in the NCBI (Bethesda, MD, USA) Sequence Read Archive under accession numbers PRJNA1472965 and PRJNA1472964, respectively.

2.5. Yield and Its Components

At the maturity stage of maize, harvesting ten ears from each plot, investigate the hundred grain weight, grain number per ear, and grain yield of summer maize. At physiological maturity, ten representative ears were hand-harvested from the central two rows of each plot to avoid border effects; grains were threshed, oven-dried at 80 °C to constant weight, and the 100-grain weight, grain number per ear and grain yield (adjusted to a 14% moisture content) were determined.

2.6. Data Analysis

All statistical analyses were performed using R software (version 4.4.2, Vienna, Austria, <http://www.r-project.org/>). A split-plot ANOVA was performed using the aov function in the stats package in R to evaluate the effects of nitrogen application regimes, waterlogging durations, and their interaction on soil nutrients and crop yield. Waterlogging duration (main-plot factor) was tested against the Block $\times$ Duration main-plot error term, while nitrogen application and the Duration $\times$ Nitrogen

interaction (sub-plot factors) were tested against the sub-plot residual error. To minimize the influence of sequencing depth on community comparisons, bacterial and fungal ASV tables were rarefied separately before downstream analyses. Rarefaction was performed using the `rarefy_even_depth()` function in the `phyloseq` package in R. Duncan's test was used for multiple comparisons of soil nutrients, and alpha diversity indices (observed ASVs and Shannon index). For the analysis of community composition of bacteria and fungi, only phyla with an average relative abundance greater than 1% in all treatments were retained for subsequent statistical comparisons. We visualized treatment clustering via Principal Coordinate Analysis (PCoA) based on Bray-Curtis distance at ASV levels using the `vegan` package, and permutational multivariate analysis of variance (PERMANOVA) with 999 permutations was applied to test the significant effects of waterlogging duration and postponed nitrogen application on community structure using the `adonis2` function. Linear discriminant analysis effect size (LEfSe) was conducted to identify differentially abundant microbial biomarkers among three nitrogen application treatments, with a logarithmic LDA score threshold of 2.0 using the `microeco` package. Random forest (RF) analysis with permutation-based significance testing was conducted using the `rfPermute` package to pinpoint the key soil physicochemical factors shaping microbial alpha diversity at the flowering and maturity stages. The variable importance was evaluated by the percent increase in mean squared error (%IncMSE), and its statistical significance was assessed via permutation tests. Redundancy analysis (RDA) was conducted based on the `vegan` package to explore linkages between soil physicochemical properties and microbial community composition, incorporating forward selection. Meanwhile, the `vegan` package's `envfit` function was employed to determine which soil properties had marked effects on bacterial and fungal communities.

For co-occurrence network construction, only ASVs detected in at least two samples were retained to avoid spurious correlations. Spearman correlations among ASVs were estimated with the `Hmisc` package. Only correlations meeting the thresholds of $|r| > 0.8$ and FDR-adjusted $p < 0.01$ were regarded as significant and incorporated into the networks. Individual co-occurrence networks were constructed for each waterlogging and nitrogen combination, yielding a total of 12 networks. For each network, three replicates from the flowering stage and three replicates from the maturity stage under the same treatment were pooled, resulting in six samples per network. Given the limited statistical power of $n = 6$, 1000 bootstrap resamplings were performed to evaluate network robustness. Only correlations that remained significant in more than 80% of bootstrap iterations were retained. The resulting networks were visualized using Gephi (v.0.10.1, University of Technology of Compiègne, Compiègne, France). Additionally, topological properties, including the number of nodes, number of edges, average degree, diameter, and modularity, were calculated with the `igraph` package. The network complexity index was calculated as follows. Four topological indices, number of nodes, number of edges, density, and average degree, were first min-max normalized across all 12 networks using:

$$X' = \frac{X - X_{min}}{X_{max} - X_{min}} \quad (1)$$

where $X_{\min}$ and $X_{\max}$ are the minimum and maximum values of each index across all networks. The complexity index was then defined as the arithmetic mean of the four normalized values:

$$Complexity = \frac{X'_{nodes} + X'_{edges} + X'_{density} + X'_{average\ degree}}{4} \quad (2)$$

The Sloan neutral community model (NCM) was fitted separately for each waterlogging $\times$ nitrogen treatment using the *neutral.fit()* function in the MicEco package in R. For each treatment, the ASV table was formatted as a sample $\times$ ASV matrix, with samples pooled across the flowering and maturity stages. The NCM was used to quantify the relative importance of stochastic processes (e.g., dispersal) versus deterministic selection in microbial community assembly. Two key parameters were derived: the migration rate (m) and the migration–drift parameter (Nm), where $Nm = m \times N$ and N is the rarefied sequencing depth. Higher values of m and Nm indicate stronger dispersal and a greater contribution of stochastic assembly. All figures were generated using the ggplot2 and ggpubr packages.

3. Results

3.1. The Effect of Postponed Nitrogen Application on Soil Nutrients after Waterlogging Stress

Waterlogging and postponed nitrogen application both changed the nutrient status of summer maize farmland soil (**Table 1**). The split-plot ANOVA results showed that nitrate nitrogen (NO_3^- -N), available phosphorus (AP), and alkali-hydrolyzable nitrogen (AN) exhibited significant two-way interaction ($D \times N$, $p < 0.05$). At the flowering stage of summer maize, under the same waterlogging treatment, N3 treatment significantly increased the contents of soil NO_3^- -N and NH_4^+ -N ($p < 0.05$). In the CK treatment, the contents of AP, AN, and AK in N3 treatment were 49.28%, 21.73%, and 43.58% higher than those in N1 treatment, respectively. In the WL9 treatment, the contents of AP, AN, and AK in N3 treatment increased by 31.43%, 77.30%, and 22.60%, respectively, compared with N1 treatment. At the mature stage of summer maize, under WL3 treatment, compared with N1 treatment, N3 treatment significantly increased the soil NO_3^- -N, NH_4^+ -N, AP, AN, and AK content ($p < 0.05$), with an increase of 6.76% and 15.74% in AP and AK content, respectively; In the WL9 treatment, N3 significantly improved the above nutrient indicators compared to N1 treatment, and the increase in AP, AN, and AK content was 21.23%, 22.07%, and 20.23%, respectively.

Table 1. Effects of postponed nitrogen application on soil physicochemical properties of summer maize after waterlogging stress at the flowering and maturity stages.

Period	Duration	Nitrogen Treatment	pH	TN ($\text{g}\cdot\text{kg}^{-1}$)	NO_3^- -N ($\text{mg}\cdot\text{kg}^{-1}$)	NH_4^+ -N ($\text{mg}\cdot\text{kg}^{-1}$)	OM ($\text{g}\cdot\text{kg}^{-1}$)	AP ($\text{mg}\cdot\text{kg}^{-1}$)	AN ($\text{mg}\cdot\text{kg}^{-1}$)	AK ($\text{mg}\cdot\text{kg}^{-1}$)
Flowering stage	CK	N1	7.85 ± 0.08^a	1.52 ± 0.03^a	11.70 ± 1.00^c	6.20 ± 0.40^b	23.32 ± 0.22^b	17.98 ± 0.55^b	75.64 ± 2.87^b	290.67 ± 9.02^b
		N2	7.82 ± 0.07^a	1.53 ± 0.04^a	17.00 ± 0.28^b	7.47 ± 0.65^a	23.57 ± 0.14^{ab}	18.01 ± 0.51^b	88.93 ± 1.57^a	318.00 ± 20.00^b
		N3	7.76 ± 0.03^a	1.55 ± 0.02^a	20.00 ± 0.39^a	7.77 ± 0.55^a	23.81 ± 0.13^a	26.75 ± 0.88^a	92.08 ± 2.84^a	417.33 ± 10.07^a
	WL3	N1	7.96 ± 0.05^b	1.52 ± 0.01^a	16.10 ± 0.53^b	4.77 ± 0.55^a	23.22 ± 0.20^b	24.77 ± 0.81^a	66.35 ± 2.70^b	288.67 ± 5.03^b
		N3								

