



Differential Responses of Soil Bacterial and Fungal Communities to Simulated Nitrogen Deposition in a Temperate Wetland of Northeastern China

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Abstract

China has experienced a notorious increase in nitrogen (N) deposition as a result of anthropogenic activities, particularly in temperate areas. While aboveground biodiversity has been extensively studied, the impact of long-term N deposition on the diversity, composition, and function of the soil microbiome remains largely unexplored. In this study, we evaluated alterations in the diversity, composition, and function of soil bacterial and fungal communities in response to varying levels of N deposition (LN = low N addition, 40 kg N ha⁻¹ yr⁻¹; HN = high N addition, 80 kg N ha⁻¹ yr⁻¹) using Illumina MiSeq sequencing technology in a temperate natural wetland. N deposition had no discernible impact on bacterial α diversity, whereas fungal α diversity exhibited a significant decrease in response to high N addition only. Additionally, N deposition led to a notable increase in the relative abundance of the bacterial phylum Patescibacteria but a decrease in Latescibacteria. The relative abundance of Epsilonbacteraeota was highest in the unamended plots and lowest in the low N addition plots. Furthermore, N addition significantly increased the relative abundance of Ascomycota while decreasing that of Mortierellomycota, with no significant effect observed on Basidiomycota. Structural equation modeling (SEM) indicated that soil organic carbon (SOC), and total and available N were the two primary drivers shaping bacterial and fungal communities. Our study demonstrated that bacterial communities were less responsive to N addition compared to fungal communities, emphasizing the significance of simultaneously evaluating the soil microbiome in response to global changes.

Keywords Sanjiang plain · Bacteria and Fungi · Nitrogen deposition · Community composition · Wetland soil

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1 Introduction

Atmospheric nitrogen (N) deposition primarily results from the emission of excessive reactive N compounds and the subsequent atmospheric transport processes. The increase in N deposition has been significantly driven by industrial and agricultural activities (Gruber and Galloway 2008; Song et al. 2014; Wang et al. 2018). This increase in N can potentially enhance plant primary productivity by increasing N content (LeBauer and Treseder 2008). However, N may also have adverse effects on ecosystems by altering vegetation composition and soil nutrient cycling (Roth et al. 2013; Zhu et al. 2015). N deposition can influence the N cycle at regional and global scales through both dry and wet deposition, thereby impacting the global ecosystem's biogeochemical cycles (Holland et al. 2005; Fowler et al. 2013). Therefore, atmospheric N deposition has garnered increasing attention in the context of global change ecology. Soil microorganisms are pivotal components within soil ecosystems (Frey et al. 2020) and play essential roles in energy flow, biogeochemical cycling, litter decomposition, and various climate-related processes (Lakshmi et al. 2020; Basu et al. 2021). Alterations in soil microbial composition and function in response to N deposition can lead to critical challenges for ecosystem function and services. Nevertheless, although several studies have characterized the responses of the soil microbiome to N deposition in grassland and forest ecosystems (Yan et al. 2017; Frey et al. 2020, 2023; He et al. 2021; Wang et al. 2021), research on wetland ecosystems subject to long-term N deposition, especially in temperate freshwater wetlands, remains relatively limited.

N deposition can directly or indirectly influence the structure and function of the soil microbiome by altering various edaphic parameters (Nie et al. 2018; Sui et al. 2022). Microorganisms reliant on inorganic N as an energy source may proliferate with increasing N deposition, potentially leading to an increase in microbial diversity (Wang et al. 2021). However, the abundance of some oligotrophic microorganisms may decrease due to increased N availability (Frey et al. 2023), resulting in decreased microbial diversity. Moreover, N deposition can indirectly impact soil microbial functions, compositions, and diversities by altering above-ground vegetation composition and soil properties (Treseder 2008). Lu et al. (2021) observed changes in the α -diversity and composition of soil microbial communities with increasing N deposition in a temperate coastal wetland of China. In contrast, Hu et al. (2022) reported that N deposition did not alter soil microbial community diversity but significantly changed the structure of the soil bacterial community in a temperate swamp meadow wetland of China. These two examples illustrate the persisting contradictions regarding the impact of N deposition on the structure, function, and diversity of soil microbial communities in temperate wetlands. Furthermore, N deposition affects

not only the soil microbial composition and abundance but also elicits varying responses in bacterial and fungal composition and abundance (Li et al. 2019). Previous studies have reported that N deposition influences the community composition of ectomycorrhizal fungi in temperate beech forests and subtropical pine plantations (De Witte et al. 2017; Ning et al. 2018). Another study reported that N deposition increased the relative abundance of Basidiomycetes and decreased that of Ascomycetes in a northern hardwood forest soil (Edwards et al. 2011). In temperate steppe and tropical forest soils, N deposition affected bacterial community composition by enhancing the relative abundance of Proteobacteria, Actinobacteria, and Firmicutes while reducing the relative abundance of Acidobacteria and Verrucomicrobia (Zeng et al. 2016; Nie et al. 2018). In contrast, DeForest et al. (2004) reported that elevated N deposition had no observable influence on the abundance and composition of bacterial or fungal communities in a hardwood forest. Weng et al. (2022) reported that N deposition significantly impacted the metabolic functional potential of bacterial and fungal communities in wetland soils, promoting various metabolic processes of the microbial community, particularly carbohydrate and amino acid-related metabolic processes and archaea-mediated ammonia oxidation. However, N deposition reduced N₂ fixation and signal transduction. Therefore, uncertainties persist regarding the effects of N deposition on the composition, abundance, function, and diversity of soil microbial communities.

Wetlands are crucial amphibious ecosystems and are widely recognized as biodiversity hotspots (Sui et al. 2021). They serve a multitude of essential ecological functions, including regulating biogeochemical cycles, preserving biodiversity, and mitigating pollution (Junk et al. 2013; Cai et al. 2021; Sui et al. 2021). The Sanjiang Plain, located in northeastern China, is one of the most important freshwater wetland ecosystems (Weng et al. 2022). It is also China's most pristine and well-preserved wetland, serving as a significant grain production region (Zhang et al. 2021). Moreover, the Sanjiang Plain plays a crucial role in ensuring national food security and participating in critical ecological processes (Li et al. 2020a, b). However, it is also severely affected by the high N deposition rates occurring in China. For example, Weng et al. (2022) observed a significant decline in soil microbial carbon utilization with increasing N deposition in the Sanjiang Plain. Zhang et al. (2018a, b, c, d) reported that N deposition notably constrained greenhouse gas emissions in *Deyeuxia angustifolia* wetlands in the Sanjiang Plain. Dai et al. (2018) demonstrated that high N addition significantly reduced plant diversity, whereas low N enhanced it. However, the influence of N addition on the soil microbial diversity, community, and function in temperate wetlands remains unclear.

In May 2010, we initiated a long-term experiment to simulate the increasing N deposition in a typical wetland located

in the Sanjiang Plain, northeastern China. Following four consecutive years of elevated N deposition, we investigated the alterations in the diversity, structure, and function of soil microbial communities in response to N addition. Particularly, our objectives were to 1) elucidate the changes in diversity, composition, and function of soil bacterial and fungal communities within the wetland ecosystem; 2) identify which phyla or genera of soil microorganisms exhibited differential responses, such as mycorrhizal or saprophytic fungi; and 3) determine the critical soil physicochemical parameters that influence the composition and diversity of soil bacteria and fungi. Therefore, our study provides a valuable theoretical framework to support the ecological restoration of wetland ecosystems in the Sanjiang Plain facing elevated N deposition.

