



Submergence in the Dry Season Alters Microbial Nitrogen Transformations in the Root Zone of *Carex cinerascens*: A Mesocosm Study in One Floodplain Lake

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Abstract

The increasing demand for water resources has triggered a series of water level regulation (WLR) projects, which exerts considerable effects on local hydrologic conditions. In particular, artificial impoundments, which may occur during the dry season in wetlands, increase the periods of waterlogging. However, little is known about their potential effects on biogeochemical cycling. To evaluate how impoundments affect nitrogen (N) cycling in the floodplain ecosystem, we conducted a mesocosm experiment to investigate N dynamics and the potential N-gene changes in the root-zone soil of the dominant plant in one large floodplain lake (Poyang Lake, China). The results indicated that, compared with the control, the 12 cm submergence treatment (SP₁₂) caused NH₄⁺-N accumulation in the root-zone soil on day 14 and day 41. On the contrary, NO₃⁻-N levels in SP₁₂ were statistically lower than those in the control from day 7 to day 28. The curve of organic N had a tendency of declining as a whole. Changes in N-gene abundances revealed that SP₁₂ significantly inhibited nitrification and enhanced denitrification in root-zone soil. Moreover, SP₁₂ enhanced the links and complexity of the N-gene network, reflecting the increased correlations among the N transformations under flooding stress. Considering the increasing demand for WLR worldwide, the study about the effects of anti-seasonal submergence on biogeochemical cycling in floodplains provides insight into the ecological impacts of anthropogenic activities.

Keywords Water level regulation · Dry season · Impoundment · Wetland · Functional genes

Introduction

Nitrogen transformation is one of the crucial biogeochemical cycles in wetlands and sediments, since reactive forms of nitrogen (such as ammonium and nitrate) are the limiting nutrients for primary productivity and organism growth (Kuypers et al. 2018; Wang et al. 2019). Currently, nitrogen (N) cycle processes are strongly impacted by varying hydrological conditions resulting from climate change and

human activities (Schmadel et al. 2018; Gao et al. 2021). Specifically, water conservation projects, e.g., sluice or dam construction, have influenced more than half of the large rivers worldwide and may concurrently have affected river-connected wetlands or lakes (Nilsson et al. 2005; Wang 2020; Li et al. 2021b). Hydrological conditions in floodplain systems are well reported, such as the temporal dynamics of the flooded area in Amazon floodplain lakes and the hydrological connectivity in Poyang Lake floodplains (Bonnet et al. 2008; Li et al. 2021b). However, little is known about the potential impacts of hydrological change on biogeochemical cycles in the Poyang Lake floodplain system, particularly N transformation.

Usually, floodplains, as river-connected wetlands, experience a clear alternation between the wet and dry seasons annually (Sasikala et al. 2009; Philippot et al. 2013). In the wet season, a high water level has adverse effects on the growth of some wetland plants (Zhang et al. 2012; Fan et al. 2019; Lan et al. 2021). As the water level drops in the dry season, the surface soil is usually aerobic due to the atmospheric oxygen supply. However, artificial regulation maintains a

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high water level in the dry season, and wetland plants are subjected to flooding stress even during their growing period. Moreover, an anoxic state is maintained in the flooded soil due to hindered oxygen transport and oxygen consumption by heterotrophic microbes (Li et al. 2021a). Thus, a variety of environmental conditions, including biogeochemical cycles, could be significantly influenced by water level regulation (WLR) in floodplain systems (Rezanezhad et al. 2014; Ding et al. 2020).

Among these potentially altered conditions, N transformation and relative microbial responses are of great significance for soil and plants (Xiang et al. 2021). Although altered N chemistry in wetland and aquatic environments under dam construction has been reported previously, the responses of microbial communities related to the N cycle under WLR were mostly neglected (Gergel et al. 2005; Hill and Duval 2009; Maavara et al. 2020). In one study, compared with the soil at control sites, dam-affected soil was shown to have a higher N fixation rate. The abundance and diversity of N fixation bacteria in dam-affected soil were higher and microbial interactions were more complex (Chen et al. 2019a). It was also found that the decreased diversity of anammox bacteria and the enhanced anammox role in N loss were induced by sediment coarsening in the dam area (Chen et al. 2019b). In another study, a higher abundance of N conversion species and function groups occurred in the bacterioplankton communities in the upper Yangtze River without direct dam impact than in the lower reach of the river, and this effect was deemed to be related to the growth of algae (Yan et al. 2015). An analysis of N-cycle genes in a dam area along a gradient of water levels suggested that water level drove the differences in microbial communities and N transformation genes; for example, *amoA* and *nirS* were the most sensitive indicators (Zhang et al. 2021). Nevertheless, the whole N cycle under WLR has rarely been taken into account, particularly its changes in the root zone of floodplains during the dry season, which may profoundly affect ecosystem function.

Poyang Lake, as the largest freshwater lake in China and a typical floodplain system, plays an important role in ecosystems and biogeochemical cycles (Feng et al. 2013). With the connection to the Yangtze River, the lake presents an apparent landscape of alternating drying-wetting in winter and summer (Hu et al. 2007; Guo et al. 2012). *Carex cinerascens* is the dominant species of wetland vegetation, with a coverage of 50% (Zhang et al. 2012). In summer, plants are submerged by flooding and their aboveground parts decay. When the water level declines and the habitat is exposed to air in October, *C. cinerascens* usually grow. Over the past few decades, drought in this lake has been aggravated in the dry season (Li et al. 2020b; Zheng et al. 2021). To cope with the adverse influences of drought, a water conservation facility was proposed to store water and maintain a certain water level to meet local needs for

production and daily life. The elevated lake water will submerge the exposed soil and *C. cinerascens* during the dry season (Wang et al. 2016; Wu et al. 2021; Yao et al. 2021). However, the ecological impacts of the proposed water project on the biogeochemical cycle, especially N transformations, in the root zone of the wetlands have not been assessed. In this study, to address the knowledge gap among the existing studies, the aims are (1) to explore the change in N contents in root-zone soil under the diverse submergence treatments in the dry season; (2) to analyze the diversity and functions of the microbial community, which are related to the N cycle; and (3) to evaluate the effects of WLR on the microbial N cycle in the root-zone soil of floodplain lake during the dry season.