Maturing stage	WL6	N2	7.91 ± 0.02 ^b	1.55 ± 0.04 ^a	18.57 ± 0.87 ^a	5.10 ± 0.44 ^a	23.59 ± 0.15 ^{ab}	25.36 ± 0.86 ^a	74.28 ± 0.97 ^a	335.33 ± 6.43 ^a
		N3	7.74 ± 0.01 ^a	1.55 ± 0.01 ^a	19.50 ± 0.69 ^a	5.50 ± 0.30 ^a	23.67 ± 0.18 ^a	26.07 ± 0.95 ^a	78.07 ± 2.12 ^a	345.33 ± 11.37 ^a
		N1	7.86 ± 0.01 ^c	1.46 ± 0.04 ^b	8.82 ± 0.20 ^b	2.57 ± 0.23 ^b	23.04 ± 0.07 ^b	17.85 ± 0.47 ^b	62.85 ± 2.24 ^c	291.33 ± 9.45 ^b
	WL9	N2	7.78 ± 0.01 ^b	1.47 ± 0.01 ^b	11.40 ± 0.17 ^b	3.00 ± 0.48 ^{ab}	23.30 ± 0.25 ^{ab}	21.60 ± 0.98 ^a	70.59 ± 1.89 ^b	300.00 ± 4.00 ^b
		N3	7.67 ± 0.01 ^a	1.51 ± 0.02 ^a	16.07 ± 3.03 ^a	3.43 ± 0.15 ^a	23.58 ± 0.21 ^a	22.87 ± 0.88 ^a	89.60 ± 2.84 ^a	322.67 ± 10.26 ^a
		N1	7.82 ± 0.01 ^c	1.40 ± 0.04 ^b	14.78 ± 0.30 ^b	3.08 ± 0.38 ^b	22.78 ± 0.14 ^b	18.61 ± 0.53 ^c	55.54 ± 1.62 ^b	256.67 ± 1.15 ^c
	CK	N2	7.77 ± 0.01 ^b	1.44 ± 0.03 ^b	15.55 ± 0.66 ^{ab}	3.28 ± 0.25 ^{ab}	23.10 ± 0.05 ^a	20.58 ± 0.83 ^b	56.70 ± 2.29 ^b	292.00 ± 9.17 ^b
		N3	7.71 ± 0.01 ^a	1.52 ± 0.01 ^a	16.38 ± 0.39 ^b	3.75 ± 0.18 ^a	23.39 ± 0.16 ^a	24.46 ± 1.15 ^a	98.47 ± 2.54 ^a	314.67 ± 11.37 ^a
		N1	7.77 ± 0.05 ^b	1.46 ± 0.00 ^b	17.30 ± 0.85 ^a	3.57 ± 0.15 ^a	23.26 ± 0.18 ^a	20.57 ± 0.36 ^b	59.21 ± 1.13 ^b	286.00 ± 5.29 ^b
	WL3	N2	7.86 ± 0.02 ^a	1.51 ± 0.03 ^a	19.07 ± 0.95 ^a	3.70 ± 0.46 ^a	23.41 ± 0.14 ^a	21.49 ± 1.09 ^b	54.74 ± 1.79 ^b	305.33 ± 17.47 ^{ab}
		N3	7.90 ± 0.01 ^a	1.54 ± 0.02 ^a	19.27 ± 1.20 ^a	4.07 ± 0.21 ^a	23.54 ± 0.35 ^a	29.47 ± 0.67 ^a	81.81 ± 2.61 ^a	318.67 ± 8.08 ^a
		N1	7.95 ± 0.01 ^a	1.50 ± 0.06 ^a	17.47 ± 0.55 ^b	2.87 ± 0.23 ^b	23.25 ± 0.23 ^b	33.57 ± 0.92 ^b	73.99 ± 2.67 ^b	360.00 ± 5.29 ^b
	WL6	N2	7.89 ± 0.03 ^{ab}	1.53 ± 0.04 ^a	17.97 ± 0.21 ^{ab}	3.57 ± 0.21 ^{ab}	23.45 ± 0.11 ^{ab}	35.13 ± 0.49 ^{ab}	90.19 ± 2.73 ^a	370.67 ± 1.15 ^b
		N3	7.86 ± 0.03 ^b	1.54 ± 0.04 ^a	18.87 ± 0.40 ^a	4.13 ± 0.95 ^a	23.70 ± 0.03 ^a	35.84 ± 0.78 ^a	85.17 ± 2.64 ^a	416.67 ± 13.32 ^a
		N1	7.94 ± 0.01 ^a	1.45 ± 0.04 ^a	17.03 ± 0.90 ^a	2.57 ± 0.47 ^b	23.17 ± 0.11 ^a	22.31 ± 1.34 ^c	76.46 ± 2.07 ^c	316.00 ± 5.29 ^c
	WL9	N2	7.88 ± 0.02 ^b	1.48 ± 0.04 ^a	17.67 ± 1.04 ^a	2.90 ± 0.20 ^{ab}	23.34 ± 0.17 ^a	26.61 ± 0.88 ^b	85.19 ± 1.40 ^b	352.67 ± 4.16 ^b
		N3	7.87 ± 0.01 ^b	1.50 ± 0.06 ^a	19.23 ± 2.37 ^a	3.37 ± 0.15 ^a	23.49 ± 0.28 ^a	31.65 ± 1.01 ^a	93.75 ± 3.19 ^a	372.67 ± 10.07 ^a
		N1	7.93 ± 0.02 ^a	1.42 ± 0.02 ^b	17.87 ± 0.76 ^b	3.50 ± 0.17 ^b	22.93 ± 0.17 ^b	22.00 ± 0.11 ^b	76.80 ± 1.99 ^a	290.00 ± 16.00 ^b
		N2	7.84 ± 0.04 ^b	1.43 ± 0.05 ^{ab}	18.33 ± 0.50 ^b	3.63 ± 0.45 ^b	23.27 ± 0.17 ^{ab}	26.05 ± 1.02 ^a	79.85 ± 3.28 ^a	294.00 ± 7.21 ^b
		N3	7.79 ± 0.01 ^b	1.48 ± 0.06 ^a	21.90 ± 0.87 ^a	4.70 ± 0.50 ^a	23.47 ± 0.22 ^a	26.67 ± 0.88 ^a	93.75 ± 3.19 ^a	348.67 ± 4.16 ^a
ANOVA										
Duration (D)			96.63***	75.37***	149.00***	353.20***	12.60**	143.90***	147.10***	487.60***
Nitrogen treatment (N)			2.51	36.19***	26.06***	0.49	0.500	54.32***	7.54**	1.85
D*N			2.72*	8.94***	4.12**	4.46**	2.94*	10.22***	7.75***	0.88

Note: Different letters within the same waterlogging treatment and stage denote significant differences ($p < 0.05$, Duncan's HSD post-hoc analysis, $n = 3$). * represents $p < 0.05$; *** represents $p < 0.001$. Values are mean ± SD.

3.2. The Effect of Postponed Nitrogen Application on Soil Microbial Diversity and Structure after Waterlogging Stress

From the 72 samples, bioinformatic processing yielded 4,531,590 high-quality bacterial reads and 6,843,207 fungal reads. To standardize subsequent analyses, we rarefied the datasets to 33,398 sequences per bacterial sample and 49,795 sequences per fungal sample. This resulted in the clustering of 55,546 bacterial ASVs and 6,978 fungal ASVs. The rarefaction curves for all samples approached a plateau, suggesting that the sequencing depth was sufficient to capture the majority of microbial diversity (**Figure S1**).

The waterlogging duration and postponed nitrogen application significantly affected the alpha diversity of soil bacterial and fungal communities in summer maize farmland, and there were significant differences in the response patterns of different microbial taxa (**Figure 1**). At the flowering stage, the observed ASVs and Shannon index of the bacterial community increased with the prolongation of waterlogging duration, and the observed ASVs and Shannon index of the bacterial community treated with WL9 reached the highest value (**Figure 1A, B**). Under WL3 treatment, compared with N1 treatment, the bacterial observed ASVs and Shannon index of N3 treatment were significantly increased by 13.97% and 2.40%, respectively ($p < 0.05$). In contrast, the alpha diversity of bacterial community in summer maize farmland soil

did not change significantly at the maturity stage (**Figure 1D, E**). Compared with CK treatment, WL6 treatment significantly increased the observed ASVs of fungal community at the flowering and maturity stages (**Figure 1G, J**). At the maturity stage, compared with N1 treatment, N2 treatment significantly increased the observed ASVs and Shannon index of fungal communities by 13.31% and 8.02%, respectively, under WL9 condition (**Figure 1J, K**).

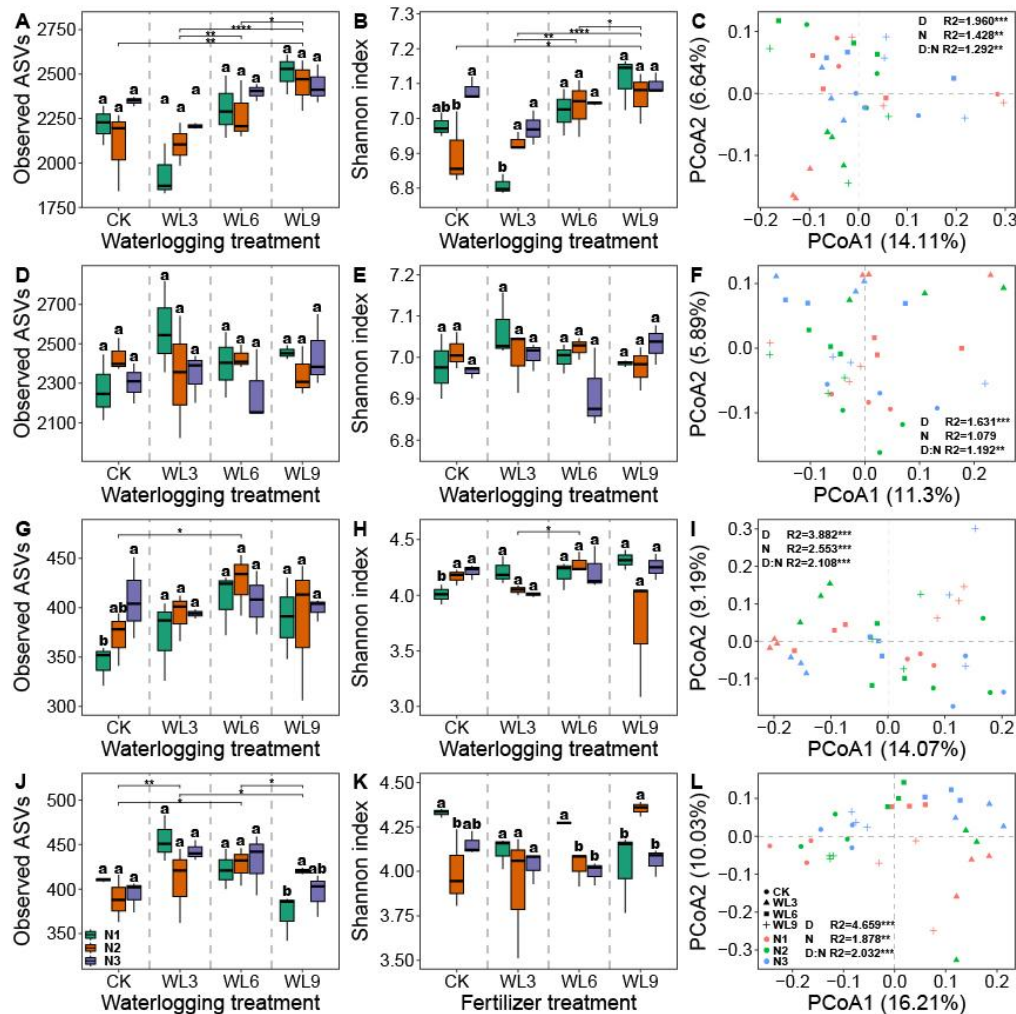


Figure 1. Effects of postponed nitrogen application on soil bacterial and fungal diversity after waterlogging stress at the flowering and maturity stages of summer maize. (**A, B, C**) Bacterial Observed ASVs, Shannon index, and Principal coordinates analysis (PCoA) at the flowering stage; (**D, E, and F**) Bacterial Observed ASVs, Shannon index, and PCoA at the maturity stage; (**G, H, and I**) Fungal Observed ASVs, Shannon index, and PCoA at the flowering stage; (**J, K, and L**) Fungal Observed ASVs, Shannon index, and PCoA at the maturity stage; CK: control (no waterlogging); WL3, WL6, WL9: waterlogging for 3, 6, and 9 days, respectively; N1, N2, N3: different postponed nitrogen application ratios (see Materials and Methods). Error bars represent standard deviation (SD). The asterisk represents significant differences (Duncan's t test, $p < 0.05$, $n = 3$). * represents $p < 0.05$; ** represents $p < 0.01$; *** represents $p < 0.0001$.