2 Materials and Methods

2.1 Research area description

This study was conducted at a field experimental station belonging to the Heilongjiang Academy of Sciences,

situated within the Honghe National Nature Reserve (47°42'18"–47°52'07"N, 133°34'38"–133°46'29"E), Heilongjiang Province, China (Fig. 1). Since 2010, this site has been used for simulating N (NH_4^+ -N and NO_3^- -N) deposition. The wetland area covers approximately 1.1×10^4 ha and is primarily dominated by *Deyeuxia angustifolia* wetlands (Zhang et al. 2018a, b, c, d). The study site exhibits a typical temperate humid/semi humid monsoon climate and exhibits an average annual temperature of 1.9 °C, with an average annual precipitation of 585 mm and evaporation of 1166 mm. The soil in this region is classified as typical bleached stagnant soil and fibrous organic soil according to the USDA soil taxonomy. The dominant vegetation species include *Deyeuxia angustifolia*, *Glyceria spiculose*, *Carex lasiocarpa*, and *Carex pseudocuraica*.

2.2 Experimental design

Three N deposition treatments were established at the field experimental station, namely, ambient [control (CK), 0 kg N ha⁻¹ year⁻¹], low N addition [(LN), 40 kg N ha⁻¹ year⁻¹], and high N addition [(HN), 80 kg N ha⁻¹ year⁻¹]. Each

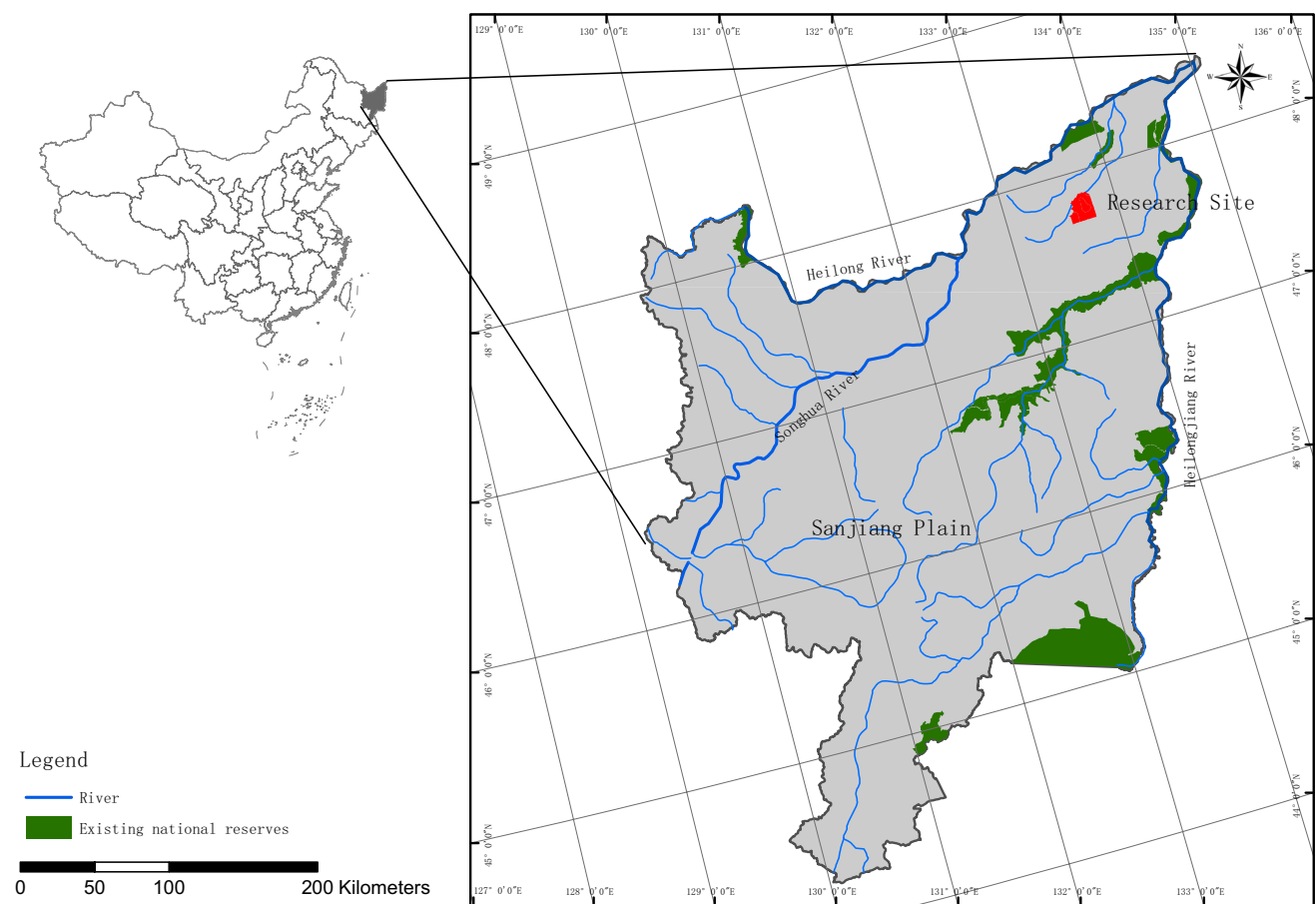


Fig. 1 The location of this study. The red indicates this research site

treatment was replicated in triplicate and randomly allocated with an area of 10 m × 10 m per treatment. The N application rates were selected to mimic a typical fertilization regimen used in local agricultural practices (Song et al. 2016). Each year in May, NH₄NO₃ was uniformly sprayed in each of the N deposition treatments, whereas the control treatment received an equivalent amount of pure water.

2.3 Soil sample collection and analysis

Following four years of simulated N deposition, soil samples from the top layer (approximately 0–20 cm depth) were collected from each N deposition treatment in 2014. Soil samples were collected using a 5-cm inner diameter auger, and 5–10 soil cores were randomly obtained from each plot after removing the litter layer. The soil samples from each plot were combined, placed in a Ziplock bag, and kept at 4 °C before being promptly transported to the laboratory. The soil samples were then homogenized using a 2-mm mesh size sieve and divided into two parts. One part was stored at –80 °C for microbial community analysis, whereas the other part was air-dried for the determination of soil physicochemical properties.

2.4 Determine the soil physicochemical properties

Soil physicochemical properties were characterized as described in our previous study (Sui et al. 2022). Briefly, soil pH was determined by preparing a soil–water (deionized water) suspension at a 1:2.5 ratio (w v⁻¹) and shaking it for 30 min. The pH was subsequently measured using a pH meter (Thermo Scientific Orion 3-Star Benchtop, Cambridge, United Kingdom). Soil organic carbon and total N were quantified using an elemental analyzer (Elementar, Langensfeld, Germany). Nitrate nitrogen (NO₃⁻-N) and ammonium nitrogen (NH₄⁺-N) levels were analyzed using a continuous flow analysis system (SAN ++, Skalar Analytical, Netherlands). Total phosphorus was determined using a spectrophotometer. Available phosphorus content was measured through a colorimetric method following extraction with 0.5 M NaHCO₃. Soil moisture content (SMC) was assessed by comparing the fresh wet weight with the dry weight obtained after drying at 120 °C for 24 h.