Materials and Methods

Study Area

Poyang Lake, a floodplain lake, is located downstream of the Yangtze River, China. Most annual precipitation occurs from April to September, i.e., the wet season. With decreasing water supplementation from upstream, the lake enters the dry season (from October to March of the following year). According to the local plan, a water conservation project was proposed. Under the project, the water level of Poyang Lake maintains at a certain height in the dry season for production and domestic needs. Thus, the plants formerly exposed to air during this season are submerged. This study was carried out around the floodplain lake from November 2019 to March 2020, corresponding to the dry season (Fig. 1a).

Submergence Experiments in the Dry Season

Soil samples and fresh *C. cinerascens* were collected in the northwestern Poyang Lake wetland (29°26'53"N, 116°3'3"E) in December 2019 (winter). The soil depth was 20 cm and the vegetation on the soil surface was *C. cinerascens*. Subsequently, the soil was sieved to remove stones and damaged plant roots through an 8 mm sieve, and the soil was well homogenized using shovels prior to the field experiments. Water samples were collected adjacent to the sediment sampling location in Poyang Lake. The field experiment was conducted in buckets (0.5 m × 1.2 m, diameter × height) with 15.5-cm mixed soil that weighed approximately 45.9 kg. According to the plant density in the field, 25 shoots were suitable for each bucket. For planted groups, 25 *C. cinerascens* shoots with a fresh weight of approximately 80 g and an initial height of approximately 10 cm were transplanted into each bucket. It was necessary to cultivate the *C. cinerascens* seedlings after collecting

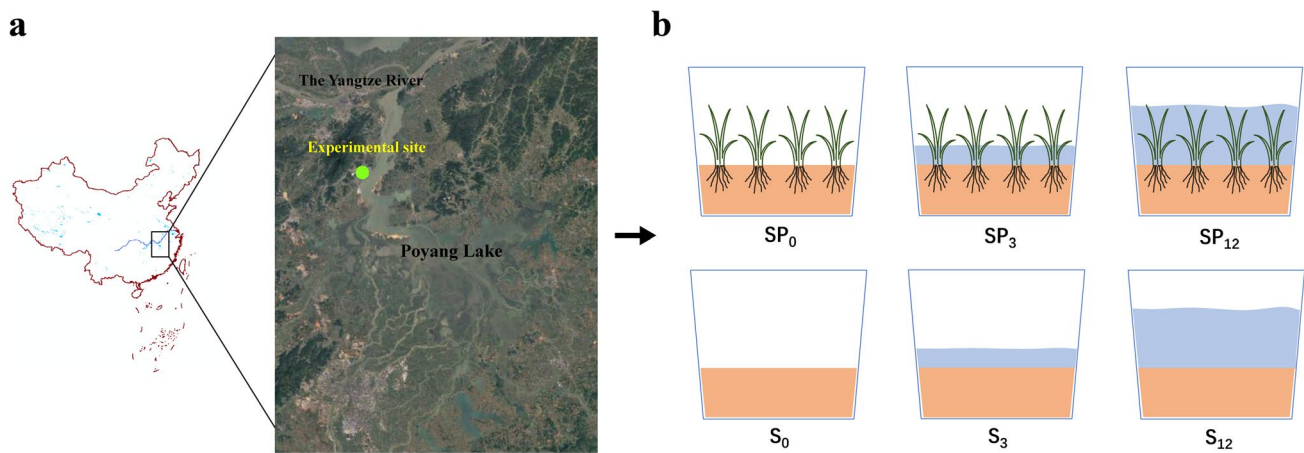


Fig. 1 Mesocosm experimental setup (a). The experimental site (29°26'53"N, 116°3'3"E) was located in Poyang Lake wetland (Map data from Google). The map shows the dry season landscape in December 2019. Schematic diagram of experiment setup (b). SP₀: wetting soil + *C. cinerascens*. SP₃: soil submerged with 3 cm water depth + *C.*

cinerascens. SP₁₂: soil submerged with 12 cm water depth + *C. cinerascens*. S₀: wetting soil. S₃: soil submerged with 3 cm water depth. S₁₂: soil submerged with 12 cm water depth. All treatments were conducted in triplicates ($n=3$)

the grass from the field in order to minish the effects of transplanting. After 25 days (equilibrium period), the groups were flooded at three water levels, respectively. A schematic diagram of the experimental design is shown in Fig. 1b. The soils planted with *C. cinerascens* were labelled as SP₀: 0 cm water depth, SP₃: 3 cm water depth (which submerged the part of the plant), SP₁₂: 12 cm water depth (which submerged the whole plant). Similarly, impoundment treatments without *C. cinerascens* were carried out, and the groups were marked as wet soil (S₀), 3 cm water depth (S₃) and 12 cm water depth treatment (S₁₂) (Photos of the experiment see Fig. S1). Each treatment was performed in three buckets.

In each bucket, soil was collected at random locations using a tube sampler (3 cm diameter) and the samples from three buckets were analyzed separately. The root-zone soils were obtained from the area influenced by the roots of *C. cinerascens*. The sampling times were on Days 0, 7, 14, 21, 28, 41 and 104 (sampling was interrupted between January and March 2020 due to COVID-19). The height of the plants in each treatment was concurrently recorded, and redox potential (Eh) of soil was measured using a platinum electrode. Aliquots of sediment were stored at -20 °C for physicochemical property measurements and were frozen at -80 °C for microbial analysis.

Soil Chemical Analysis

Before follow-up analysis, the samples were first sieved to remove roots. The wet soil was dried in an oven at 105 °C for the moisture measurement. Ions were obtained from the wet soil by the KCl extraction method, and

the contents of NH₄⁺-N, NO₂⁻-N and NO₃⁻-N were determined using a Smartchem DiscreteAuto Analyzer (McCrary 2011; Zhang et al. 2021). The content of total nitrogen (TN) was analyzed by the alkaline potassium persulfate digestion method through a spectrophotometer, and the content of organic N was obtained by the difference between TN and inorganic N. The total organic carbon (TOC) was measured by the potassium dichromate oxidation method (Santi et al. 2006).