PCoA analysis showed that different treatments significantly changed the soil microbial community structure. The first two axes of flowering stage explained 20.89% and 17.11% of the variation in bacterial and fungal communities (**Figure 1C, F**), respectively, and the interpretation of maturity stage increased to 23.24% and 26.18% (**Figure 1I, L**). The results of PERMANOVA further showed that waterlogging

treatment significantly affected the bacterial and fungal community structure ($p < 0.001$), and the postponed nitrogen application and its interaction with waterlogging also reached a significant level (**Table 2**).

Table 2. Topological properties of co-occurrence networks of bacterial and fungal communities under waterlogging and postponed nitrogen application at the flowering and maturity stage of summer maize.

Waterlogging	Fertilizer	Nodes			Edge		Modularity	Density	Average Degree	Complexity	
		Total	Bacteria	Fungi	Total	Positive					Negative
CK	N1	2480	2071	409	14,633	14,284	349	0.923	0.00465	11.52	0.15
	N2	2494	2083	411	13,780	13,440	340	0.954	0.00432	10.78	0.06
	N3	2695	2268	427	17,153	16,759	394	0.938	0.00462	12.44	0.43
WL3	N1	2488	2040	448	14,223	13,794	429	0.961	0.00446	11.09	0.10
	N2	2546	2117	429	13,899	13,338	561	0.958	0.00412	10.48	0.05
	N3	2593	2150	443	14,506	14,171	335	0.943	0.00422	10.93	0.15
WL6	N1	2752	2299	453	22,215	21,786	429	0.919	0.00576	15.83	0.96
	N2	2706	2247	458	18,100	17,716	384	0.936	0.00484	13.09	0.53
	N3	2634	2182	452	17,494	17,149	345	0.932	0.00495	13.02	0.47
WL9	N1	2700	2282	418	17,731	17,285	446	0.925	0.00474	12.8	0.49
	N2	2769	2324	445	19,122	18,773	349	0.934	0.0049	13.56	0.64
	N3	2803	2372	431	19,926	19,515	411	0.937	0.00497	13.92	0.72

The study of bacterial and fungal community composition showed that the dominant bacterial phyla mainly included Pseudomonadota, Acidobacteriota, Chloroflexi, and Actinobacteriota, and the fungal community was dominated by Ascomycota (**Figure S2**). Compared with WL3 treatment, WL6 treatment significantly increased the relative abundances of Glomeromycota and *Septoglomus* at the flowering stage of summer maize. Under WL6 treatment, the relative abundances of Glomeromycota and *Septoglomus* in the N2 treatment were significantly increased by 488.89% and 310.95%, respectively, compared with those in the N1 treatment (**Figure S3**). LEfSe analysis showed that under WL9 treatment, N3 treatment enriched more biomarkers than N1 and N2 treatments. For example, N3 significantly enriched functional bacteria such as *Microcoleus*, *Nitrolancea*, *Luteimonas*, and *Defluviicoccus* in the bacterial community (**Figure 2D**), and N3 treatment significantly enriched *Cercophora*, *Acrophialophora*, *Epicoccum*, and *Blastocladiiales* in the fungal community (**Figure 2G**).

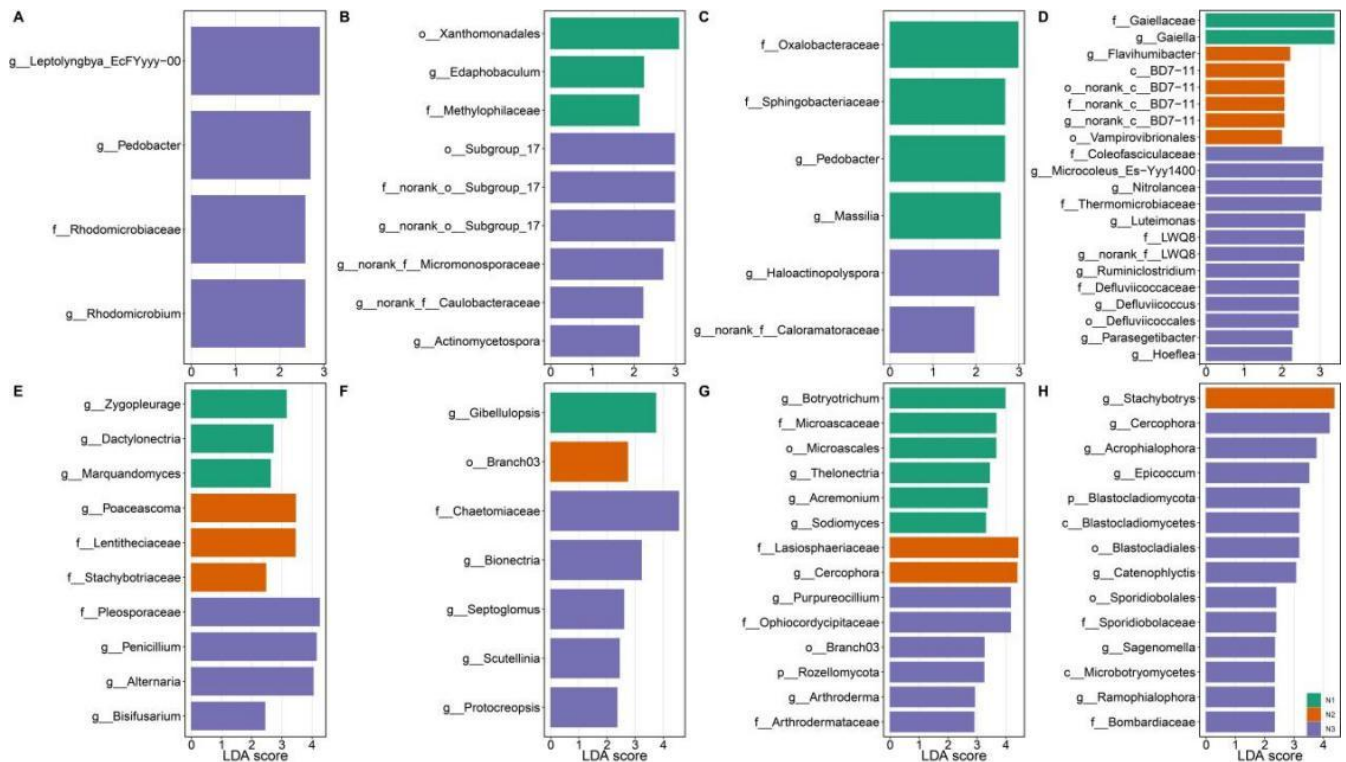


Figure 2. LEfSe analysis showing differentially enriched key taxa in soil bacterial and fungal communities within three postponed nitrogen application after waterlogging stress. **(A–D)** Enriched bacterial taxa at waterlogging for 0, 3, 6, and 9 days, respectively; **(E–H)** Enriched fungal taxa at waterlogging for 0, 3, 6, and 9 days, respectively.

3.3. The Driving Effect of Soil Nutrients on Bacterial and Fungal Microbial α -Diversity

Random forest analysis showed that soil inorganic nitrogen was an important environmental factor driving the change in microbial alpha diversities (**Figure 3**). At the flowering stage, NO_3^- -N was the dominant driver of bacterial observed ASVs and Shannon index (**Figure 3A, B**), whereas NH_4^+ -N primarily shaped fungal richness and diversity. Specifically, NH_4^+ -N was the key environmental factor influencing fungal Shannon index at both the flowering and maturity stages (**Figure 3F, H**). The results of RDA analysis showed that NO_3^- -N and NH_4^+ -N were important environmental variables affecting the bacterial and fungal community structure at the maturity stage (**Figures 4 and S4B, D**).

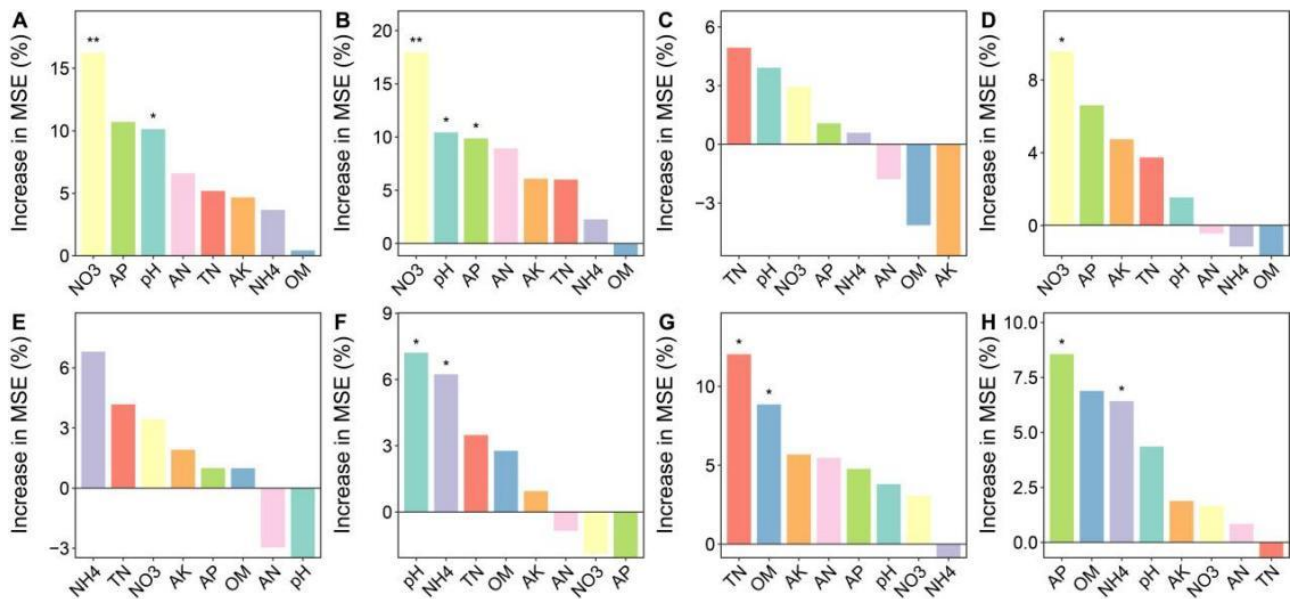


Figure 3. Random forest analysis identifying key environmental factors influencing soil microbial α -diversity. (A, B) Bacterial observed ASVs and Shannon index at the flowering stage; (C, D) Bacterial Observed ASVs and Shannon index at the maturity stage; (E, F) Fungal Observed ASVs and Shannon index at the flowering stage; (G, H) Fungal Observed ASVs and Shannon index at the maturity stage. * represents $p < 0.05$; ** represents $p < 0.01$.