2.5 DNA extraction and PCR amplification

Soil genomic DNA was extracted using the MOBIO soil DNA Isolation Kit (Catalog Number: 12888) from MOBIO (Carlsbad, CA, USA). For each sample, 0.5 g of fresh soil was used to extract total DNA according to the

manufacturer's instructions. The purity and concentration of the extracted DNA were determined prior to PCR amplification using a NanoDrop Spectrophotometer (NanoDrop Technologies Inc., DE, USA). All DNA concentrations were adjusted to 50 (ng μL⁻¹) for subsequent PCR amplification. The amplifications were conducted using the 338F/806R primer pair for bacterial 16S rRNA and the ITS1F/ITS2R primer pair for fungal ITS1 rRNA. The PCR reaction mixture volume was 25 μL and consisted of 12.5 μL PCR Master Mix (TAKARA, Japan), 2.0 μL forward and reverse primers (5 μmol L⁻¹), 2.0 μL DNA, and 6.5 μL of H₂O. The PCR amplification program consisted of an initial denaturation step at 95 °C for 45 s, followed by 30 cycles at 95 °C for 45 s, 52 °C (for bacteria) for 45 s or 55 °C (for fungi) for 50 s, and 72 °C for 60 s, and a final extension step at 72 °C for 10 min. Each PCR was performed in triplicate. The PCR products were visualized using 2% agarose gel electrophoresis and then purified using the AXYPrep DNA purification Kit from Axygen Biosciences (CA, United States). Subsequently, the three replicate PCR products for each sample were pooled together and sequenced using the Illumina MiSeq platform (2 × 300 bp) at Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). All raw sequence data were deposited into the NCBI Sequence Read Archive database under the following BioProject accession numbers: PRJNA960933.

2.6 Bioinformatic analysis

The bioinformatic analyses of the raw fasta sequences from bacteria and fungi was conducted using the online platform provided by Majorbio Cloud Platform (www.majorbio.com) (Ren et al. 2022). Briefly, the raw PE reads were merged using the Flash software (version 1.2.11). The barcode primers were trimmed from the merged PE reads, and dereplication was performed using QIIME (version 1.9.1). Chimeric sequences were identified and removed from the dataset using Vsearch (Rognes et al. 2016). Operational taxonomic units (OTUs) were detected at a 97% similarity threshold using the Uparse (Edgar 2010) software. Taxonomic classification was carried out using the SILVA (version 138.1) and UNITE (version 8.0) databases for bacteria and fungi, respectively. A threshold value of 0.8 was used for classification in QIIME1 (Caporaso et al. 2010). Prior to estimating α-diversity, the dataset was rarefied to the lowest number of reads present in all soil samples.

2.7 Statistical analysis

The data analysis was also conducted on the Majorbio Cloud Platform (www.majorbio.com). Briefly, soil bacterial and fungal alpha diversity indices (Chao1, Shannon,

and Simpson) were computed using QIIME1 (Caporaso et al. 2010). Principal coordinates analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA) were conducted based on Bray–Curtis dissimilarity at the OTU level on the online platform. This analysis enables the visualization and assessment of differences in community composition between samples. Venn diagrams representing shared and unique OTUs were generated at the OTU level on the online platform. Cladogram diagrams illustrating taxonomic relationships from phylum to species were generated on the online platform. Heatmaps displaying the 30 most abundant bacterial and fungal genera were generated using online tools (<https://www.bioincloud.tech/>). Correlation heatmaps showing relationships between soil physicochemical parameters and the 30 most abundant bacterial and fungal genera were also generated using online tools (<https://www.bioincloud.tech/>). Structural equation modeling (SEM), a multivariate statistical method for testing complex path-relation networks, was utilized to examine the direct and indirect effects of N deposition on soil bacterial and fungal community diversity and composition in the R software. An a priori model was constructed based on a literature review and our knowledge of how these predictors are related. Diversity was assessed using the Shannon diversity index, whereas composition was assessed via non-metric multidimensional scaling (NMDS). Model adequacy was determined by χ^2 tests ($P > 0.05$), the goodness-of-fit index (CFI, > 0.9), and Akaike information criteria (AIC). Functional prediction of bacterial communities at the OTU level using FAPROTAX was conducted as described by Louca et al. (2016). Fungal guild predictions at the OTU level were performed using FUNGuild (Nguyen et al. 2016). Both FAPROTAX and FUNGuild analyses were conducted using online tools (<https://cloud.majorbio.com/>). Statistical significance was determined using Duncan's multiple comparison method via one-way analysis of variance (ANOVA) with a significance level of 0.05. All relevant statistical analyses were conducted using the SPSS 19.0 software.

Table 2 α diversity of bacterial and fungal communities with increasing N addition

N levels	Sobs	Shannon	ACE	Chao1
Bacteria				
CK	4764 ± 952	6.9 ± 0.2	6762 ± 1518	6543 ± 1139
LN	4957 ± 804	6.9 ± 0.2	7177 ± 1256	6802 ± 939
HN	4689 ± 201	6.8 ± 0.2	6676 ± 157	6469 ± 221
Fungi				
CK	725 ± 68a	4.4 ± 0.2a	778 ± 65a	774 ± 67a
LN	587 ± 98ab	4.3 ± 0.1a	638 ± 71ab	664 ± 58ab
HN	499 ± 42b	4.0 ± 0.3a	534 ± 57b	548 ± 59b

Sobs=Number of observed OTUs. All results reported as mean ± standard deviation (n=3). Different letters within a row indicate significant difference ($P < 0.05$; ANOVA) among different N addition treatments tested in this study. Control: (CK), 0 kg N ha⁻¹ year⁻¹; low N addition: (LN), 40 kg N ha⁻¹ year⁻¹; high N addition (HN), 80 kg N ha⁻¹ year⁻¹

3 Results

3.1 Effects of simulated N deposition on soil physicochemical properties

N deposition significantly changed the soil physicochemical properties except TP (Table 1). Specifically, pH, AP, and SMC decreased in response to N, whereas TN, NO₃⁻-N and NH₄⁺-N increased with increased N deposition (Table 1). Furthermore, LN did not have any observable effects on SOC, but HN significantly increased the SOC levels compared to CK (Table 1).

3.2 Variations in the α - and β -diversities of soil bacterial and fungal communities under simulated N deposition

The bacterial α -indices, including Shannon, Chao1, and number of observed OTUs (Sobs), were not significantly different between the N levels (Table 2). However, the Chao1,

Table 1 Soil physicochemical properties in response to different N additions

N level	pH	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	TP (g kg ⁻¹)	AP (mg kg ⁻¹)	SMC (%)
CK	5.9 ± 0.03a	35.3 ± 1.14b	2.5 ± 0.12c	4.8 ± 0.07c	16.3 ± 0.10c	1.3 ± 0.06a	58.7 ± 1.33a	16.0 ± 0.35a
LN	5.1 ± 0.05b	36.7 ± 2.03ab	2.8 ± 0.13b	5.2 ± 0.04b	19.6 ± 0.49b	1.2 ± 0.07a	51.7 ± 2.15b	12.3 ± 0.59b
HN	4.7 ± 0.08c	38.5 ± 0.68a	3.5 ± 0.11a	6.2 ± 0.06a	24.5 ± 0.66a	1.1 ± 0.06a	47.3 ± 0.96c	12.5 ± 1.36b

All results reported as mean ± standard deviation (n=3). Different letters within a row indicate significant difference ($P < 0.05$; ANOVA) among different N addition treatments tested in this study. Control: (CK), 0 kg N ha⁻¹ year⁻¹; low N addition: (LN), 40 kg N ha⁻¹ year⁻¹; high N addition (HN), 80 kg N ha⁻¹ year⁻¹. SOC, soil organic carbon; TN, total nitrogen; NO₃⁻-N, nitrate nitrogen; NH₄⁺-N, ammonium nitrogen; TP, total phosphorus. AP, available phosphorus; SMC, soil moisture content

ACE, and Sobs of fungal diversity were affected by N addition (Table 2). The fungal Chao1, ACE, and Sobs decreased significantly with increasing N addition ($P < 0.05$), whereas the fungal Shannon index did not change significantly ($P > 0.05$).

PCoA was conducted to visualize the β diversities of soil microbial communities (Fig. 2). Our findings demonstrated that N addition had a significant impact on the β diversity of fungal communities (Fig. 2b; PERMANOVA: $P < 0.05$). In contrast, the β diversity of bacterial communities (Fig. 2a) did not exhibit significant changes among the different levels of N addition (Fig. 2a, PERMANOVA: $P > 0.05$).