Microbial 16S rDNA Amplification, Illumina Sequencing and Community Analysis

Extraction of soil microbial DNA was conducted using the PowerSoil DNA Isolation Kit (QIAGEN, U.S.A.) following the manufacturer's instructions. To confirm the microbial taxonomy, the V3V4 region of microbial 16S rDNA was subsequently amplified using primers 338F and 806R (Liu et al. 2016). DNA sequencing was carried out by Majorbio Biopharm Technology Co., Ltd. (Shanghai, China). The raw sequences were assembled based on the overlap region, and then filtered for quality control. On the basis of 97% similarity, the clean sequences were clustered into operational taxonomic units (OTUs) using UPARSE (Edgar 2013). Each OTU was represented by the highest abundance sequence, and species annotation was obtained according to the SILVA database (<https://www.arb-silva.de/>). The microbial OTUs were assigned to various taxa covering phylum, class, order, family and genus. Based on the OTU table of soil microbes, species abundance and diversity information were measured. Principle coordinate analysis (PCoA) was used to

determine the differences among submergence treatments on the basis of a weighted UniFrac dissimilarity matrix (Chen et al. 2020). To determine the potential N cycle function of the microorganisms in the soil samples, the relationships between species and genes were established using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The abundance and metabolism pathways of the genes related to N transformation were then summarized. The co-occurrence network was plotted based on Spearman correlation, and the strong connections ($p < 0.05$ and $R > 0.6$) were retained. The microbial 16S rDNA data of this study were deposited in the Sequence Read Archive (SRA) under study accession number PRJNA725048.

Statistical Analysis

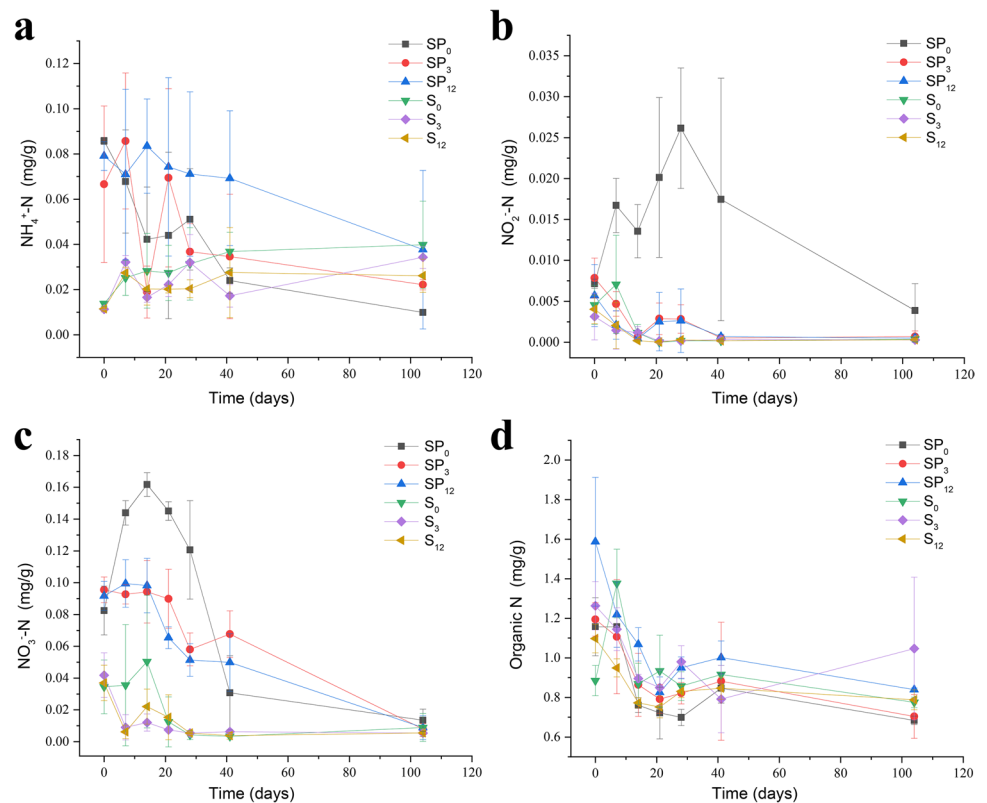
Significance tests of differences among the physico-chemical factors, compositions and functions of the microbial community were conducted using one-way analysis of variance (ANOVA). The significance of the microbial community differences was calculated using Anosim, Adonis and MRPP methods. MRPP analysis was executed based on the similarity matrix of species abundance. Data analysis and visualization were conducted in R, Origin and SPSS.

Results

Changes of N Contents and Soil Properties in the Root Zone Soil

The contents of inorganic N ($\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$) and organic N in the soil were determined during the experiments. Overall, the $\text{NH}_4^+\text{-N}$ contents in root zone soil ranged from 0.0099 ± 0.0008 (average \pm SD) to 0.0858 ± 0.0016 mg/g (Fig. 2a). The $\text{NH}_4^+\text{-N}$ content in SP_{12} was significantly ($p < 0.05$) higher than those in SP_3 and SP_0 on days 14 and 41 (Table S1). From days 28 to 104, the $\text{NH}_4^+\text{-N}$ content underwent a long-term decline. In addition, the $\text{NO}_2^-\text{-N}$ curve of SP_0 presented an increase followed by a decrease (Fig. 2b). The $\text{NO}_2^-\text{-N}$ contents in SP_3 and SP_{12} were lower than those in SP_0 after 7 days. A significant test showed that the $\text{NO}_3^-\text{-N}$ levels in SP_{12} and SP_3 were statistically lower ($p < 0.05$) than those in SP_0 from day 7 to day 28 (Fig. 2c and Table S1). It should be noted that SP_{12} had a significantly lower $\text{NO}_3^-\text{-N}$ content ($p < 0.05$) than SP_3 and SP_0 on day 21. This result implied a larger consumption of $\text{NO}_3^-\text{-N}$ in this treatment than in the other submergence treatments. Ultimately, contents of $\text{NO}_3^-\text{-N}$ in root zone soils dropped to a low level of 0.0101 ± 0.0060 mg/g. The organic N curve had a tendency of declining as a whole, with a slight increase from days 21 to 41 (Fig. 2d). In the