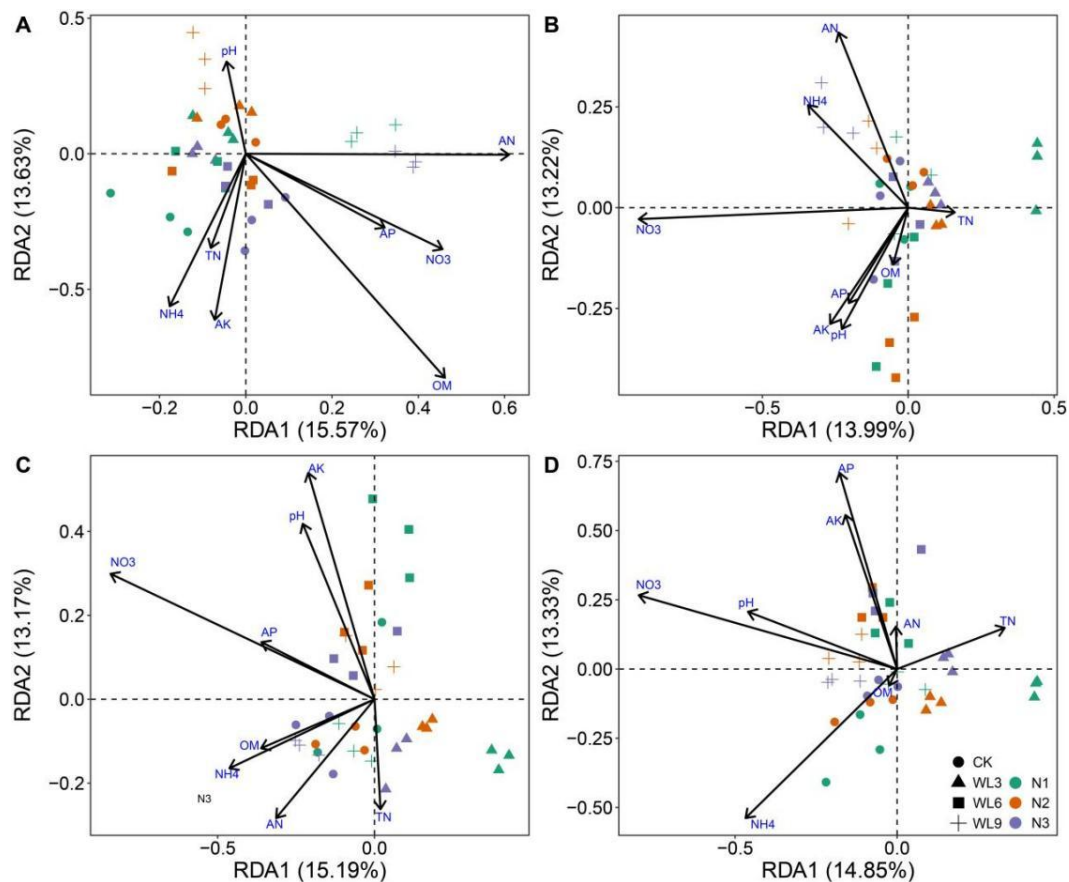


Figure 4. Redundancy analysis (RDA) revealing relationships between soil environmental factors and microbial community structure. (A) Bacterial community at the flowering stage; (B) Bacterial community at the maturity stage; (C) Fungal community at the flowering stage; (D) Fungal community at the maturity stage.

3.4. The Effect of Postponed Nitrogen Application on Co-Occurrence Network after Waterlogging Stress

The co-occurrence network analysis results indicated that waterlogging and postponed nitrogen application altered the interactions between microorganisms (**Figure 5**). Under the WL6 condition, the postponed nitrogen application reduced the complexity of the microbial network. Compared with N1 treatment, the number of edges, nodes, density, average degree, and complexity of N2 and N3 treatments decreased, indicating that postponed nitrogen application under moderate waterlogging conditions weakened the connection between microorganisms (**Table 2**). However, the opposite trend was observed under WL9 conditions. With the increase in the postponed nitrogen ratio, the number of nodes, edges, average degree, and complexity of the network increased gradually. Compared with N1 treatment, the complexity of N2 and N3 treatments increased by 30.61% and 46.94%, respectively, indicating that postponed nitrogen application enhanced the synergy between microbial communities and improved the network complexity under waterlogging conditions (**Table 2**).

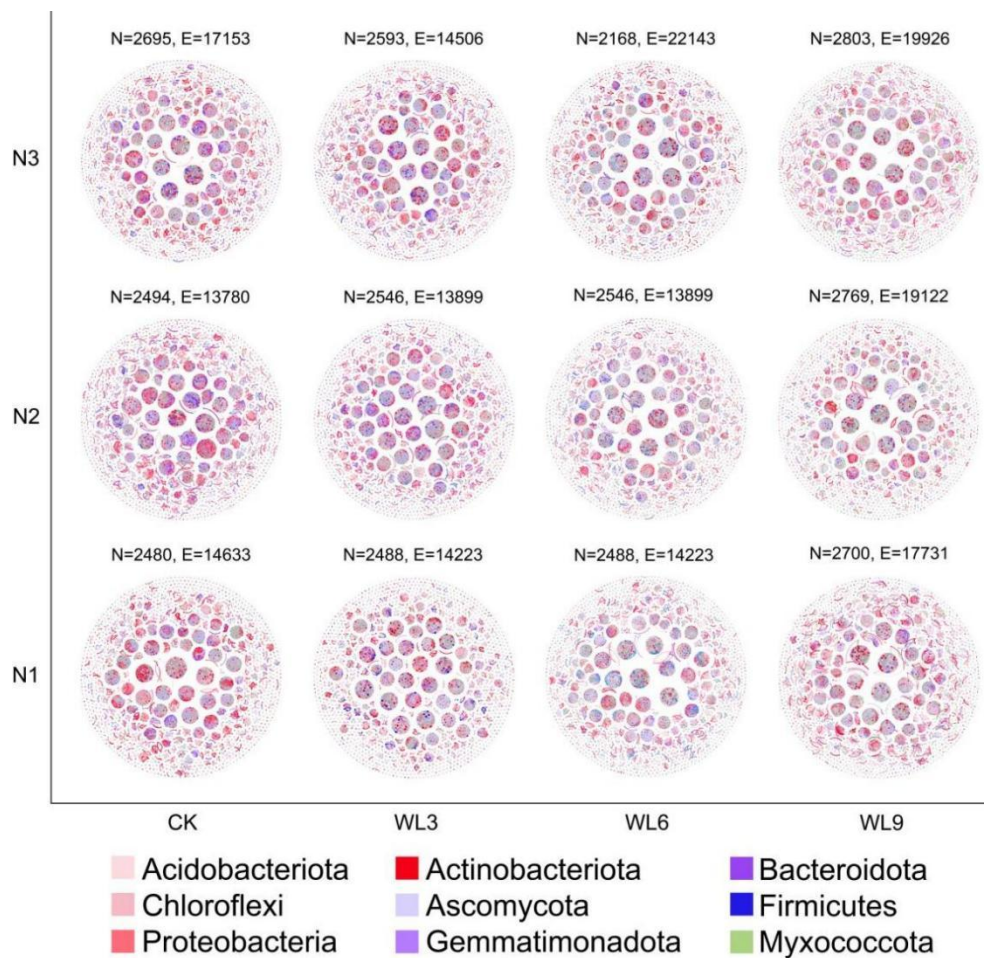


Figure 5. Co-occurrence networks of soil microorganisms under different waterlogging durations and postponed nitrogen applications. The size of each node is proportional to the number of connections (i.e., degree). Each bacterial and fungal node is marked at the phylum level, respectively. The edges indicate the correlation between two nodes.

3.5. The Effect of Postponed Nitrogen Application on Neutral Community Model after Waterlogging Stress

The results of the neutral community model showed that both waterlogging and postponed nitrogen application affected the strength of the random diffusion process during microbial community construction (**Figure 6** and **Figure 7**). For bacterial communities, under CK and WL3 conditions, as the proportion of postponed nitrogen increased, the m and N_m values in the neutral model gradually increased, indicating an enhanced process of microbial migration and diffusion. Under WL9 conditions, there was a trend of first increasing and then decreasing, indicating that the postponed nitrogen strategy increased the environmental screening effect under severe waterlogging conditions (**Figure 6**). For the fungal community, in CK, WL3, and WL6 treatments, m and N_m increased with the increase of postponed nitrogen ratio, but under N3 condition, m and N_m decreased gradually with the increase of waterlogging duration, indicating that moderate nitrogen postponing was beneficial to enhance microbial diffusion ability and community regeneration, while an excessively high proportion of late-season topdressing limits microbial migration and weakens random processes (**Figure 7**).

