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Mechanisms of Irrigation Water Levels on Nitrogen Transformation and Microbial Activity in Paddy Fields

Yunqing Fang [1], Jiangping Qiu [1,2] and Xudong Li [1,2,*]

[1] School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China; fangyunqing100@126.com (Y.F.); jpq@sjtu.edu.cn (J.Q.)
[2] Yunnan Erhai Lake Ecosystem Observation and Research Station, Dali 671000, China
* Correspondence: lixudong@sjtu.edu.cn

Abstract: Nitrogen is a vital nutrient for rice growth; however, its inefficient use often results in nutrient loss, environmental degradation, and the emission of greenhouse gases. In this study, a rice paddy simulation was conducted under different water levels (1–4 cm), incorporating a comprehensive analysis of nitrogen dynamics, environmental factors, and microbial communities to evaluate the impact of water levels on nitrogen concentrations and microbial composition. The results indicated that the water level had a greater impact on nitrogen concentrations in surface water than in soil water. Compared to low water level conditions (1 cm), the average concentrations of ammonium nitrogen, nitrate nitrogen, and nitrite nitrogen in surface water under 2–4 cm water levels decreased by approximately 53.8%, 36.7%, and 78.9%, respectively. Water levels also influenced the microbial composition and nitrogen cycling in paddy soil, with lower water levels promoting aerobic processes such as nitrification, while higher water levels facilitated anaerobic processes such as denitrification and dissimilatory nitrate reduction to ammonium. Correspondingly, microbial composition shifted, with aerobic bacteria predominating in shallow water conditions and anaerobic bacteria flourishing under deeper water. These findings suggest that optimized water management, particularly through shallow irrigation, may mitigate nitrogen loss and improve nitrogen use efficiency. Nevertheless, additional field studies are necessary to validate these results and explore their interaction with other agricultural practices.

Keywords: nitrogen dynamics; water level management; nitrogen cycle; microbial communities

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1. Introduction

Rice is one of the most important food crops globally, serving as the primary food source for over half of the world's population. As the global population continues to grow, there is an increasing demand for rice production in order to ensure global food security [1]. However, rice cultivation is highly dependent on nitrogen fertilizer, which is a critical nutrient for promoting rice growth and achieving high yields. The application of nitrogen fertilizer in large quantities to paddy fields is a common practice with the objective of enhancing rice yields. Nevertheless, the efficiency of nitrogen fertilizer utilization in rice fields is relatively low, with only 30–50% of the applied nitrogen being absorbed and utilized by the crop. This inefficiency results in a considerable loss of nitrogen [2]. Nitrogen losses occur through a number of processes, including leaching, runoff, ammonia volatilization, and denitrification. These not only result in the waste of nitrogen fertilizer resources but also trigger a series of environmental problems. These include the eutrophication of surface water bodies, contamination of groundwater with nitrogen, and the emission of nitrogen-based greenhouse gases such as nitrous oxide (N_2O) and nitrogen oxides [3–5]. The environmental consequences of this practice not only result in a significant deterioration of the quality of water bodies in the vicinity of paddy fields but also contribute to biodiversity loss and exacerbate climate change risks.

Due to the significant environmental problems caused by nitrogen loss, optimizing nitrogen management in paddy fields to mitigate nitrogen loss has become a critical focus of current agricultural research. Among the various strategies, water level management has garnered widespread attention as an effective method for regulating both water and fertilizer use in agricultural fields [6]. The regulatory impact of field water levels on nitrogen cycling is multifaceted. Specifically, water levels influence soil moisture content and redox conditions, which in turn significantly affect the nitrogen cycle in rice fields [7]. By adjusting the depth and frequency of flooding in paddy fields, water level management can regulate oxygen supply, nitrogen conversion rates, and the emission of nitrogenous gases from the soil [8]. Studies have demonstrated that proper water level management strategies can effectively reduce nitrogen loss while improving nitrogen fertilizer utilization efficiency. For example, Qiu et al. conducted a meta-analysis on the impact of irrigation and fertilization management on nitrogen loss in paddy fields. The results indicated that most water-saving irrigation methods could enhance nitrogen use efficiency, with controlled irrigation improving nitrogen use efficiency by 1.06%. Additionally, nitrogen fertilizer use efficiency exhibited a trend of initially increasing and then decreasing with varying nitrogen fertilizer input [9]. Xu et al. reported that shallow water level management can significantly reduce both water consumption and nitrogen loss, as well as lower greenhouse gas emissions. Seasonal ammonia volatilization losses in non-diffusely irrigated rice were reduced by 18–20% compared to diffusely irrigated rice [10]. Furthermore, Chen et al. showed that intermittent irrigation, which alternates between flooding and drying phases, can reduce nitrogenous gas emissions and conserve water resources when compared to the traditional method of continuous flooding irrigation, thereby achieving the dual objectives of water conservation and yield enhancement. Nitrogen use efficiency was affected by the amount of nitrogen applied and increased with decreasing levels of nitrogen applied [11].