3.3 Effects of simulated N deposition on the soil bacterial and fungal compositions

The most dominant bacterial phyla in the LN and HN treatments did not exhibit significant changes compared to the CK (Fig. 3a). However, the relative abundance of Patescibacteria, Firmicutes, Latescibacteria, and Epsilonbacteraeota in the LN and HN treatments did show significant alterations in comparison to the CK (Fig. 3a). Specifically, the relative abundance of Patescibacteria increased significantly, whereas Firmicutes and Latescibacteria decreased significantly with N deposition (Fig. 3a). Notably, the relative abundance of Epsilonbacteraeota was highest in the CK and lowest in the LN treatment (Fig. 3a). Among the bacterial genera, *Candidatus_Solibacter*, *Candidatus_Udaebacter*, *Bryobacter*, *HSB_of53-F07*, *Candidatus_Koribacter*, *Bradyrhizobium*, *Acidotherium*, *Geobacter*, *Euin6067*, and

Halliangium were the most dominant genera across all N deposition levels (Fig. S3 a, relative abundance of top 10 genera). Interestingly, the relative abundance of *Acidothermus*, *Anaeromyxobacter*, *Gaiella*, *Bacillus*, and *Opitutus* in the LN and HN treatments exhibited significant differences compared to the CK (Fig. 4a). Specifically, the relative abundance of *Acidothermus*, *Anaeromyxobacter*, and *Gaiella* decreased significantly, whereas that of *Opitutus* increased with N addition (Fig. 4a).

Regarding the fungal communities, the relative abundance of Ascomycota and Mortierellomycota in the LN and HN treatments exhibited significant changes compared to the CK (Fig. 3b). Notably, N addition significantly increased the abundance of Ascomycota, while decreasing that of Mortierellomycota (Fig. 3b). *Mortierella*, *Archaeorhizomyces*, *Clavaria*, *Lachnum*, *Penicillium*, *Pseudeurotium*, *Trichoderma*, *Acephala*, *Sordaria*, and *Arniium* were the most dominant genera across all N deposition levels (Fig. S3b, relative abundance of top 10 genera). The relative abundance of *Mortierella*, *Trichoderma*, *Sordaria*, *Leohumicola*, *Paraphaeosphaeria*, *Coniochaeta*, *Dimorphospora*, *Fayodia*, *Entoloma*, and *Metarhizium* in the LN and HN treatments were significantly different compared to the CK group (Fig. 4b). Particularly, the relative abundances of *Mortierella*, *Sordaria*, *Leohumicola*, *Paraphaeosphaeria*, *Coniochaeta*, and *Entoloma* were higher in the CK group than those in the LN and HN treatments, whereas the relative abundances of *Trichoderma*, *Fayodia*, and *Dimorphospora* were lower in the CK group compared to those in the LN and HN treatments (Fig. 4b).

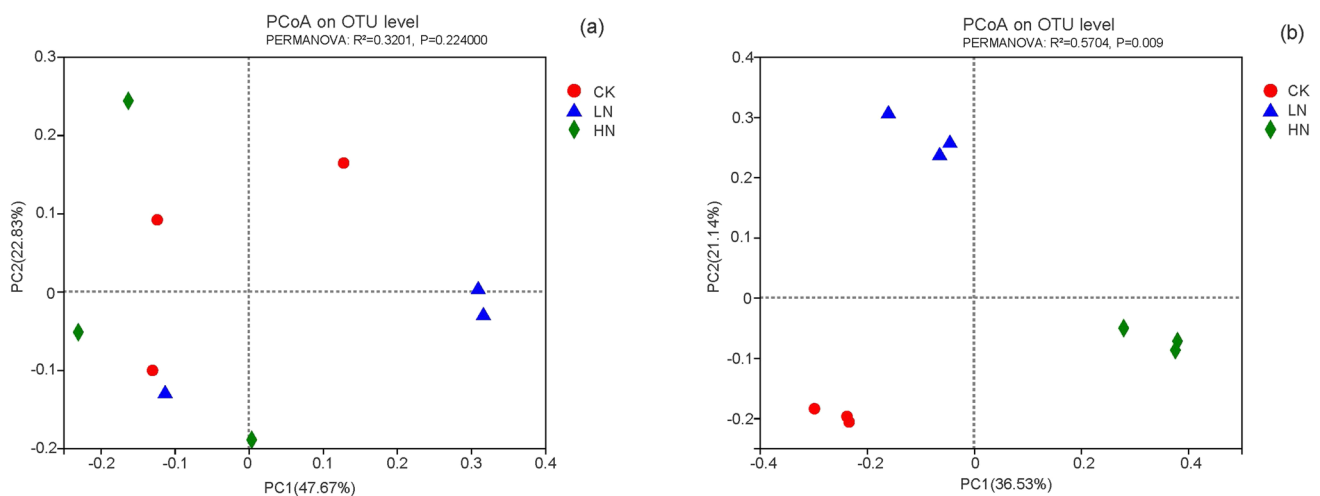
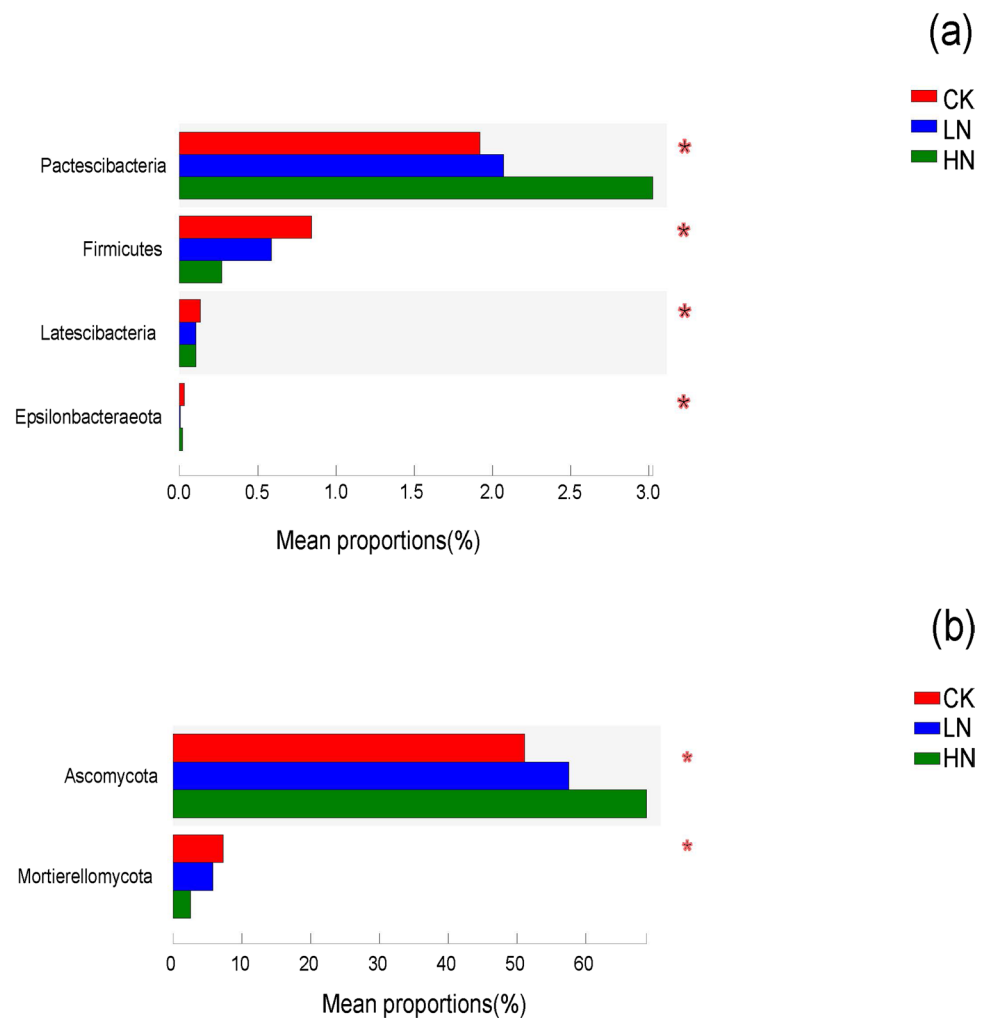