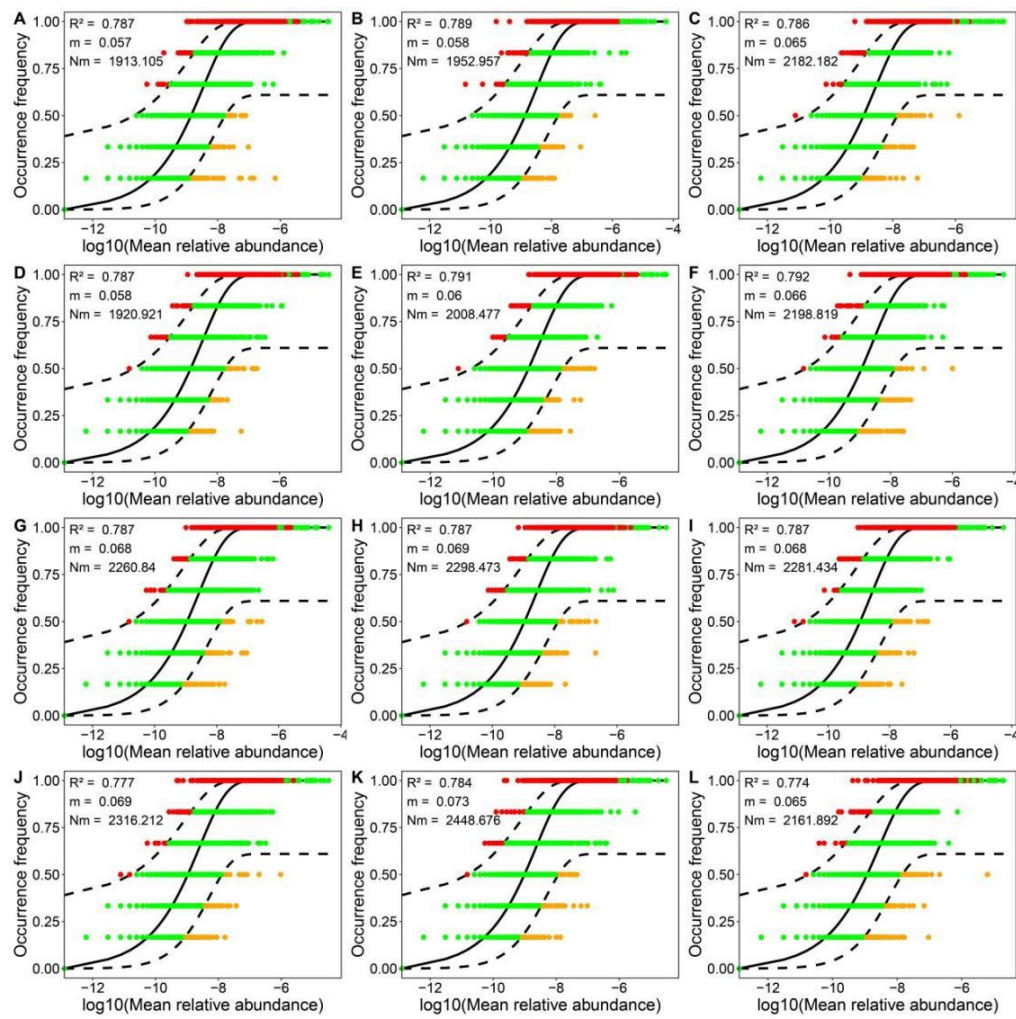


Figure 6. Neutral community model fitting for bacterial communities under different waterlogging durations and postponed nitrogen applications. Each panel shows the relationship between ASV occurrence frequency and relative abundance under CK (A–C), WL3(D–F), WL6 (G–I), and WL9 (J–L) with N1 (A, D, G, J), N2(B, E, H, K), N3(C, F, I, L). Solid line: model prediction; dashed lines: 95% confidence intervals. R^2 indicates model fit; m is the migration rate; Nm is the migration-drift parameter.

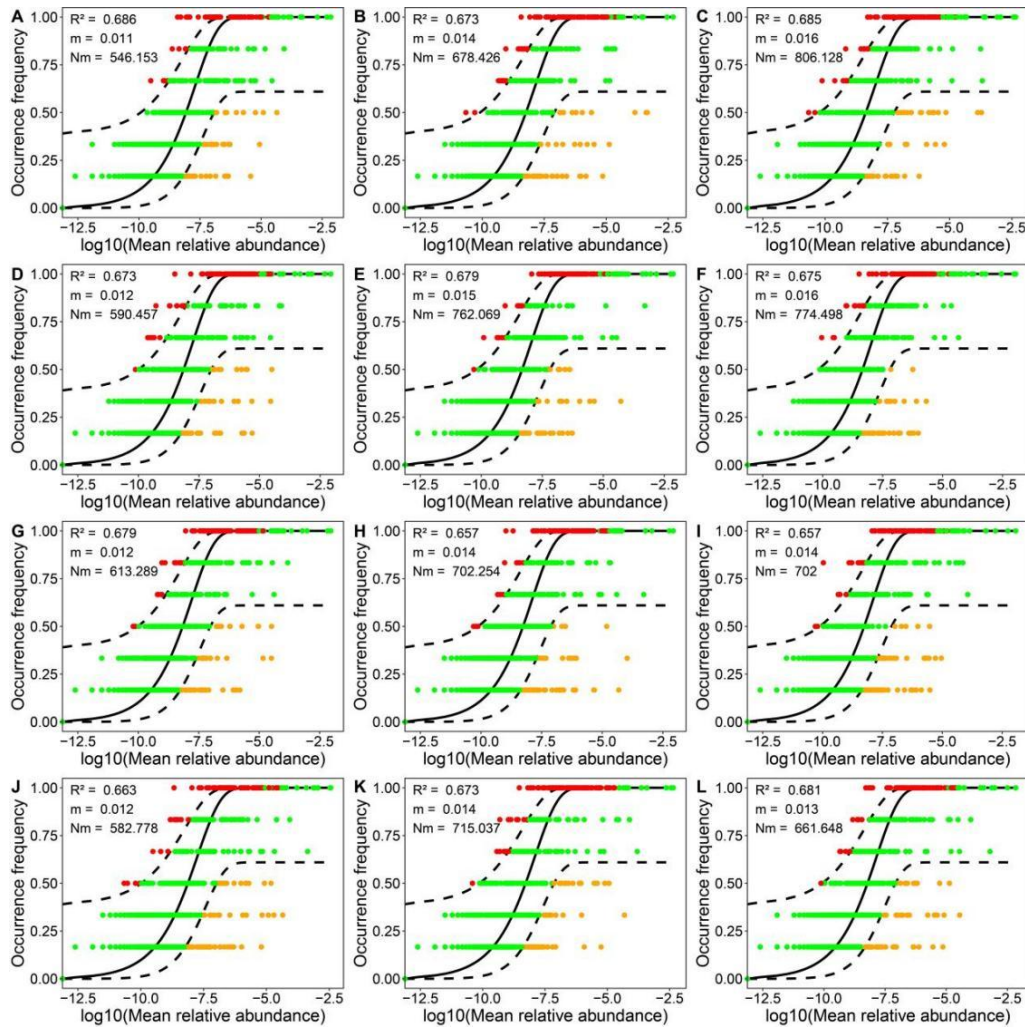


Figure 7. Neutral community model fitting for fungal communities under different waterlogging durations and postponed nitrogen applications. Each panel shows the relationship between ASV occurrence frequency and relative abundance under CK (A–C), WL3(D–F), WL6 (G–I), and WL9 (J–L) with N1 (A, D, G, J), N2(B, E, H, K), N3(C, F, I, L). Solid line: model prediction; dashed lines: 95% confidence intervals. R^2 indicates model fit; m is migration rate; Nm is migration-drift parameter.

3.6. Effects of Postponed Nitrogen Application on Summer Maize Yield after Waterlogging Stress

Waterlogging stress and postponed nitrogen application both significantly regulated the yield of summer maize (**Table 3**). In the CK treatment, compared with the N1 treatment, the 100-grain weight, grain number per ear, and yield of the N3 treatment increased by 9.39%, 8.86%, and 20.89%, respectively. In the WL3 treatment, the N3 treatment increased the 100-grain weight, grain number per ear, and yield by 13.59%, 14.20%, and 33.75%, respectively. In the WL6 treatment, the N3 treatment increased the 100-grain weight, grain number per ear, and yield by 11.26%, 12.10%, and 27.29%, respectively. In the WL9 treatment, the 100-grain weight, grain number per ear, and yield of the N3 treatment increased by 13.01%, 17.29%, and 30.54%, respectively. Two-way ANOVA results showed that both waterlogging duration and nitrogen application treatments had highly significant main effects on the 100-grain weight, grain number per ear, and yield of summer maize ($p < 0.001$).

Table 3. Effects of postponed nitrogen application on summer maize yield under waterlogging stress.

Waterlogging Duration	Fertilizer	100-Grain Weight (g)	Grain Number per Ear	Yield (kg·hm ⁻²)
CK	N1	30.55 ± 0.18 ^c	515.33 ± 6.66 ^c	9591.36 ± 231.88 ^c
	N2	31.68 ± 0.40 ^b	535.67 ± 6.43 ^b	10360.43 ± 241.66 ^b
	N3	33.42 ± 0.49 ^a	561.00 ± 5.29 ^a	11594.97 ± 360.49 ^a
WL3	N1	27.16 ± 0.27 ^c	455.33 ± 14.47 ^b	7680.04 ± 171.92 ^c
	N2	28.69 ± 0.63 ^b	474.67 ± 7.57 ^b	8459.78 ± 185.57 ^b
	N3	30.85 ± 0.47 ^a	520.00 ± 10.82 ^a	10272.37 ± 342.25 ^a
WL6	N1	26.12 ± 0.24 ^c	446.33 ± 6.11 ^c	7256.13 ± 238.79 ^c
	N2	27.31 ± 0.18 ^b	464.00 ± 4.00 ^b	8087.65 ± 158.62 ^b
	N3	29.06 ± 0.30 ^a	500.33 ± 3.06 ^a	9236.47 ± 260.89 ^a
WL9	N1	23.99 ± 0.48 ^c	401.00 ± 10.44 ^c	6138.14 ± 241.04 ^c
	N2	25.49 ± 0.63 ^b	436.00 ± 9.17 ^b	6850.35 ± 274.95 ^b
	N3	27.11 ± 0.13 ^a	470.33 ± 15.31 ^a	8012.55 ± 216.23 ^a
ANOVA				
Duration (D)		878.60 ^{***}	174.10 ^{***}	795.50 ^{***}
Nitrogen treatment (N)		2.01	1.45	0.81
D*N		0.12	0.29	0.06

Note: Different letters within the same waterlogging treatment and stage denote significant differences ($p < 0.05$, Duncan's HSD post-hoc analysis, $n = 3$). * represents $p < 0.05$; *** represents $p < 0.001$. Values are mean ± SD.

4. Discussion

4.1. The Regulatory Effect of Postponed Nitrogen Application on Soil Microbial Community Diversity and Key Functional Microbial Communities under Waterlogging Stress

Soil microbial diversity is the core foundation for maintaining the functions of agricultural ecosystems, and waterlogging stress often has a significant inhibitory effect on microbial communities by changing soil aeration and reducing redox potential. Previous studies showed that water stress significantly altered the structure of rhizosphere microbial communities and promoted the enrichment of stress tolerant functional microbial taxa, thereby increasing the community niche differentiation and microbial richness [30–32]. This study found that with prolonged waterlogging