Nitrogen transformation in paddy fields is a complex biogeochemical process involving the transport and transformation of nitrogen among soil, plants, water bodies, and the atmosphere [12]. This transformation is primarily driven by microorganisms and involves various biochemical processes, including nitrification, denitrification, mineralization, sequestration, dissimilatory nitrate reduction to ammonium (DNRA), and anaerobic ammonia oxidation [13]. These microbial processes are influenced by nutrient concentrations and various environmental factors, such as soil moisture content, oxygen availability, temperature, and pH. In paddy systems, changes in water levels directly affect the redox state of the soil, which in turn controls the microbial activities responsible for nitrogen transformation [14]. For example, under deep flooding conditions, the soil environment tends to become anaerobic, thereby promoting denitrification. This process allows nitrate nitrogen (NO_3^--N) to be reduced to nitrogen gas (N_2) or N_2O, leading to nitrogen loss from the system in gaseous form [15]. Conversely, intermittent irrigation promotes aerobic conditions in the soil by periodically lowering the field water level, thereby enhancing nitrification, which is the oxidation of ammonium nitrogen (NH_4^+-N) to nitrate nitrogen (NO_3^--N), and consequently reducing ammonia volatilization and nitrogen loss in gaseous forms [16].

The soil microbial community plays a crucial role in the nitrogen cycle, and different water level conditions can significantly influence microbial activity [17]. Under deep flooding and irrigation conditions, the activity of denitrifying bacteria is enhanced in anaerobic environments, resulting in NO_3^--N reduction and the production of significant amounts of greenhouse gases, such as N_2O and N_2 [18]. In contrast, shallow water management or intermittent irrigation promotes an alternating aerobic–anaerobic environment, which not only promotes the balance between nitrification and denitrification but also regulates nitrogen transformations within the paddy field, thereby reducing total nitrogen (TN) losses [8]. In addition, the alternation between wet and dry conditions affects the microbial community structure at different soil depths, which further influences the vertical transport and distribution of nitrogen [4]. Changes in water levels during nitrogen cycling particularly affect the structure and function of microbial communities. Under continuous

flooding, anaerobic microorganisms, such as denitrifying and methanogenic bacteria, proliferate, whereas aerobic microorganisms, including nitrifying bacteria, dominate under shallow or dry conditions [19]. This dynamic shift in microbial community composition has a direct impact on nitrogen transformations, thereby influencing the final form and fate of nitrogen [3]. Despite this progress, there is still a limited understanding of how different field water levels specifically affect microbial nitrogen transformation processes. In particular, the interaction mechanisms between different water level conditions and microbial community structures at different soil depths during sustained inundation remain insufficiently explored [5,17].

Based on this, the present study aimed to investigate the mechanisms by which different field water levels influence the nitrogen transformation processes in paddy fields, with a particular focus on the role of water level fluctuations in regulating nitrogen transformation pathways and microbial communities in paddy soils. To achieve this, a simulated soil column experiment was designed to systematically investigate the dynamic changes in nitrogen cycling within the soil and water environment of the paddy field under different water level conditions. This simulation was combined with high-throughput sequencing technology to provide a detailed analysis. By examining nitrogen levels and microbial community structures at different soil depths, this study sought to elucidate the interactions between water levels, nitrogen-transforming microorganisms, and environmental factors. Ultimately, this study not only sheds light on the mechanisms by which field water levels affect nitrogen-cycling processes in paddy fields but also provides theoretical support for optimizing irrigation management in rice production, with the aim of improving nitrogen use efficiency, reducing nitrogen losses, and promoting sustainable agricultural development.