Fig. 2 Effects of submergence treatments on N contents including $\text{NH}_4^+\text{-N}$ (a), $\text{NO}_2^-\text{-N}$ (b), $\text{NO}_3^-\text{-N}$ (c) and organic N (d) in root-zone and nonplanted soils. SP_0 : wetting soil with the plant *C. cinerascens*; SP_3 : soil submerged under 3 cm of water + *C. cinerascens*; SP_{12} : soil submerged under 12 cm of water + *C. cinerascens*; S_0 : wetting soil; S_3 : soil submerged under 3 cm of water; S_{12} : soil submerged under 12 cm of water



experiment, N contents in the nonplanted soil were also measured. Compared to the root zone soil, the unplanted groups had lower $\text{NH}_4^+\text{-N}$ contents which ranged from 0.0113 ± 0.0001 mg/g to 0.0398 ± 0.0193 mg/g (Fig. 2). And the tendency of curve remained stable from days 14 to 104. Besides, the $\text{NO}_3^-\text{-N}$ in nonplanted groups had lower contents than those in the root zone groups. On day 7, the concentrations of $\text{NO}_3^-\text{-N}$ in S_3 and S_{12} obviously decreased, whereas the S_0 treatment still maintained at the initial level. All the $\text{NO}_3^-\text{-N}$ contents in the nonplanted treatments dropped to the extremely low levels after day 28.

In addition, Eh in deep submerged root zone soil had an obvious decline from 75 mV to -15 mV during these periods. However, SP_0 and SP_3 had fluctuating Eh values and were maintained at about 100 mV (Fig. S2). The TOC content in the soil and the growth of *C. cinerascens* were also recorded in the experiments (Figs. S3 and S4). The reduction of the mean plant height in SP_{12} before day 21 implied a decrease in biomass of *C. cinerascens*. From day 41 to day 104, most plants in the root zone groups had obvious growth, and the final height reached 14–20 cm.

Microbial Community Structure in the Root-Zone Soil of *C. cinerascens*

From days 14 to 41, a higher $\text{NH}_4^+\text{-N}$ content was found in SP_{12} compared to that in SP_0 and SP_3 . To investigate the underlying mechanism of the change in N transformation process under submergence condition, the samples on day 21 were used for microbial analysis as well as the initial samples on day 0. The number of OTUs in all treatments ranged from 1749 to 2754, with a mean of 48,537 sequences per sample. According to the Simpson and Shannon indices, the microbial diversity was highest in SP_{12} , followed by in SP_3 and SP_0 on day 0 in the root zone groups (Fig. S5). The richness of the microbial communities in the

SP_3 and SP_{12} groups was high after 21 days. The PCoA and hierarchical clustering based on Bray–Curtis distance showed strong dissimilarity between SP_0 and SP_3 and SP_{12} in the microbial community on day 21 (Figs. 3a and S6). Nonparametric multivariate statistical algorithms (Anosim, Adonis and MRPP) showed that the difference among various submergence treatments was greater than that within groups ($R > 0$, $p < 0.05$) (Table S2).

In the root zone and nonplanted soils, there were obvious differences in microbial composition between the different water depth treatments (Fig. S7). After 21 days of incubation, *Micrococcaceae* exhibited a significantly lower relative abundance in SP_3 and SP_{12} than in SP_0 , while *Sphingomonadaceae*, *Chitinophagaceae*, *Beijerinckiaceae*, Order_SJA-15, SC-I-84 and *Caulobacteraceae* were significantly higher in the root-zone soil in the deeper water submergence treatments (SP_3 and SP_{12}) than in the control. The heatmap illustration based on the top 30 OTUs presented strong clustering (Fig. 3b). The shallow- and deep-water submergence treatments, including SP_3 , SP_{12} , S_3 and S_{12} on day 0 were obviously clustered, and the situation was similar for SP_3 , SP_{12} , S_3 and S_{12} on day 21. The dominant organisms in SP_3 and SP_{12} were *Streptomyces*, *Nocardioideae*, *Marmoricola*, *Nocardioidea*, *Massilia*, *Pedobacter* and *Methylocystis* on day 0. In contrast, most of these microbes were relatively low on day 21.

Effect of Submergence on the Abundances of N Cycle Genes

To investigate the mechanism of N transformation, the change in microbe-related N functional genes in all treatments was analyzed according to the KEGG database. Dominant N functional genes were affiliated with N fixation (*nifDHK*), nitrification (*amoABC*, *hao* and

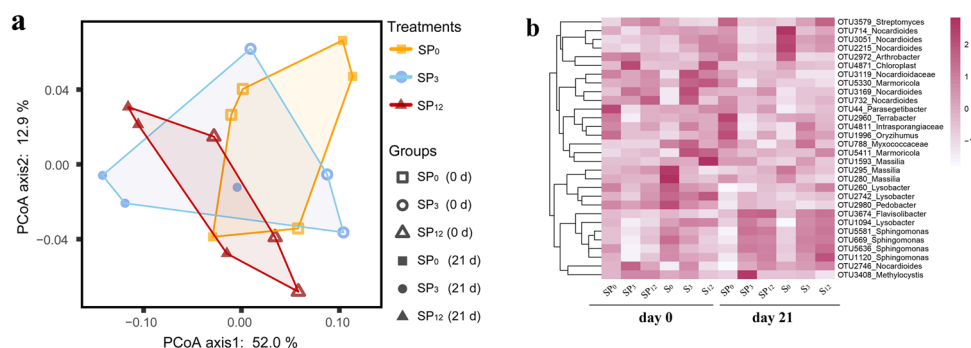


Fig. 3 Differences in microbial community structures among submergence treatments by PCoA (a). Beta diversity is determined by weighted UniFrac distance and is plotted for three treatments: SP_0 (orange polygon), SP_3 (skyblue polygon), and SP_{12} (red polygon) and all sample groups: initial SP_0 (hollow square), initial SP_3 (hollow cir-

cle), initial SP_{12} (hollow triangle), SP_0 on day 21 (square), SP_3 on day 21 (circle), and SP_{12} on day 21 (triangle). Heatmap of top 30 species in all treatments on day 0 and day 21 (b). The relative abundance of microbes was displayed after standardization