duration, the alpha diversity of soil bacteria at the flowering stage of summer maize exhibited an increasing trend, whereas no significant difference was observed at the maturity stage. The possible reason is that the hypoxic environment under waterlogging stress promoted the enrichment of anaerobic or facultative anaerobic bacteria. At the maturity stage, however, soil aeration and nutrient conditions tended to stabilize, the legacy effects of stress attenuated, and the microbial community structure approached a steady state; as a result, bacterial diversity showed no significant change. Bacterial diversity under seasonal flooding conditions was higher than that under perennial flooding and perennial drought treatments [33], confirming that appropriate flooding disturbance may increase microbial diversity by increasing niche heterogeneity. Meanwhile, we also noted that 6 days of waterlogging stress significantly increased the richness of soil fungal communities compared to normal growth conditions. This phenomenon can be attributed to the fact that moderate waterlogging stress may activate the germination of hypoxic-tolerant resting spores and increase the species composition of the community; secondly, waterlogging stress might recruit more arbuscular mycorrhizal fungi to enhance nutrient acquisition, thereby promoting the increase of fungal diversity (**Figure S2**). This study found that the bacterial alpha diversity at the flowering stage increased significantly with the increase in the proportion of postponed nitrogen application under the short-term waterlogging conditions, indicating that a higher proportion of postponed nitrogen application led to a more abundant supply of inorganic nitrogen in the soil, thereby providing more diverse niches for bacteria with different nutritional strategies. Notably, under the WL9 treatment, the N2 treatment significantly increased the alpha diversity of the fungal community at the maturity stage, suggesting that the N2 treatment provided appropriate nitrogen allocation that not only alleviated nitrogen loss caused by waterlogging but also avoided the toxicity of over-applying late-season N, thus creating a more favorable micro-environment for the recovery of the fungal community. Microbial response patterns to nutrient management may vary considerably across different soil environments. In a granite quarry restoration study under a humid subtropical climate, biochar application increased bacterial richness but reduced bacterial diversity by 7% [34]. In the same quarry system, by contrast, biochar enhanced fungal diversity and co-occurrence network complexity only in vegetated plots, whereas fungal diversity declined in bare soil [35]. However, these sites were characterized by acidic, nutrient-poor sandy parent material with no prior agricultural history, and biochar served as the primary amendment — conditions that differ notably from the agricultural soil settings in the present study. The maize-cropped soil in this work is a near-neutral Vertisol with well-established organic matter and nutrient pools, and receives continuous root-derived carbon inputs throughout the growing season. Postponed nitrogen application mitigates post-waterlogging nutrient limitation primarily via delayed topdressing, which is distinct from the action mechanism of biochar. Accordingly, when generalizing the microbial response patterns observed in this study to other soil types, differences in parent material, soil pH, climatic conditions, vegetation cover, and amendment type should be fully accounted for. Notably, although postponed nitrogen application significantly altered bacterial community structure at the flowering stage (PERMANOVA, $p = 0.007$), its effect became non-significant at maturity ($p = 0.162$), whereas its effect on fungal

communities remained significant at both stages. This difference likely reflects the stronger resilience and faster recovery of the bacterial community after stress recession, together with the narrower nutrient window during grain filling, by which time the legacy of the early topdressing had weakened; fungi, being more dependent on soil organic matter and residual nutrient pools, retained a detectable response to N allocation throughout the growing season.

This study found that N3 treatment significantly enriched *Nitrolancea* and *Defluviicoccus* in the bacterial community, and *Blastocladales*, *Acrophialophora*, and *Epicoccum* in the fungal community under WL9 treatment. Among them, *Nitrolancea*, an important member of the nitrite-oxidizing bacteria (NOB), performed the key step of oxidizing nitrite to nitrate in the nitrogen cycle; its enrichment may help alleviate the excessive accumulation of ammonium nitrogen in the soil under prolonged waterlogging conditions [36,37]. *Acrophialophora* and *Epicoccum* are considered to have the ability to adapt to adversity and maintain activity in extreme environments such as waterlogging. Moreover, they are saprophytic fungi, and waterlogging-induced root damage and residue accumulation provided abundant carbon substrates [38], and the higher proportion of post-stress topdressing in N3 maintained greater soil inorganic-N availability to meet their nitrogen demands for metabolic activity and reproduction, thereby promoting their enrichment. Increasing the proportion of topdressed nitrogen alleviated nutrient limitation after waterlogging stress, shifting the soil microbial community from a stress-suppressed state toward a functional recovery stage [33]. Many opportunistic taxa within Blastocladiomycota become more competitive when resources are enriched by the postponed nitrogen treatment. Waterlogging stress for 9 days created an anaerobic environment, selectively enriching the anaerobic or facultative anaerobic functional taxa *Defluviicoccales*. The N3 treatment significantly increased soil nitrate and ammonium nitrogen contents, providing ample electron acceptors and nitrogen sources for the denitrification and nitrogen assimilation metabolism of these taxa, and thereby promoted the recovery and re-balancing of soil nitrogen cycling after waterlogging stress. These results suggest that postponed nitrogen application may promote the functional recovery of soil microbial communities after waterlogging stress, by selectively enriching taxa putatively associated with nitrogen cycling and organic matter degradation.

4.2. Regulatory Effects of Postponed Nitrogen Application on Soil Nutrients under Waterlogging Stress and Key Environmental Driving Factors

Waterlogging stress significantly altered the soil nutrient levels of summer maize farmland, and postponed nitrogen application, as an active nutrient management practice, played a key role in alleviating the nutrient imbalance caused by waterlogging [39]. This study confirmed that across waterlogging durations and growth stages, N3 treatment significantly increased the soil nutrient contents. Postponed nitrogen application reduced the nitrogen loss caused by denitrification and leaching under waterlogging conditions by delaying part of the nitrogen fertilizer to the application after the release of waterlogging stress. Meanwhile, delayed

topdressing made the nitrogen supply more matched with the peak period of nitrogen demand of maize, and improved the nitrogen use efficiency [40,41].

This study confirmed that soil inorganic nitrogen forms were key environmental factors affecting microbial diversity, but they exhibited a distinct divergent pattern between bacteria and fungi. At the flowering stage, NO_3^- -N was the key factor influencing bacterial community richness and diversity, whereas NH_4^+ -N primarily drove the fungal community diversity. This difference reflected the fundamental distinctions in nitrogen acquisition strategies and metabolic preferences between bacteria and fungi. Bacteria are known to harbor a broad range of nitrogen-transforming metabolic pathways, which may underlie their greater sensitivity to changes in NO_3^- -N [42,43]. In contrast, fungi, as heterotrophic microorganisms, depended more on the mineralization of organic nitrogen in soil for their growth and metabolic activities, and were consequently more closely associated with NH_4^+ -N [44,45]. Previous studies also found that NH_4^+ -N content was an important factor driving changes in total bacterial and ammonia-oxidizing bacterial community structure, while NO_3^- -N played a more important role in driving compositional shifts in ammonia-oxidizing archaea [46,47]. RDA further confirmed that NO_3^- -N and NH_4^+ -N were important environmental variables influencing bacterial and fungal community structure at the maturity stage. Furthermore, Soil moisture and available nitrogen were the primary factors predicting the stability of nitrogen-cycling microbial communities [48]. Taken together, postponed nitrogen application enhanced the regulation of microbial communities by increasing soil NO_3^- -N and NH_4^+ -N contents, thereby promoting the reconstruction of microbial community structure.

4.3. Regulation Effects of Postponed Nitrogen Application on Soil Microbial Co-Occurrence Networks and Community Assembly Processes under Waterlogging Stress

Microbial co-occurrence networks can reflect interspecific interactions within a community and ecological stability [49]. This study found that postponed nitrogen application reduced the complexity of microbial co-occurrence networks under WL6 conditions, mainly due to the fact that moderate waterlogging exerted strong selection pressure on microorganisms, so that only specific functional taxa that could adapt to anaerobic conditions were retained, and the synergistic interaction between species was weakened, so the number of network edges and average degree decreased. Moderate waterlogging combined with nitrogen management reshaped microbial interspecific relationships [33], resulting in a simplified network structure. In contrast, long-term waterlogging promoted microbial dispersal and colonization, driving the microbial community composition toward specific functional groups adapted to anaerobic environments. Meanwhile, delayed nitrogen application provided re-supplied nutrients when crop N demand recovered, alleviating resource limitations under extreme conditions, and thereby enabled the formation of closer cooperative relationships among different microbial taxa [50,51]. Microbial network complexity increased with greater N addition rates in their N-rate experiment, and network stability and ecological resilience were enhanced under waterlogged conditions [51]. Moreover, the increase in positive correlations within the network under severe

waterlogging conditions indicated the formation of new synergistic relationships among different anaerobic microbial taxa, thereby reconstructing more complex network interactions [52,53]. This intensity-dependent regulation pattern of waterlogging stress revealed the dual effects of postponed nitrogen application on soil microecology restoration after waterlogging, that is, moderate postponed nitrogen fertilizer helps to restore the network stability, while excessive or insufficient nitrogen fertilizer may destroy the balance of interspecific interactions [54].

The migration probability (m) and migration intensity (Nm) in the neutral community model reflect the relative contributions of stochastic dispersal and environmental filtering during microbial community assembly [55]. In this study, under normal or mild waterlogging conditions, the m and Nm values of bacterial communities increased with the increasing ratio of postponed nitrogen application, indicating that appropriate nitrogen allocation alleviated resource limitations, enhanced microbial dispersal connectivity, and allowed stochastic processes to play a more important role in community assembly. However, the values of m and Nm showed a trend of increasing first and then decreasing, indicating that an overly high proportion of late-season N in the long-term anaerobic environment may re-dominate community construction by strengthening environmental filtration. Nitrogen input can shift the assembly pattern of bacterial communities from stochasticity-dominated to determinism-dominated [56]. Fungal communities exhibited a different response pattern: the values of m and Nm increased continuously with the increasing ratio of postponed nitrogen application in the CK, WL3, and WL6 treatments, indicating that fungal dispersal ability was more sensitive to appropriate nitrogen allocation; however, under the N3 condition, m and Nm values gradually decreased with prolonged waterlogging duration, suggesting that long-term waterlogging superimposed with the highest proportion of late-season topdressing (N3) may limit the dispersal efficiency of fungal spores or disrupt their suitable microhabitats. It should be noted that the neutral community model was fitted at the treatment level, yielding a single m and Nm value. Therefore, formal correlation between these parameters and soil physicochemical variables was not performed, and the assembly patterns were interpreted as qualitative trends rather than quantitative mechanistic evidence. Similarly, riparian zone studies found that flood-driven deterministic processes dominated bacterial community assembly [57], whereas stochastic processes played a more prominent role in fungal community assembly, and the assembly processes were primarily driven by factors such as soil pH, SOC, and AK. In summary, the postponed nitrogen application enhanced community resilience and stability by modulating the relative contributions of stochastic and deterministic processes in the assembly process under waterlogging stress. However, under extreme waterlogging conditions, environmental filtering effects become dominant, and moderate postponed nitrogen application was more conducive to maintaining the stochastic components in community assembly, thereby promoting the functional recovery of microbial communities.