Fig. 2 Principal coordinates analysis (PCoA) of soil bacterial (a) and fungal (b) communities under the different N addition treatments. Control: (CK), 0 kg N ha⁻¹ year⁻¹; low N addition: (LN), 40 kg N ha⁻¹ year⁻¹; high N addition (HN), 80 kg N ha⁻¹ year⁻¹

Fig. 3 Differences in the relative abundance of bacterial (a) and fungal (b) phyla under different N deposition treatments. The asterisks indicate significant differences between three N depositions at the 0.05 level. Control: (CK), 0 kg N ha⁻¹ year⁻¹; low N addition: (LN), 40 kg N ha⁻¹ year⁻¹; high N addition (HN), 80 kg N ha⁻¹ year⁻¹



3.4 Effects of simulated N deposition on soil bacterial and fungal indicator species

The LEfSe analysis identified a total of 7 bacterial and 14 fungal taxa that displayed significant differences in relative abundance (as indicated by LDA effect size scores exceeding 3) among the three N addition treatments (Fig. 5).

3.5 Effects of different soil physicochemical parameters on soil bacterial and fungal communities

SEM was employed to evaluate both the direct and indirect effects of N deposition on the diversity and composition of soil bacterial and fungal communities (Fig. 6). The final model for soil bacteria met our significance criteria ($\chi^2 = 14.65$, $df = 8$, $P = 0.07$, $CFI = 0.94$, $AIC = -10.32$). Notably, N deposition did not exhibit any significant direct effects on the diversity and composition of the soil bacterial community. However, it did show significant indirect effects through SOC ($\lambda = 0.87$, $p < 0.05$), NH_4^+ ($\lambda = -3.67$,

$p < 0.05$), NO_3^- ($\lambda = 3.19$, $p < 0.05$), and TN ($\lambda = -3.36$, $p < 0.05$) on diversity and composition of soil bacterial community (Fig. 6a). Similarly, the final fungi model met our significance criteria ($\chi^2 = 13.56$, $df = 9$, $P = 0.13$, $CFI = 0.96$, $AIC = 4.63$). Moreover, N deposition exhibited significant indirect effects through SOC ($\lambda = -0.62$, $p < 0.05$), NH_4^+ ($\lambda = 2.62$, $p < 0.05$), NO_3^- ($\lambda = -2.71$, $p < 0.05$), and TN ($\lambda = 1.79$, $p < 0.05$) on the diversity and composition of the soil fungal community (Fig. 6b).

3.6 Impact of simulated N deposition on the predictions of potential functions

A total of 50 functional potential functions of soil bacterial communities were annotated using FAPROTAX (Fig. 7a), which were primarily associated with carbon (C) and nitrogen (N) processes. Among these functions, only the abundance of anoxygenic_photoautotrophy_H2_oxidizing bacteria within the soil bacterial communities exhibited significant changes across different N additions (Fig. 7a). Conversely, the other potential functions of soil

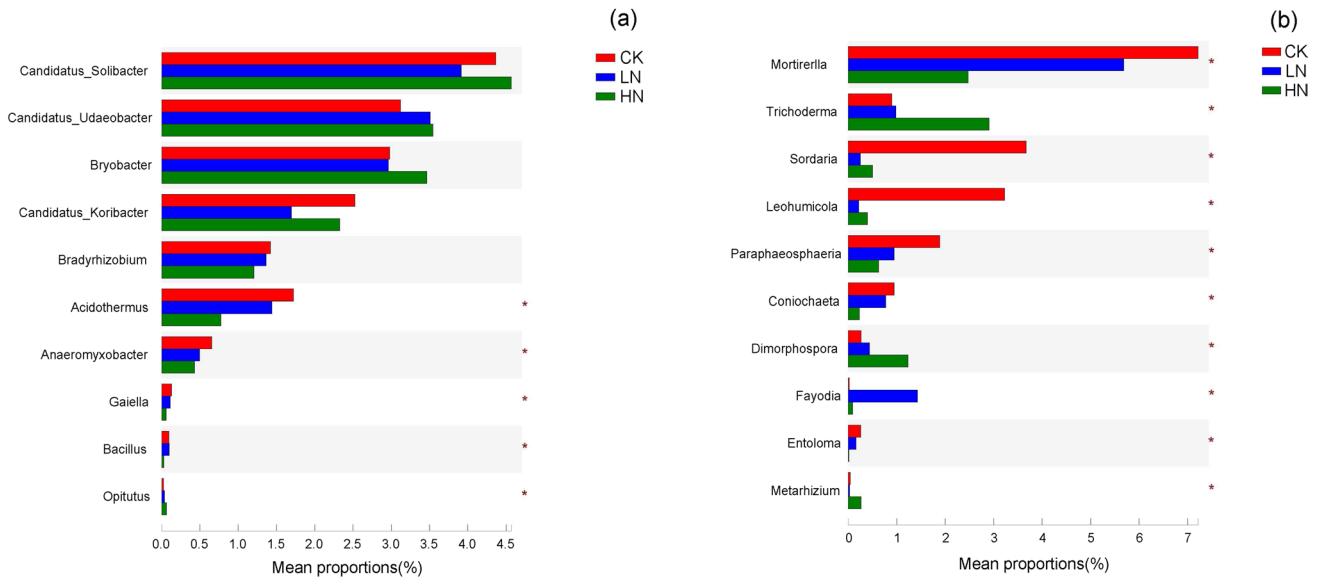


Fig. 4 Differences in the relative abundance of bacterial (a) and fungal (b) genera under N deposition treatment. The asterisks indicate significant differences between three N depositions at the 0.05 level.

Control: (CK), 0 kg N ha⁻¹ year⁻¹; low N addition: (LN), 40 kg N ha⁻¹ year⁻¹; high N addition (HN), 80 kg N ha⁻¹ year⁻¹

bacterial communities did not show significant alterations (Fig. 7a). Notably, the abundance of anoxygenic_photoautotrophy_H2_oxidizing bacteria tended to decrease with increasing N addition (Fig. 7a).

Regarding the soil fungal communities, a total of 19 potential guilds were annotated using the FUNGuild database (Fig. 7b), primarily falling into the “Saprotrophs,” “Pathogens,” “Endophytes,” “Endomycorrhizal,” and “Arbuscular Mycorrhizal” categories. However, only the “Dung Saprotrophs” and “Litter Saprotrophs” categories exhibited significant changes among the different N additions (Fig. 7b, $P < 0.05$), whereas the other guilds did not exhibit significant differences among the three N additions (Fig. 7b). Interestingly, both the abundance of “Dung Saprotrophs” and “Litter Saprotrophs” decreased significantly from the CK to the HN treatment (Fig. 7b).