nrxAB), denitrification (*narGH*, *napAB*, *nirSK*, *norBC* and *nosZ*), dissimilatory nitrate reduction (*nirBD* and *nrfH*) and assimilatory nitrate reduction (*narB*, *nasAB* and *nirA*) (Kuypers et al. 2018). At the early stage, *nifHDK*, *amoABC*, *hao* and *nrxAB* of SP₃ and SP₁₂ had higher relative abundances than those of SP₀ (Fig. 4a). However, these N cycle genes decreased to varying degrees in SP₁₂ after 21 days of incubation, especially in the hydroxylamine oxidation process. The relative abundances of *hao* in SP₃ and SP₁₂ were significantly lower than those in SP₀, with 41.32% and 53.24% reductions, respectively. It was found that the proportion of amoxidation-related microbes decreased, and nitrification was likely weakened by high water levels. For the denitrification process, increases in *napAB*, *nirSK*, and *norBC* were found in shallow- and deep-water submerged treatments. The relative abundance of *napAB* in SP₁₂ was 56.38% and 72.67% significantly higher than that in SP₀ at the two time points, respectively, which illustrated the increase in nitrate reduction to nitrite in the deep-water submerged treatment.

In addition, 50% higher abundance of nitrite reductase *nirS* gene was also detected in SP₁₂ on day 0. Deep-water submergence improved *nosZ* (nitrous oxide reductase) gene by 39.13% on day 46. Several nitrate reduction-related genes significantly decreased in SP₃ and SP₁₂ compared to those in SP₀. The abundance of *nirBD* and *nrfH* in dissimilatory nitrate reduction (DNRA), as well as *nasAB* in assimilatory nitrate reduction (ANR), was significantly lower in SP₃/SP₁₂ than in SP₀. Furthermore, the relative

changes in N cycling genes in root zone groups compared to those in nonplanted soils were also summarized (Fig. S8). Although the abundance changes were not evident, *amoABC* and *hao* in SP₃ were significantly higher than those in S₃. The increased gene abundance implied that the presence of *C. cinerascens* might enhance the nitrification processes in soil.

Networks can reveal the interactions of microbes associated with diverse N conversion processes and the stability of N cycle (Fig. 5). In the root-zone soil, the average weighted degree obviously increased in the submergence treatments (2.075 for SP₃ and 3.37 for SP₁₂, respectively), compared to an average weighted degree of 0.41 in SP₀ (Table S3). The average weighted degree commonly showed the complexity of microbial interactions, and a larger value reflected higher complexity in SP₃ and SP₁₂ than in SP₀. Moreover, the network of SP₁₂ contained 106 edges in total, which was higher than the number of edges in SP₀. These results indicated that the deep submergence treatment improved, to a large extent, the connections among the N conversion genes of the microbial community in the root-zone. Moreover, the networks of nonplanted soils also exhibited a similar tendency: a stronger connection in S₁₂ (77 edges) than in S₃ (65) and S₀ (31) (Table S3 and Fig. S9). The elevated connection suggested that the complexity of the N cycle was enhanced under submergence stress, which implied the ability of the root zone microbiota to maintain the N balance under ambient pressure.

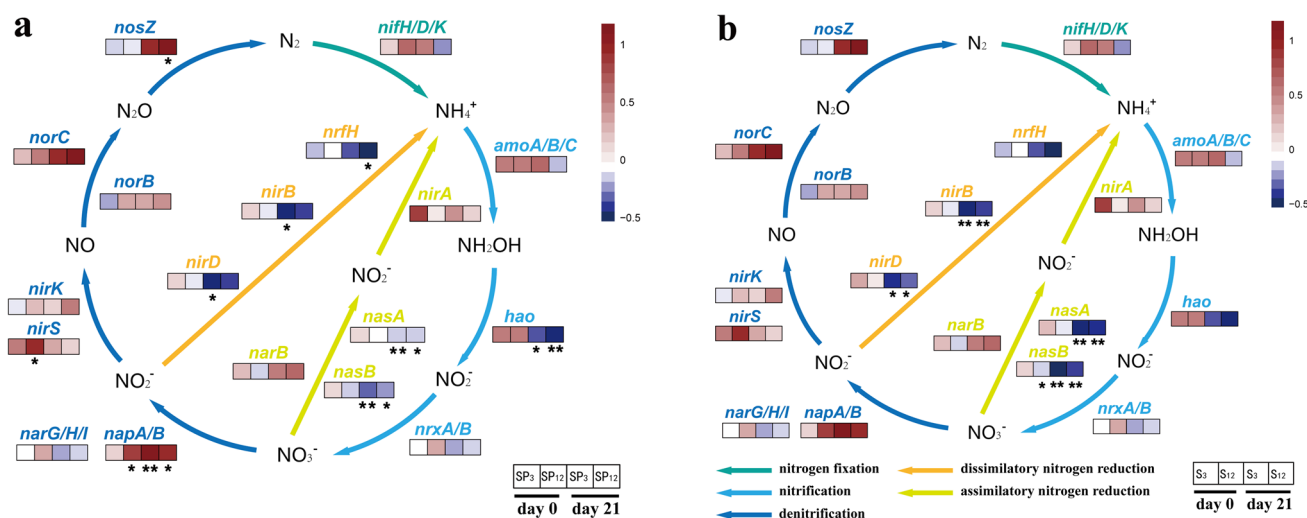


Fig. 4 Changes in the relative abundances of potential N cycling genes in the root zone groups (a) and nonplanted soil groups (b). Arrows show the main N transformation processes that were driven by microorganisms. Colors of the squares indicate the relative changes in gene abundance in the submergence groups compared to

those in control group, and the index was calculated as the following: $(SP_3-SP_0)/SP_0$, $(SP_{12}-SP_0)/SP_0$, $(S_3-S_0)/S_0$, and $(S_{12}-S_0)/S_0$. Red represents the increase in the abundance of N gene in soil, and blue represents the reduction in the abundance of gene. *, $p < 0.05$; **, $p < 0.01$