5. Conclusion

This study demonstrated that increasing the proportion of postponed nitrogen application generally improved soil nutrient status and grain yield under waterlogging stress, but the optimal nitrogen allocation strategy depended on waterlogging severity. Bacterial alpha diversity at the flowering stage increased with prolonged waterlogging duration. Compared with the N1 treatment, N3 significantly increased bacterial alpha diversity under WL3 at the flowering stage, while N2 markedly enhanced fungal alpha diversity under WL9 at the maturity stage. These results indicated that the optimal proportion of postponed nitrogen application was dependent on waterlogging severity. Specifically, under WL9 conditions, the moderate N2 strategy was preferable, as it improved fungal diversity and avoided potential adverse effects caused by excessive late nitrogen input. In contrast, under CK, WL3, and WL6 conditions, the high-proportion postponed N3 strategy was more beneficial when prioritizing bacterial community recovery and grain yield formation. Postponed nitrogen application effectively enriched putative nitrogen-cycling bacteria and saprophytic fungi. NO_3^- -N and NH_4^+ -N were the primary environmental correlates shaping bacterial and fungal community structures, respectively. The microbial co-occurrence network complexity continuously increased with the rising postponed nitrogen proportion under WL9, while it exhibited an initial increase followed by a decline under WL6. Neutral model analysis revealed that postponed nitrogen application strengthened stochastic dispersal processes of bacterial and fungal communities under CK and WL3 conditions. Overall, postponed nitrogen application is a promising field management strategy for mitigating waterlogging stress damage, restoring soil micro-ecological stability, and potentially enhancing nitrogen use efficiency in summer maize production in the Huang-Huai-Hai region.

Supplementary materials: Figure S1: Rarefaction curve explores the number of bacterial (A) and fungal (B) ASVs in all analyzed samples. The curves of different colors represent different samples, and there are a total of 72 samples in the figure; Figure S2: The relative abundance of the dominant phyla of the bacterial and fungal community under waterlogging stress and postponed nitrogen applications. (A, B) Bacterial community at flowering and maturity stage; (C, D) Fungal community at flowering and maturity stage; Figure S3: Effects of postponed nitrogen application on the relative abundances of Glomeromycota (A) and *Septoglomus* (B) after waterlogging stress at the flowering and maturity stages of summer maize farmland. The asterisk represents significant differences (Duncan's t test, $p < 0.05$, $n = 3$). * represents $p < 0.05$; ** represents $p < 0.01$; ns represents not significant; Figure S4: The significance of each soil property on the bacterial and fungal community structures using *Envfit* function. (A, B) Bacterial community at flowering and maturity stage; (C, D) Fungal community at flowering and maturity stage.

Author contributions: D. J. performed the experiments, analyzed the data, and wrote the original draft; L. T. conceived and designed the research; D. J. collected soil samples with the assistance of G. W., L. M., Z. W., C. Z., Y. Z. (Yue Zhuo) and M.

G.; X. C. was responsible for project administration; J. L., Y. Z. (Yang Zhao), H. L. and L. T. provided supervision. Y. Z. (Yang Zhao) was responsible for funding acquisition. All authors have read and agreed to the published version of the manuscript.

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References

1. Zou Y., Pan S., Yang F., Zhang D., Han Y., Zhao X., Wang K., Zhao C. Precise Recommendation Method of Suitable Planting Areas of Maize Varieties Based on Knowledge Graph. *Agriculture*. 2023, 13, 526. <https://doi.org/10.3390/agriculture13030526>
2. Zhao J., Chen P. High-resolution cropland mapping in China's Huang-Huai-Hai Plain: The coupling of machine learning methods and prior information. *Computers and Electronics in Agriculture*. 2024, 224, 109225. <https://doi.org/10.1016/j.compag.2024.109225>
3. Han D., Wang P., Li Y., Zhang Y., Guo J. Mapping the Main Phenological Spatiotemporal Changes of Summer Maize in the Huang-Huai-Hai Region Based on Multiple Remote Sensing Indices. *Agronomy*. 2025, 15, 1182. <https://doi.org/10.3390/agronomy15051182>
4. Zhao Y., Zheng R., Zheng F., Zhong K., Fu J., Zhang J., Flanagan D.C., Xu X., Li Z. Spatiotemporal distribution of agrometeorological disasters in China and its impact on grain yield under climate change. *International Journal of Disaster Risk Reduction*. 2023, 95, 103823. <https://doi.org/10.1016/j.ijdrr.2023.103823>
5. Islam M.Z., Wang C. Cost of high-level flooding as a consequence of climate change driver?: A case study of China's flood-prone regions. *Ecological Indicators*. 2024, 160, 111944. <https://doi.org/10.1016/j.ecolind.2024.111944>
6. Wang W., Tang S., Han H., Xu Y. Spatiotemporal Changes in Precipitation during the Summer Maize Growing Season in the North China Plain and Analysis of Its Causes. *Atmosphere*. 2022, 13, 1288. <https://doi.org/10.3390/atmos13081288>
7. Wu W., Yue W., Bi J., Zhang L., Xu D., Peng C., Chen X., Wang S. Influence of climatic variables on maize grain yield and its components by adjusting the sowing date. *Frontiers in Plant Science*. 2024, 15, 1411009. <https://doi.org/10.3389/fpls.2024.1411009>
8. Tian L., Li J., Bi W., Zuo S., Li L., Li W., Sun L. Effects of waterlogging stress at different growth stages on the photosynthetic characteristics and grain yield of spring maize (*Zea mays* L.) Under field conditions. *Agricultural Water Management*. 2019, 218, 250–258. <https://doi.org/10.1016/j.agwat.2019.03.054>
9. Ren B., Zhang J., Li X., Fan X., Dong S., Liu P., Zhao B. Effects of waterlogging on the yield and growth of summer maize under field conditions. *Canadian Journal of Plant Science*. 2014, 94(1), 23–31. <https://doi.org/10.4141/cjps2013-175>
10. Wang X., Li X., Gu J., Shi W., Zhao H., Sun C., You S. Drought and Waterlogging Status and Dominant Meteorological Factors Affecting Maize (*Zea mays* L.) in Different Growth and Development Stages in Northeast China. *Agronomy*. 2023, 13, 374. <https://doi.org/10.3390/agronomy13020374>
11. Ju X.-T., Xing G.-X., Chen X.-P., Zhang S.-L., Zhang L.-J., Liu X.-J., Cui Z.-L., Yin B., Christie P., Zhu Z.-L., et al. Reducing environmental risk by improving N management in intensive Chinese agricultural systems. *Proceedings of the National Academy of Sciences*. 2009, 106(9), 3041–3046. <https://doi.org/10.1073/pnas.0813417106>
12. Chen B., Ren C., Wang C., Duan J., Reis S., Gu B. Driving forces of nitrogen use efficiency in Chinese croplands on county scale. *Environmental Pollution*. 2023, 316, 120610. <https://doi.org/10.1016/j.envpol.2022.120610>
13. Raun W.R., Johnson G.V. Improving Nitrogen Use Efficiency for Cereal Production. *Agronomy Journal*. 1999, 91(3), 357–363. <https://doi.org/10.2134/agronj1999.00021962009100030001x>
14. Kaur G., Singh G., Motavalli P.P., Nelson K.A., Orlowski J.M., Golden B.R. Management Practices to Reduce Crop Production and Nitrogen Losses from Waterlogging. *Crops & Soils*. 2020, 53(4), 33–45. <https://doi.org/10.1002/crso.20053>
15. Walsh O., Raun W., Klatt A., Solie J. Effect of delayed nitrogen fertilization on maize (*Zea mays* L.) grain yields and nitrogen use efficiency. *Journal of Plant Nutrition*. 2012, 35(4), 538–555. <https://doi.org/10.1080/01904167.2012.644373>

16. Preza-Fontes G., Pittelkow C.M., Greer K.D., Bhattarai R., Christianson L.E. Split-nitrogen application with cover cropping reduces subsurface nitrate losses while maintaining corn yields. *Journal of Environmental Quality*. 2021, 50(6), 1408–1418. <https://doi.org/10.1002/jeq2.20283>
17. Xia Z., Gong Y., Lyu X., Lin J., Yang Y., Lu H. Split nitrogen application increases maize root growth, yield, and nitrogen use efficiency under soil warming conditions. *The Crop Journal*. 2025, 13(2), 565–575. <https://doi.org/10.1016/j.cj.2025.01.010>
18. Huang C., Gao Y., Qin A., Liu Z., Zhao B., Ning D., Ma S., Duan A., Liu Z. Effects of waterlogging at different stages and durations on maize growth and grain yields. *Agricultural Water Management*. 2022, 261, 107334. <https://doi.org/10.1016/j.agwat.2021.107334>
19. Ren B., Zhang J., Dong S., Liu P., Zhao B. Effects of Waterlogging on Leaf Mesophyll Cell Ultrastructure and Photosynthetic Characteristics of Summer Maize. *PLOS ONE*. 2016, 11, e0161424. <https://doi.org/10.1371/journal.pone.0161424>
20. Sun Y., Wang S., Chen M., Hu X., Ahmed R.M.M., Meng F., Tang Y., Yang Q., Yang S., Shao R. Development stage-specific recovery of remedial nitrogen fertilizer application after waterlogging in maize through photosynthetic capacity and nitrogen allocation. *Journal of Integrative Agriculture*. 2025. <https://doi.org/10.1016/j.jia.2025.11.015>
21. Bertola M., Ferrarini A., Visioli G. Improvement of Soil Microbial Diversity through Sustainable Agricultural Practices and Its Evaluation by -Omics Approaches: A Perspective for the Environment, Food Quality and Human Safety. *Microorganisms*. 2021, 9, 1400. <https://doi.org/10.3390/microorganisms9071400>
22. Chen Q., Song Y., An Y., Lu Y., Zhong G. Soil Microorganisms: Their Role in Enhancing Crop Nutrition and Health. *Diversity*. 2024, 16, 734. <https://doi.org/10.3390/d16120734>
23. Wang Z., Yin D., Wang H., Zhao C., Li Z. Effects of biochar on waterlogging and the associated change in micro-ecological environment of maize rhizosphere soil in saline-alkali land. *BioResources*. 2020, 15(4), 9303–9323. <https://doi.org/10.15376/biores.15.4.9303-9323>
24. Zeng Q., Hu H., Ge A., Xiong C., Zhai C., Duan G., Han L., Huang S., Zhang L. Plant–microbiome interactions and their impacts on plant adaptation to climate change. *Journal of Integrative Plant Biology*. 2025, 67(3), 826–844. <https://doi.org/10.1111/jipb.13863>
25. Nguyen Linh T.T., Osanai Y., Anderson I.C., Bange M.P., Braunack M., Tissue D.T., Singh B.K. Impacts of waterlogging on soil nitrification and ammonia-oxidizing communities in farming system. *Plant and Soil*. 2018, 426(1–2), 299–311. <https://doi.org/10.1007/s11104-018-3584-y>
26. Lian T., Cheng L., Liu Q., Yu T., Cai Z., Nian H., Hartmann M. Potential relevance between soybean nitrogen uptake and rhizosphere prokaryotic communities under waterlogging stress. *ISME Communications*. 2023, 3, 71. <https://doi.org/10.1038/s43705-023-00282-0>
27. Sun P., Zhao Z., Fan P., Chen W., Ruan Y., Wang Q. Ammonia- and Nitrite-Oxidizing Bacteria are Dominant in Nitrification of Maize Rhizosphere Soil Following Combined Application of Biochar and Chemical Fertilizer. *Frontiers in Microbiology*. 2021, 12, 715070. <https://doi.org/10.3389/fmicb.2021.715070>
28. Deng T., Wang J.-H., Gao Z., Shen S., Liang X.-G., Zhao X., Chen X.-M., Wu G., Wang X., Zhou S.-L. Late Split-Application with Reduced Nitrogen Fertilizer Increases Yield by Mediating Source–Sink Relations during the Grain Filling Stage in Summer Maize. *Plants*. 2023, 12, 625. <https://doi.org/10.3390/plants12030625>
29. Li Y., Zou N., Liang X., Zhou X., Guo S., Wang Y., Qin X., Tian Y., Lin J. Effects of nitrogen input on soil bacterial community structure and soil nitrogen cycling in the rhizosphere soil of *Lycium barbarum* L. *Frontiers in Microbiology*. 2023, 13, 1070817. <https://doi.org/10.3389/fmicb.2022.1070817>
30. Yu T., Cheng L., Liu Q., Wang S., Zhou Y., Zhong H., Tang M., Nian H., Lian T. Effects of Waterlogging on Soybean Rhizosphere Bacterial Community Using V4, LoopSeq, and PacBio 16S rRNA Sequence. *Microbiology Spectrum*. 2022, 10(1), e02011–21. <https://doi.org/10.1128/spectrum.02011-21>
31. Mo Y., Bier R., Li X., Daniels M., Smith A., Yu L., Kan J. Agricultural practices influence soil microbiome assembly and interactions at different depths identified by machine learning. *Communications Biology*. 2024, 7, 1349. <https://doi.org/10.1038/s42003-024-07059-8>
32. Naasko K., Martin T., Zakolski E., Mann M., Malacrinò A., Novais W., Lindsey A., Sprunger C. The impact of short-term flooding on soil microbial communities, soil nitrogen and maize productivity in clay loam soils of Ohio, United States. *Annals of Applied Biology*. 2026, 188(3), 971–982. <https://doi.org/10.1111/aab.70107>