4 Discussion

In contrast to our initial hypothesis, our results demonstrated that elevated N deposition did not significantly alter soil bacterial α and β diversities, but it did lead to a significant reduction in fungal α diversity under high N (HN) deposition, as well as changes in fungal β diversities across the three N deposition levels. These results are inconsistent with previous studies that demonstrated a significant decrease in both soil bacterial and fungal α diversities under N deposition

concentrations in coastal wetland ecosystems (Lu et al. 2021; Sun et al. 2022) and wetland forests (Li et al. 2019). However, there have been other conflicting findings, such as those reported by Bledsoe et al. (2020), who observed an increase in soil bacterial α diversity in oligotroph-dominated wetlands; Sun et al. (2022), who reported an increase in soil microbial α diversity in coastal wetlands; and Lee et al. (2015) and Hu et al. (2022), who reported no change in soil microbial α diversity under N deposition in marsh wetland ecosystems and swamp meadow wetland ecosystems. These variations suggest that the responses of α diversity in bacteria and fungi to N deposition can vary among different wetland ecosystems. Firstly, the Sanjiang plain wetland, a typical freshwater wetland, possesses distinct characteristics in terms of vegetation species and soil properties compared to other wetland ecosystems. This variation could lead to differences in the sensitivity of soil microorganisms to external N input (Hu et al. 2022). In nutrient-limited conditions, plants often rely on soil microorganisms to facilitate nutrient acquisition, and in return, plants provide carbon resources to support these soil-associated microorganisms (Bledsoe et al. 2020). Consequently, soil microbial α diversity could increase under N deposition in coastal wetlands due to their poor soil nutrient conditions. However, the Sanjiang plain wetland is not nutrient-limited (Chen et al. 2023), and exogenous N input may primarily be absorbed by above-ground vegetation, with limited facilitation by soil microorganisms. Secondly, the duration of N deposition can have a noticeable

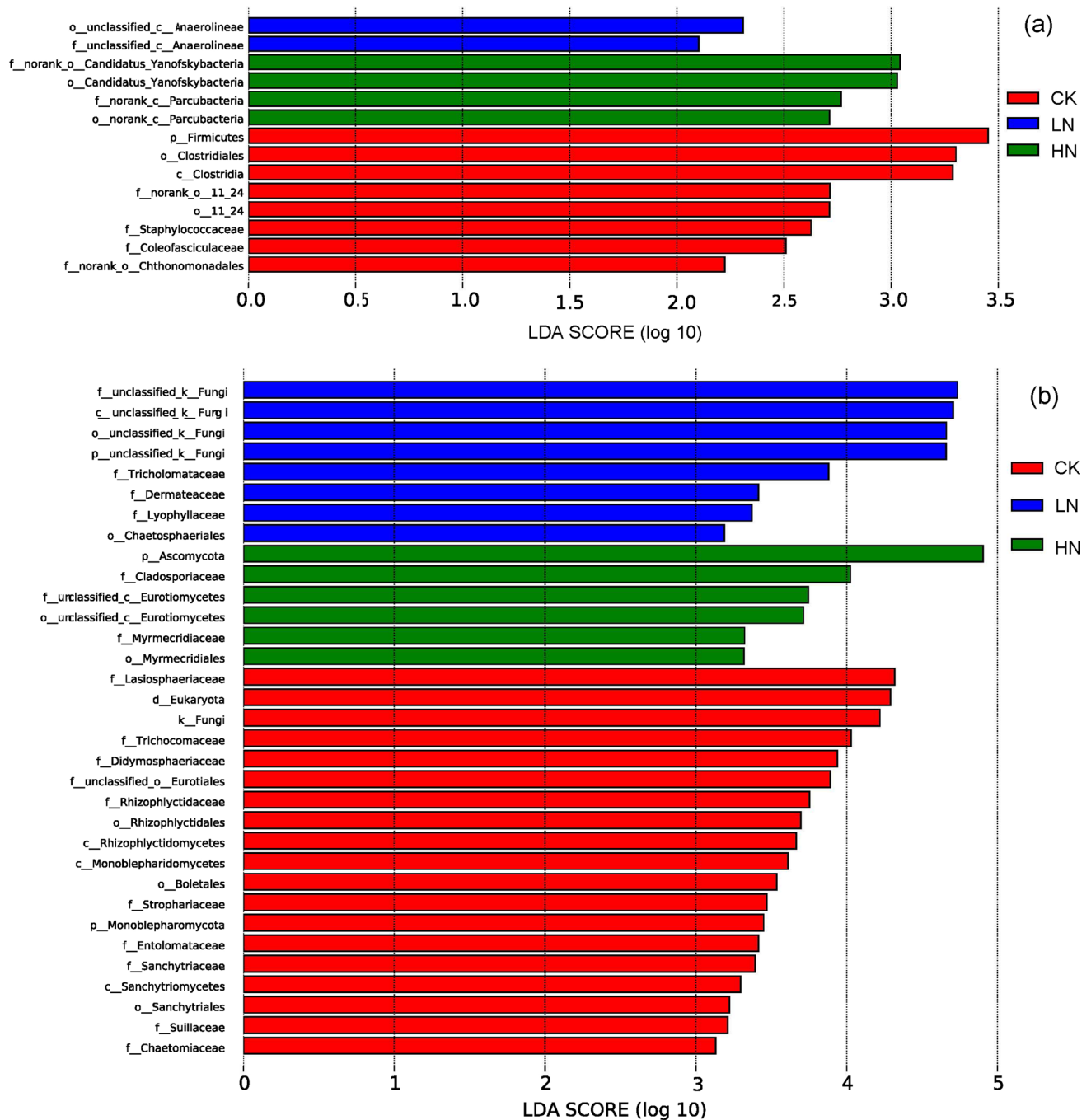


Fig. 5 Lefse graph of the bacterial (a) and fungal (b) communities with LDA > 3.0 among different N deposition treatments. Control: (CK), 0 kg N ha⁻¹ year⁻¹; low N addition: (LN), 40 kg N ha⁻¹ year⁻¹; high N addition (HN), 80 kg N ha⁻¹ year⁻¹

impact on soil microbial community diversity. For instance, soil bacterial community diversity may remain stable under short-term N deposition (McHugh et al. 2017) but decrease with long-term N deposition (Zhang et al. 2022). In our case, a four-year N deposition period may not have been sufficient to induce changes in soil bacterial community diversity. Thirdly, the rate of N deposition may also influence soil microbial community diversity. For example, Liu

et al. (2020) observed that soil bacterial community diversity remained unchanged when N deposition levels were below 160 g·N·m⁻²·a⁻¹. In our study, the N deposition levels (i.e. 40 g and 80 g N·m⁻²·a⁻¹) were somewhat lower, suggesting that these amounts of N application had no significant effect on bacterial diversity. This could be attributed to the greater plasticity, higher population density, and greater adaptability of microbial communities. Lastly, prior research has

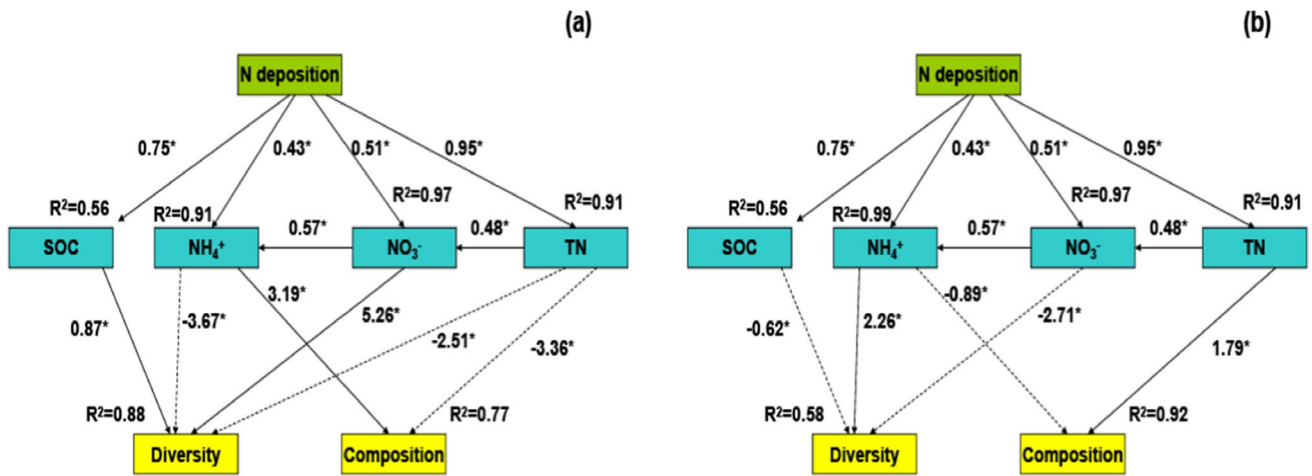


Fig. 6 Path diagrams of the structural equation modeling (SEM) for the relationship of N deposition on the diversity (Shannon index) and composition (NMDS) of soil bacteria **(a)** and fungi **(b)**. SOC, soil organic carbon; TN, total nitrogen; NH₄⁺, ammonium nitrogen; NO₃⁻, nitrate nitrogen. The values above the line represent the path