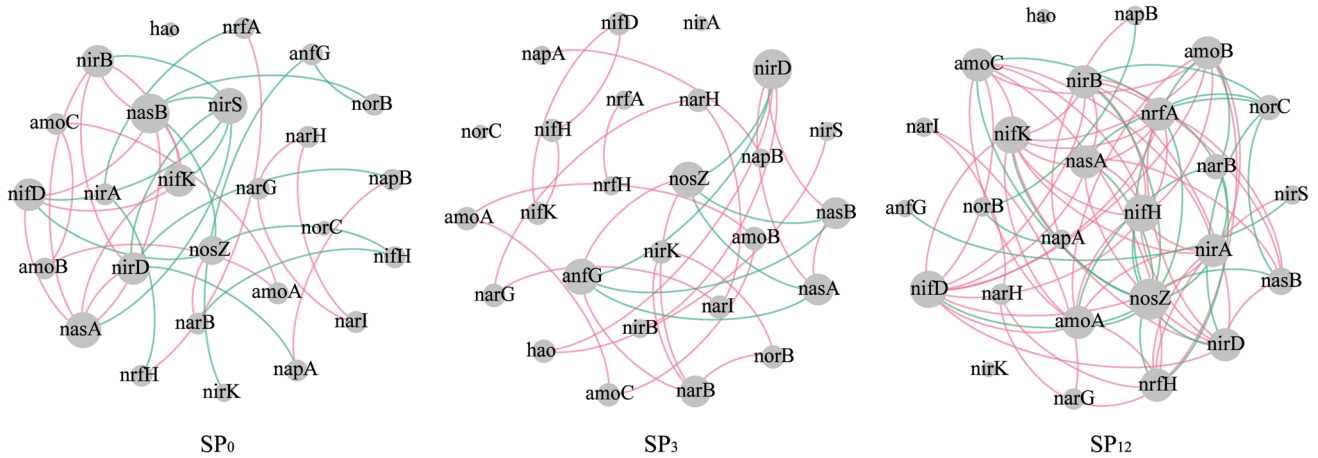


Fig. 5 Networks of N cycle genes in the root zone groups under different submergence conditions. Nodes represent the diverse N genes, node size represents the node degrees (the number of other genes that

are connected to the single gene), and edge color reflects the positive (red) or negative (green) correlation between nodes

Composition of Putative Nitrifiers and Denitrifiers

In view of the changes in nitrification and denitrification processes under the submergence treatments, the relevant microbial community was investigated. Overall, the abundance of putative nitrifiers accounted for 27% of the community, and the dominant nitrifying bacteria were *Nocardioidea*, *Intrasporangiaceae*, *Massilia*, *Nitrospira* and *Bacillus* (Fig. S10). The relative abundance of nitrifiers on day 21 was lower in SP₃ (24.98%) and SP₁₂ (26.22%) than that in the SP₀ (28.89%). Among these nitrifiers, three taxa with significantly reduced abundance, including *Nitrosospira*, *Intrasporangiaceae* and *Bacillus*, were observed in SP₃ and SP₁₂ (Fig. 6a). The decreased proportion of these microorganisms in SP₁₂ ranged from 0.3% to 73.2% compared to those in SP₀ on day 21. In contrast, the abundance of putative denitrifying microbes in SP₁₂ (43.4%) was generally higher than that in SP₀ (42.5%). The denitrifiers with significantly higher abundance in SP₃ and SP₁₂ were *Flavisolibacter*, *Massilia*, *Ferruginibacter*, *Bradyrhizobium* and *Arenimonas* (Fig. 6b). Moreover, the relative abundance of these species in SP₃ and SP₁₂ showed

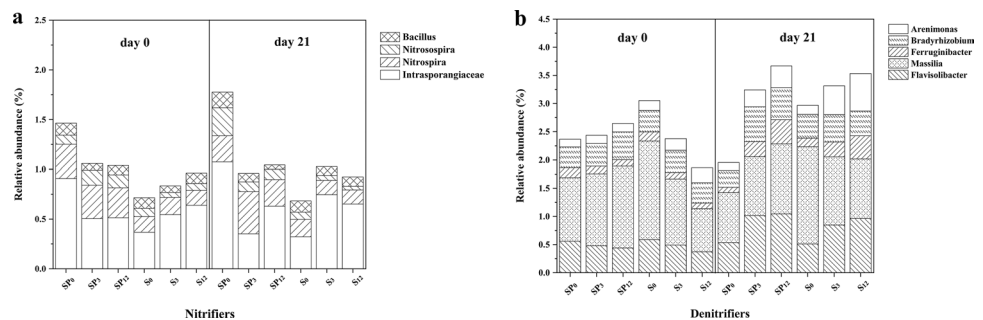
a substantial increase of 39.3%-367.2% on day 21, and the increases were also found in S₃ and S₁₂.

Discussion

NH₄⁺-N Accumulation and the Changes of Potential N-genes in the Deep-Submergence Treatment in the Early Phase

The N content profiles indicated that, compared with SP₃ and SP₀, deep submergence led to NH₄⁺-N accumulation in the root-zone soils from day 14 to day 41 (Fig. 2a). Although the growth of plants in equilibrium period could be the reason that caused the minor difference of N contents among groups on day 0, the NH₄⁺-N, NO₂⁻-N and NO₃⁻-N contents were statistically (*p* > 0.05) same (Table S1). The only variable in SP₃ and SP₁₂ was water level throughout the experiment compared to SP₀. The high NH₄⁺-N content was likely attributed to the change in N conversion processes under submergence conditions. According to the potential N cycling pathways, a significantly lower abundance (*p* < 0.05)