33. Han Y., Chen K., Shen Z., Li K., Chen M., Hu Y., Wang J., Jia H., Zhu X., Yang Z. Effects of water tables and nitrogen application on soil bacterial community diversity, network structure, and function in an alpine wetland, China. *Journal of Arid Land*. 2024, 16(11), 1584–1603. <https://doi.org/10.1007/s40333-024-0031-1>
34. Liao J.X., Bordoloi S., Wang Y.C., Hau B.C.H., Ng C.W.W. Field study of biochar effects on vegetation and bacterial communities in a restored mine. *Applied Soil Ecology*. 2025, 211, 106141. <https://doi.org/10.1016/j.apsoil.2025.106141>
35. Liao J.X., So P.S., Bordoloi S., Guo H., Hau B.C.H., Hu L., Ng C.W.W. Dynamics of soil fungal communities restored with biochar from a quarry site. *Journal of Environmental Sciences*. 2026, 168, 558–570. <https://doi.org/10.1016/j.jes.2026.02.015>
36. Daims H., Lückner S., Wagner M. A New Perspective on Microbes Formerly Known as Nitrite-Oxidizing Bacteria. *Trends in Microbiology*. 2016, 24(9), 699–712. <https://doi.org/10.1016/j.tim.2016.05.004>
37. Spieck E., Sass K., Keuter S., Hirschmann S., Spohn M., Indenbirken D., Kop L.F.M., Lückner S., Giaveno A. Defining culture conditions for the hidden nitrite-oxidizing bacterium *nitrolancea*. *Frontiers in Microbiology*. 2020, 11, 1522. <https://doi.org/10.3389/fmicb.2020.01522>
38. Bachmann J., Söffker S., Sepehrnia N., Goebel M., Woche S.K. The effect of temperature and wetting–drying cycles on soil wettability: Dynamic molecular restructuring processes at the solid–water–air interface. *European Journal of Soil Science*. 2021, 72(5), 2180–2198. <https://doi.org/10.1111/ejss.13102>
39. Hamonts K., Clough T.J., Stewart A., Clinton P.W., Richardson A.E., Wakelin S.A., O’Callaghan M., Condon L.M. Effect of nitrogen and waterlogging on denitrifier gene abundance, community structure and activity in the rhizosphere of wheat. *FEMS Microbiology Ecology*. 2013, 83(3), 568–584. <https://doi.org/10.1111/1574-6941.12015>
40. Asibi A.E., Chai Q., Coulter A. Mechanisms of Nitrogen Use in Maize. *Agronomy*. 2019, 9, 775. <https://doi.org/10.3390/agronomy9120775>
41. Motasim A.M., Wahid-Samsuri A., Abdul-Sukor A.S., Amin A.M., Motasim A.M., Wahid-Samsuri A., Abdul-Sukor A.S., Amin A.M. Split application of liquid urea as a tool to nitrogen loss minimization and NUE improvement of corn-A review. *Chilean journal of agricultural research*. 2022, 82(4), 645–657. <https://doi.org/10.4067/s0718-58392022000400645>
42. Meng P., Pei H., Hu W., Shao Y., Li Z. How to increase microbial degradation in constructed wetlands: Influencing factors and improvement measures. *Bioresource Technology*. 2014, 157, 316–326. <https://doi.org/10.1016/j.biortech.2014.01.095>
43. Kuypers M.M.M., Marchant H.K., Kartal B. The microbial nitrogen-cycling network. *Nature Reviews Microbiology*. 2018, 16, 263–276. <http://doi.org/10.1038/nrmicro.2018.9>
44. Cline L.C., Huggins J.A., Hobbie S.E., Kennedy P.G. Organic nitrogen addition suppresses fungal richness and alters community composition in temperate forest soils. *Soil Biology and Biochemistry*. 2018, 125, 222–230. <https://doi.org/10.1016/j.soilbio.2018.07.008>
45. Li D., Meng M., Ren B., Ma X., Bai L., Li J., Bai G., Yao F., Tan C. Different responses of soil fungal and bacterial communities to nitrogen addition in a forest grassland ecotone. *Frontiers in Microbiology*. 2023, 14, 1211768. <https://doi.org/10.3389/fmicb.2023.1211768>
46. Xu A., Li L., Xie J., Gopalakrishnan S., Zhang R., Luo Z., Cai L., Liu C., Wang L., Anwar S., et al. Changes in Ammonia-Oxidizing Archaea and Bacterial Communities and Soil Nitrogen Dynamics in Response to Long-Term Nitrogen Fertilization. *International Journal of Environmental Research and Public Health*. 2022, 19, 2732. <https://doi.org/10.3390/ijerph19052732>
47. Sun J., Rengel Z., Zhou Y., Li H., Zhang A. Ammonia-oxidizing archaea bacteria (AOB) and comammox drive the nitrification in alkaline soil under long-term biochar and N fertilizer applications. *Applied Soil Ecology*. 2024, 193, 105124. <https://doi.org/10.1016/j.apsoil.2023.105124>
48. Zhao Y., Wang Z., Cai K., Wang S., Wright A.L., Jiang X. Stability of nitrogen-cycling microbial communities and impact on microbial nitrogen function under different land use practices. *Applied Soil Ecology*. 2024, 204, 105729. <https://doi.org/10.1016/j.apsoil.2024.105729>
49. Feng Y., Lv C., Wu T., Li J., Wang L., Zhao C. Microbial Co-Occurrence Network Robustness, Not Diversity, Is a Key Predictor of Soil Organic Carbon in High-Altitude Mountain Forests. *Forests*. 2025, 16, 1876. <https://doi.org/10.3390/f16121876>
50. Nguyen Linh T.T., Osanai Y., Lai K., Anderson I.C., Bange M.P., Tissue D.T., Singh B.K. Responses of the soil microbial community to nitrogen fertilizer regimes and historical exposure to extreme weather events: Flooding or prolonged-drought. *Soil Biology and Biochemistry*. 2018, 118, 227–236. <https://doi.org/10.1016/j.soilbio.2017.12.016>

51. Liao X., Tang T., Li J., Wang J., Neher D.A., Zhang W., Xiao J., Xiao D., Hu P., Wang K., et al. Nitrogen fertilization increases the niche breadth of soil nitrogen-cycling microbes and stabilizes their co-occurrence network in a karst agroecosystem. *Agriculture, Ecosystems & Environment*. 2024, 374, 109177. <https://doi.org/10.1016/j.agee.2024.109177>
52. Shade A., Peter H., Allison S.D., Baho D., Berga M., Buergermann H., Huber D.H., Langenheder S., Lennon J.T., Martiny J.B., et al. Fundamentals of microbial community resistance and resilience. *Frontiers in Microbiology*. 2012, 3, 417. <https://doi.org/10.3389/fmicb.2012.00417>
53. Ye C., Gong Y., Chen M., Delgado-Baquerizo M., Che R., Liu S., Zhang Q. Revegetation promotes soil microbial network stability in a novel riparian ecosystem. *Journal of Applied Ecology*. 2023, 60(8), 1572–1586. <https://doi.org/10.1111/1365-2664.14449>
54. Sun M., Li M., Zhou Y., Liu J., Shi W., Wu X., Xie B., Deng Y., Gao Z. Nitrogen deposition enhances the deterministic process of the prokaryotic community and increases the complexity of the microbial co-network in coastal wetlands. *Science of The Total Environment*. 2023, 856, 158939. <https://doi.org/10.1016/j.scitotenv.2022.158939>
55. Wang Y., Zhu K., Chen X., Wei K., Wu R., Wang G. Stochastic and deterministic assembly processes of bacterial communities in different soil aggregates. *Applied Soil Ecology*. 2024, 193, 105153. <https://doi.org/10.1016/j.apsoil.2023.105153>
56. Chen J., Ma Y., Yin J., Qiu H., Tao X., Zheng P., Sun Y. Deterministic assembly processes of sediment bacterial communities regulate the effect of nitrogen input on water quality. *Environmental Science: Water Research & Technology*. 2025, 11(12), 3180–3192. <https://doi.org/10.1039/d5ew00727e>
57. Zhu K., Ye F., Mei Y., Jia W., Zhu X., Li S., Wu S., Zhang S., Huang P. The complex interplay of flooding intensity and land use on soil microbial communities in riparian zones: Insights for ecological restoration. *CATENA*. 2025, 248, 108549. <https://doi.org/10.1016/j.catena.2024.108549>