coefficients. Solid lines indicate positive path coefficients, whereas dashed lines indicate negative path coefficients. * P < 0.05. Control: (CK), 0 kg N ha⁻¹ year⁻¹; low N addition: (LN), 40 kg N ha⁻¹ year⁻¹; high N addition (HN), 80 kg N ha⁻¹ year⁻¹

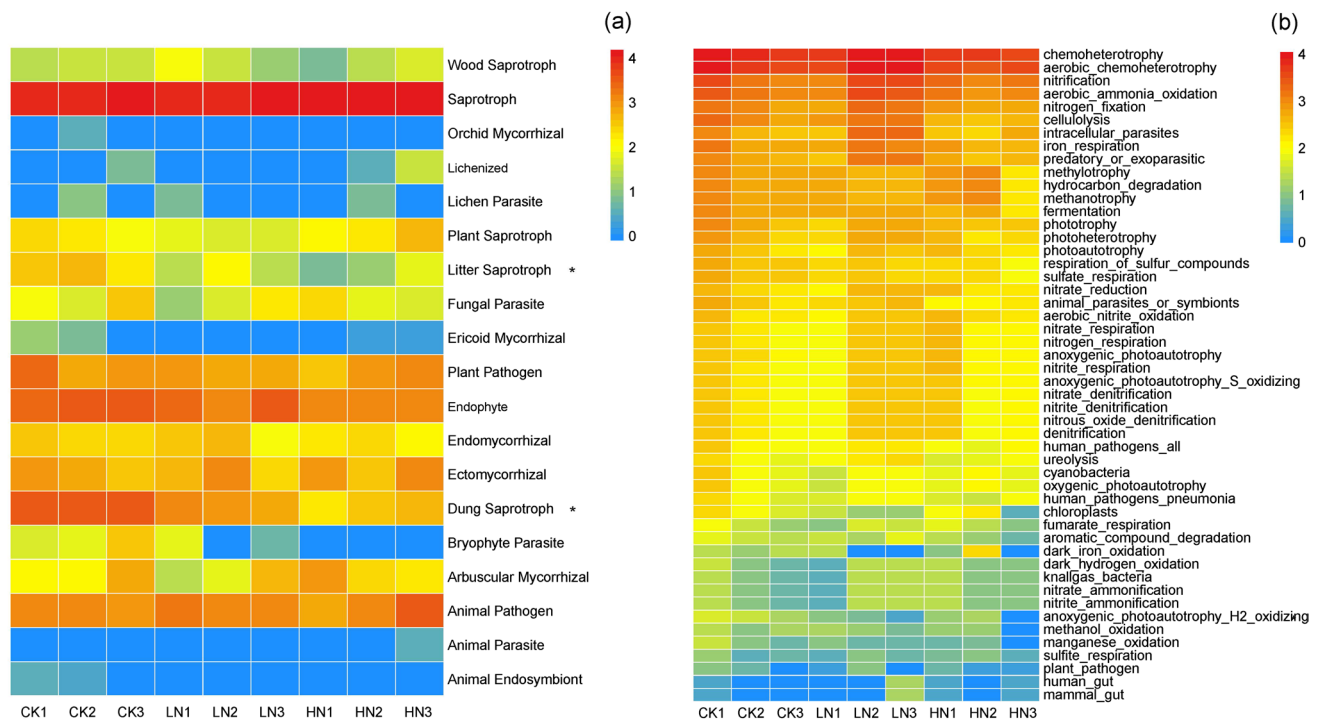


Fig. 7 Impact of N addition on the predictions of potential functions based FAPROTAX analysis for bacteria **(a)** and FUNGuild analysis for fungi **(b)**. The color gradient (blue = low, red = high) represents the relative abundance of the soil bacterial and fungal functions among the different N addition treatments. The asterisks indi-

cate the differences in different N addition treatments, according to one-way ANOVA at a 0.05 significance level. Control: (CK), 0 kg N ha⁻¹ year⁻¹; low N addition: (LN), 40 kg N ha⁻¹ year⁻¹; high N addition (HN), 80 kg N ha⁻¹ year⁻¹

established that soil pH plays a critical role in soil bacterial community diversity (Sui et al. 2022), and specific microbial strains exhibit optimal pH ranges (Bledsoe et al. 2020). However, our study demonstrated that pH did not change significantly, remaining within a range of 4.7 to 5.9. Consequently, this lack of significant pH variation may explain why N deposition did not impact soil bacterial community diversity. Conversely, soil fungal diversity appeared to be more sensitive to N deposition, likely due to soil fungi's higher carbon and nutrient assimilation efficiency compared to soil bacteria (Yang et al. 2020). Furthermore, the level of N addition directly affected soil fungal diversity. As indicated by Freitag et al. (2005), N addition effects on bacterial diversity can be either positive or negative, contingent upon the concentration and type of N added. Our study revealed that wetland soil fungal communities responded nonlinearly to N addition; specifically, they remained unaffected by N input below $40 \text{ g N}\cdot\text{m}^{-2}\cdot\text{a}^{-1}$, but decreased significantly when N input reached $80 \text{ g N}\cdot\text{m}^{-2}\cdot\text{a}^{-1}$. This phenomenon suggests that although wetland fungal communities can withstand low-level N inputs, further increases in N input may surpass a threshold, leading to a shift toward lower diversity within the fungal community.

As anticipated in our second hypothesis, the prevalence of the bacterial phyla Acidobacteria and Proteobacteria, consistent across all N levels, aligns with previous findings in various wetland ecosystems (Hartmann et al. 2017; Zhang et al. 2020a, b; Lu et al. 2021; Zhang et al. 2022). This reaffirms the notion that Acidobacteria and Proteobacteria exhibit a definitive adaptability to different rates of N deposition (Huang et al. 2020). These bacterial phyla play crucial roles in energy metabolism, nutrient cycling, and other ecological functions (Deng et al. 2019; Zhang et al. 2020a, b; Sui et al. 2021; Guo et al. 2022). Our previous study has also highlighted the importance of these bacterial phyla in maintaining soil bacterial communities in wetland ecosystems (Sui et al. 2022) and their dominance in wetland soils (Wang et al. 2019; Sui et al. 2019). However, the abundance of these predominant bacterial phyla did not exhibit significant changes. Only Patescibacteria increased, whereas Firmicutes and Latescibacteria decreased significantly with elevated N deposition.