Fig. 6 Putative nitrifiers (a) and denitrifiers (b) with significantly different abundances between SP₀ and SP₃ and SP₁₂ on day 0 and day 21, respectively (*p* < 0.05). Microbes with relative abundance above 0.01% are exhibited



of *hao*, *nasAB*, *nirBD* and *nrfH* and a higher abundance of *napAB* and *nosZ* in SP₃ and SP₁₂ appeared on day 21 (Fig. 4a). Of these N cycle genes, *hao* is responsible for the conversion of hydroxylamine to nitrite in the nitrification process (Banerjee et al. 2016). *nirBD*, *nrfH* and *nasAB* participate in DNRA and ANR processes. *napAB* is capable of nitrate reduction to nitrite and *nosZ* converts nitrous oxide to dinitrogen in denitrification (Peralta et al. 2013). The decreasing relative abundance of *hao* and increasing *napAB* and *nosZ* likely reflected the inhibited nitrification and the enhanced denitrification by the submergence treatments in planted soil. Under normal soil moisture conditions, e.g., those in grassland soil for grazing, ammonia-oxidizing bacterial abundance and potential nitrification activity increased with increasing soil moisture (Wang et al. 2006; Avrahami and Bohannan 2009). These phenomena suggest that suitable moisture condition promote the growth of nitrifying microbes (Di et al. 2014). However, flooding-induced submergence maintains a high water level above the ground. Previous studies have indicated that after a few weeks of flooding, submerged soil tends to be anaerobic, and nitrification is reduced (Yano et al. 2014; Sánchez-Rodríguez et al. 2019). Here, the deep submergence treatment where the water level was above *C. cinerascens* decreased the Eh below 0 mV (Fig. S2), revealing anoxic soil condition on day 21 (Jong et al. 2020). The nitrification process was hence depressed, which led to the impeded transformation from NH₄⁺-N to NO₃⁻-N. This result explained the NH₄⁺-N accumulation in SP₁₂ during that time.

Nitrifying microbes are generally aerobic and live in aerated soil (Li et al. 2018). Here, dominant nitrifiers such as *Nitrosospora*, *Intrasporangiaceae* and *Bacillus* in SP₃ and SP₁₂ were less abundant than those in SP₀ on day 21 (Fig. 6a). *Nitrosospora* is an ammonia-oxidizing bacterium (AOB) that converts NH₄⁺ to NO₂⁻, and the hypoxic environment does not support its development (Zhang et al. 2019). For example, in the anoxic hypolimnion of deep lakes, *Nitrosospora* accounts for a minor proportion compared with that in the oxygenated zone of smaller lakes (Alfreider et al. 2017). The decreasing abundance of *Nitrosospora* seemed to cause weaker nitrification activity. AOB appeared to be positively correlated with the potential nitrification rate ($R = 0.56$, $p < 0.001$) in the drought and rewetting seasons (Krüger et al. 2021). Thus, the 21-day deep submergence treatment had adverse impacts on *Nitrosospora* and thus decreased nitrification activity. Other nitrifying bacteria experienced a similar situation. Some members of *Intrasporangiaceae* have previously been found to be involved in nitrite oxidizing processes and lived in oxygen-rich places (Nielsen et al. 2012; Lee and Whang 2020). Although the abundance of *Intrasporangiaceae* was different between SP₀ and SP₁₂ at first, its extent of increase in SP₁₂ was lower than that in SP₀ from day 0 to day 21, revealing growth inhibition.

In contrast, denitrifiers commonly live under the anaerobic conditions and are promoted under high soil moisture (Miller et al. 2008). Putative denitrifiers also presented different abundances between SP₁₂ group and the control on day 21, mainly consisting of *Flavisolibacter*, *Ferruginibacter*, *Bradyrhizobium* and *Arenimonas* (Fig. 6b). A metagenome-assembled genome derived from *Flavisolibacter* showed that this bacterium encoded the denitrification genes *nirK*, *norBC* and *nosZ* in paddy soil (Li et al. 2020a). Thus, the *Flavisolibacter* genus was expected to be involved in the conversion of NO₂⁻ to NO and N₂O and ultimately to N₂. *Ferruginibacter*, as a denitrifier, contributes to nitrate reduction in a wastewater system (Wang et al. 2020b). Several strains of *Bradyrhizobium* are rhizobia and can grow with nitrate as electron acceptor in the denitrification process (Sameshima-Saito et al. 2004). Moreover, Wang et al. (2020a) found that nitrate and nitrite reductases were significantly upregulated in an *Arenimonas*-dominated anoxic environment by transcriptomic analysis. In summary, the SP₁₂ treatment provided deep submergence for *C. cinerascens* planted in soil, leading to hypoxic condition for microorganisms. This scenario appeared to inhibit nitrifiers and favor denitrifiers, and then affected the N cycling processes in the soil.

In addition, the mineralization of organic N and the fixation of atmospheric N are also vital sources of NH₄⁺-N in soil. Unlike the root zone groups, there were no significant differences among the NH₄⁺-N contents of S₀, S₃ and S₁₂ treatments in nonplanted soil (Table S1). The existence of *C. cinerascens* provided abundant organic matters which could decompose into NH₄⁺-N under submergence condition (Fig. S3). An earlier study suggested that *C. cinerascens* suitably grew in low-moisture (optimum at 22%) soil and that prolonged flooding caused its decay (Yuan et al. 2019). In the SP₁₂ treatment (12 cm of water depth), *C. cinerascens* was almost completely submerged and the soil moisture content reached 34.8%-45.3%. The reduction in plant height in SP₁₂ before day 21 implied the decomposition of *C. cinerascens* (Fig. S4). This kind of hydrologic condition generated an adverse environment for *C. cinerascens* and then aggravated its decay. Additionally, the decline in organic N from day 14 to day 21 verified its transformation to NH₄⁺-N (Fig. 2d). Therefore, the decomposition of plants in the root zone groups possibly produced extra NH₄⁺-N in the early stage. These results were in accordance with those in the literature that the degradation of organic N can release NH₄⁺-N under submerged conditions (Sánchez-Rodríguez et al. 2019; Lu et al. 2020). For N fixation, the potential abundances of nitrogen-fixing genes (*nifHDK*) were not significantly different between SP₁₂ and SP₀ on day 21 (Fig. 4a). This result showed that microbial N fixation did not seem to be the reason for high NH₄⁺-N content in the deep submergence treatment during that period.