Patescibacteria, consisting of many unknown bacteria, are often found in anoxic environments and are involved in hydrogen production and sulfur cycling (Frey et al. 2016). Studies by Ren et al. (2020) and Ma et al. (2021) have reported an increase in Patescibacteria with N addition, positively correlating with soil N concentration. This could explain the increase in the abundance of Patescibacteria with elevated N deposition. Firmicutes, traditionally considered copiotrophic bacterial phyla (Hartmann et al. 2017; Perez-Mon et al. 2022), have shown varying responses to N addition in previous research, with some studies reporting

an increase (Freedman et al. 2013; Zhang et al. 2014a, b) and others reporting the opposite trend (Zhang et al. 2018a, b, c, d). This study, however, observed a decrease in Firmicutes with N addition. Therefore, these findings suggest that Firmicutes possess characteristics of both copiotrophs and oligotrophs. Firmicutes appear to be opportunistic bacteria capable of adjusting their trophic strategy according to varying nutritional concentrations across a range of ecosystems (Frey et al. 2016; Arcand et al. 2017). These opportunistic Firmicutes possibly have low competitiveness in utilizing N resources, and instead, their survival depends on various substrates, regardless of their availability.

Contrary to our second hypothesis, this study found that the relative abundance of Ascomycota significantly increased with N addition, while Basidiomycota remained unchanged. Some previous studies have reported negative effects of N deposition on the abundance of Ascomycota and positive effects on Basidiomycota in wetland ecosystems (Hu et al. 2022). Conversely, another study found that N deposition had no effect on Ascomycota but decreased the abundance of Basidiomycota in permafrost wetlands (Ma et al. 2021). These discrepancies are not entirely surprising, given the substantial differences in climate, vegetation, and soil properties across the studied ecosystems. The Sanjiang wetland, in particular, differs from coastal and permafrost wetlands not only in climate but also in characteristics such as being phosphorus-limited rather than N-limited (Song et al. 2016; Zhang et al. 2018a, b, c, d) and having lower pH and base cation concentrations compared to the other two wetlands (Li et al. 2020a, b).

In this study, the FAPROTAX method revealed that only anoxygenic_photoautotrophy_H2_oxidizing significantly decreased with N deposition, while other functions did not exhibit significant changes. Simultaneously, the FUNGuild method indicated significant differences in Saprotrophs among the N deposition levels. These findings suggest that alterations in bacterial and fungal compositions can influence soil carbon cycling functions and fungal activity (Frey et al. 2016). However, many studies have reported that N deposition can affect numerous soil microbial functions (Zheng and He 2020; Ma et al. 2021; Zhang et al. 2023). There are several potential explanations for these observations. Firstly, the high soil moisture content in wetland ecosystems, along with lower microbial activity compared to forest and grassland ecosystems, could contribute to these results. The high soil moisture content leads to low soil oxygen levels, which in turn promotes the predominance of saprotrophs among soil fungi. Secondly, the methods based on the FAPROTAX and FUNGuild databases for functional analysis may not capture the functions of certain microorganisms with unique roles, which could explain the inability to detect significant changes in various functions.

In summary, N deposition primarily affects the abundance of soil microorganisms by altering the life cycle strategies of relevant microorganisms, either directly or indirectly, through changes in soil nutrient concentrations (Lu et al. 2021; Ma et al. 2021). For instance, in the case of the Sanjiang plain, which faces a high risk of N saturation and aggravated phosphorus (P) limitation, substantial N addition may directly impact the abundance of copiotrophic fungi such as Ascomycota while inhibiting oligotrophic fungi such as Basidiomycota. Alternatively, N deposition may also alter the decomposition rate of litter, indirectly influencing the composition of the soil fungal community. Therefore, in the context of N addition in temperate wetlands, changes in the composition and diversity of soil microbial communities reflect shifts in the structure and function of wetland ecosystems.

Consistent with our third hypothesis, the soil physicochemical parameters that influence the composition of soil bacterial and fungal communities varied across different N deposition levels. Soil TN and NH_4^+ -N significantly influenced the structure of the soil bacterial community, whereas soil SOC, NH_4^+ -N, NO_3^- -N, and TN significantly affected the soil bacterial diversity (Fig. 6a). In contrast, the soil fungal community was significantly influenced by NH_4^+ -N, NO_3^- -N, SOC, and TN (Fig. 6b). These results indicate that both soil bacteria and fungi exhibit close correlations with soil SOC, TN, NH_4^+ -N, and NO_3^- -N. Additionally, the effects of soil physicochemical parameters on the abundance of dominant soil bacterial and fungal genera were also clearly different. The impact of N deposition on soil microbial community composition remains uncertain. N addition can directly or indirectly alter soil properties by influencing soil microbial communities (Shi et al. 2018). On one hand, soil acidification resulting from N deposition can negatively affect soil microbial community composition and reduce the overall biomass of soil bacteria and fungi (Treseder 2008; Zhang et al. 2018a, b, c, d). On the other hand, N deposition can stimulate plant growth, leading to increased carbon input into the soil (from litter and roots) and providing abundant substrates for microbial growth and reproduction (Wu et al. 2013), thereby promoting microbial proliferation.

In this study, N addition led to increased soil SOC concentrations, indicating a response in active organic carbon in the soil, which is a crucial energy source for microbial growth. These changes are likely due to the stimulatory effects of N addition on microbial growth and the alteration of carbon use efficiency through the increase in active carbon substrates (Song et al. 2017). This finding aligns with Wang et al. (2021), who reported that N deposition significantly influenced bacterial and fungal communities in N deposition conditions by increasing SOC concentrations. Additionally, previous research on soil microbial characteristics in the Sanjiang Plain wetland has indicated that soil pH,

NO_3^- -N, and NH_4^+ -N were also key factors affecting soil microbial community abundance and composition (Weng et al. 2022; Zhang et al. 2022), which aligns with our results. This suggests that soil pH and available N concentration are important factors influencing soil bacterial and fungal communities. The shift in soil pH and alteration of the soil C:N:P stoichiometric ratio, likely induced by N deposition, negatively affects both above- and below-ground communities, altering soil microbial community structure. The mechanism by which soil acidification changes soil bacterial communities includes ecological selection of the bacterial community in response to acidification and changes in the bacterial community composition within the acidified environment (Chen et al. 2015). However, the soil fungal community is also influenced by SOC, TP, and AP. Compared to bacteria, fungi possess a stronger capacity to absorb N and phosphorus (Wu 2011), and they are closely associated with soil nutrients. Ding et al. (2007) found that soil fungal community structure is closely related to available phosphorus, and Xia et al. (2020) and Ma et al. (2021) also reported significant associations between soil fungal community structure and available phosphorus and total phosphorus. These findings align with our results, indicating that the soil physicochemical parameters influencing the fungal community differ from those influencing bacteria.

5 Conclusion

As expected, N deposition led to changes in the structural composition of soil bacterial and fungal communities at both the phyla and genera levels. Long-term and high levels of N deposition had the effect of reducing fungal diversity (as indicated by Shannon and Chao1 indices) and altering fungal community structure, while bacterial diversity remained unaffected in a temperate wetland ecosystem. N deposition exerted significant and dominant effects on the composition and abundance of the soil microbiome, with fungal communities showing greater sensitivity compared to soil bacteria. Exogenous N input significantly increased soil N (total nitrogen = TN, Nitrate nitrogen = NO_3^- -N, and ammonium nitrogen = NH_4^+ -N) contents and soil organic carbon (SOC), with nitrogen and carbon being the key environmental factors influencing the structure of the soil microbial community. These findings underscore the crucial role of microbes in regulating soil ecological processes within temperate wetland ecosystems under N addition. However, it is important to note that this study focused solely on the effects of N addition on the belowground microbiome, and therefore further research is needed to gain a more comprehensive understanding of the mechanisms through which soil microbiomes respond to global N deposition in wetland ecosystems.

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Data Availability All raw sequence data were deposited into the NCBI Sequence Read Archive database under accession number PRJNA960933.

Declarations

Conflicts of Interest The authors have no relevant financial or non-financial interests to disclose.

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