In terms of NO_3^- -N content, the change in nitrification and denitrification could explain the low level of NO_3^- -N in the submergence treatments during the initial period. The NO_3^- -N content of SP_3 and SP_{12} was significantly lower ($p < 0.05$) than that of SP_0 from day 7 to day 28, and that of SP_3 and SP_{12} was approximately 65% of the highest value of the latter (Fig. 2c and Table S1). A similar situation occurred in the nonplanted groups, where the mean NO_3^- -N content of S_3 and S_{12} were lower than that of S_0 on days 7 and 28. The deep submergence treatment led to the high consumption of NO_3^- -N in both root zone and nonplanted soils. These results verified that NH_4^+ oxidization to NO_2^- and subsequent conversion to NO_3^- were possibly inhibited in the root-zone soil in the deep submergence treatment. In addition, the intensifying denitrification process seemed to be one of the prime reasons for the NO_3^- -N decline in SP_3 and SP_{12} from day 14 to day 41. The submergence-induced anoxic conditions supported the increase in denitrifying bacteria and thus improved the consumption of NO_3^- -N.

Influence of Submergence on Microbial Communities and Networks in *C. cinerascens* Groups

The Simpson and Shannon indices of microbes were significantly higher in SP_{12} soil than in S_{12} soil at day 25, which confirmed the fact that the presence of plants facilitated microbial diversity (Fig. S5). The degradation of *C. cinerascens* as organic matter could provide adequate carbon and N sources for microbial development under submerged conditions. These results are consistent with those of previous investigations showing that nutrients in the flooding zone were obviously higher than those in the drying zone due to the decomposition of plants (Lan et al. 2021). Moreover, the microbial community of SP_{12} exhibited relatively higher diversity than those of SP_3 and SP_0 . In comparison to the low water-level treatment, the deep submergence treatment seemed to increase the release of organic matter in root-zone soil. Clustering and PCoA analysis showed that the microbial communities between SP_0 and SP_3 and SP_{12} were significantly different in root-zone soil on day 21 (Figs. 3a and S6). The dominant families including *Sphingomonadaceae*, *Chitinophagaceae*, *Beijerinckiaceae*, Order_SJA-15, SC-I-84 and *Caulobacteraceae*, had obviously higher abundances in SP_3 and SP_{12} after day 21, suggesting that deeper submergence conditions led to a suitably anoxic environment for these microbes. In contrast, both shallow and deep submergence reduced the relative proportion of *Micrococcaceae*. Furthermore, genera with the highest abundance exhibited apparent clustering in SP_3 , SP_{12} , S_3 and S_{12} on day 21. This result indicated that the submergence treatments largely influenced dominant microbes, likely causing the growth of some specific genera, such as *Flavisolibacter*,

Lysobacter, *Sphingomonas*, *Nocardioides* and *Methylocystis*. Whereas, *Streptomyces*, three species of *Nocardioidaceae*, *Marmoricola*, *Massilia* and *Pedobacter* were inhibited under submerged conditions.

Co-occurrence networks indicated that the N genes of SP_0 and SP_3 linked simply, while the network of SP_{12} showed a more complex pattern (Fig. 5). In the root-zone soil, the average weighted degrees of SP_3 and SP_{12} were obviously higher than those of SP_0 (Table S3). The networks for the nonplanted soil also presented similar patterns in S_0 , S_3 and S_{12} (Fig. S9), demonstrating that N-gene connectivity in deeply submerged soil was more complex than that in the control soil. A prior study showed that molecular ecological networks became more robust under an ambient environmental change in grassland soil microbial communities, and the complexity of the network was strongly related to its stability (Yuan et al. 2021). In this study, submergence pressure enhanced the network complexity of N genes, e.g., edge number and average weighted degree. N transformation genes connected more tightly and had stronger stability to resist hydrological condition changes in both the *C. cinerascens* root zone and the nonplanted zone. This result implied that microorganisms tended to maintain the N balance under flooding stress.

Long-term Changes of N Contents in the Mesocosm System

In this study, the submergence of wetland plants in the dry season (winter) was simulated and analyzed. Nevertheless, with the spring coming and the temperature increasing on day 104, *C. cinerascens* grew substantially and were even above the water at that time. The initial height of *C. cinerascens* was 10 cm, and the average height of plants in SP_0 , SP_3 , and SP_{12} reached 20 cm, 16 cm, and 14 cm after 104 days, respectively (Fig. S4). These facts showed that the submergence degree had been changed. NH_4^+ -N in planted groups was obviously reduced in the long term (Fig. 2a). Moreover, the content of NH_4^+ -N in S_{12} increased from days 28 to 104, which was contrary to the change in SP_{12} during the later period of the trial. From days 41 to 104, NO_3^- -N contents also decreased in planted groups. These were possibly attributed to the utilization of active N by plants. It was reported that the plants uptake nitrogen from soil during their growing (Bai et al. 2017; Piao et al. 2017; Liang et al. 2022). Plant growth could exert great influences on local soil environment. Whereas under natural condition, being submerged for a long time usually led to the decay of wetland plants (Zak et al. 2015). Thus, to reflect the real long-term situation in the large Poyang Lake after the construction of water project, the

hydrodynamics need further studied. The NH_4^+ -N accumulation phenomenon which was demonstrated by this study, could give an insight into the impacts of submergence on nitrogen cycling in root zone.

Conclusion

Water level regulation could lead to a high water level in the root zone of a floodplain even during the dry season. This study showed that, in comparison to the control, deep submergence treatment led to more anoxic conditions and NH_4^+ -N accumulation in the root-zone soil at the early stage. The NO_3^- -N content of SP_{12} was lower than that of SP_0 . Correspondingly, the changes in N-related microbes suggested that the potential nitrification process was significantly inhibited, and denitrification was promoted in SP_{12} . The significantly different nitrifiers among groups were *Nitrospira*, *Intrasporangiaceae* and *Bacillus*, and the key denitrifiers were *Flavisolibacter*, *Massilia*, *Feruginibacter*. Deep submergence caused the decay of the wetland plant *C. cinerascens* and the release of nutrients from plants, which possibly contributed to the increase of NH_4^+ -N in SP_{12} . Moreover, deep submergence enhanced the link and complexity of N-gene networks, implying the adaptive capacity of microbial N-cycling networks facing the changed hydrological conditions. With the increase of drought events caused by climate change, the demands of WLR are promoted worldwide. The potential impacts of submergence on microbial N cycling after impoundments need further attention.

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Declarations

Conflicts of Interest/Competing Interests The authors declare that they have no conflicts of interest and no competing interests.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication As Corresponding Author, I confirm that the manuscript has been read and approved for submission by all the named authors.

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