2. Materials and Methods

2.1. Study Site and Soil Samples

The experiment was conducted in 2019 at the experimental site of Shanghai Jiao Tong University (121.45° E, 31.03° N), which is characterized by a northern subtropical monsoon climate, with an average annual temperature of 17.1 °C. Soil samples were collected from the same experimental site. During the rice growing season, the region is influenced by summer monsoons and subtropical high pressure, resulting in high solar altitude angles, hot temperatures, and abundant sunshine. To accurately reflect the nitrogen-cycling processes in paddy soil, and to ensure that the simulated paddy soil's hydrodynamic and microbial environments closely resemble actual production conditions, this study utilized submerged paddy soil collected from paddy fields in Huayuan Village, Fengxian District, Shanghai, China. After collection, the soil was air-dried, crushed, and sieved for use in the experiments. The basic physical and chemical properties of the soil are presented in Table 1.

Table 1. Properties of soil in experiment (g/kg).

pH	TOC	TN	TP	TK	NH_4^+-N	Available K	Available P
8.45 ± 0.08	5.31 ± 0.77	0.58 ± 0.01	706.63 ± 3.88	1.86 ± 0.03	218.9 ± 4.5	250.8 ± 0.10	2.35 ± 0.05

2.2. Experimental Setup

In this study, a paddy field simulation experimental system was constructed, and the system structure is illustrated in Figure 1. The main body of the soil column module consists of a hollow cylinder made of opaque acrylic material, measuring 80 cm in height, with an inner diameter of 30 cm and a wall thickness of 1 cm, and open at the top. Water extraction holes, soil extraction holes, a CD-1 oxidation–reduction potential (ORP) sensor (Chuandi, China), and a WT-11 soil moisture (FDR) sensor (Chuandi, China) were mounted around the soil column. These four holes were spaced equally from top to bottom at 10 cm intervals. Additionally, a ruler was placed at the top of the column, positioned longitudinally at

15 cm, to facilitate visual measurement of the water level. The water extraction holes were connected to multi-channel micro-flow peristaltic pumps through water extraction tubes to absorb soil water samples. The water supply module consisted of a water supply bucket, a peristaltic pump, and a water supply pipe, which was connected to the soil column module through the water supply holes during irrigation. The online monitoring module included sensors, a data collector, and a computer for real-time monitoring of soil temperature, redox potential, and soil moisture. To avoid the influence of rainfall on the water levels, the experiment was conducted inside a covered glass room.

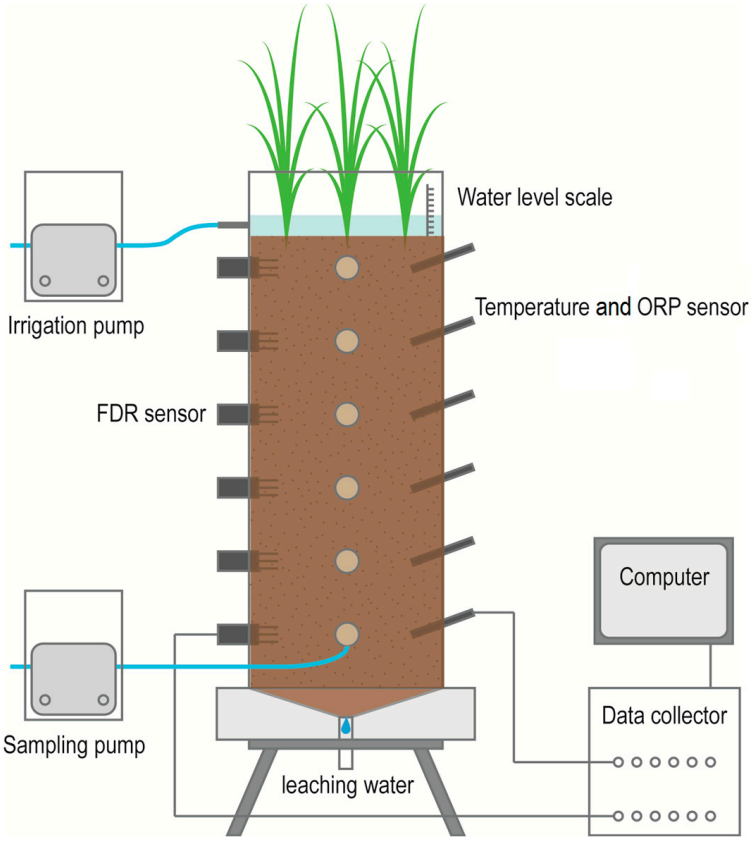


Figure 1. Experimental setup.

2.3. Experiments

Widely used chemical fertilizers were applied for fertilization, with nitrogen fertilizer administered at a strength of 100 kg N/ha in the form of urea. The ratio of base fertilizer, tiller fertilizer, and spike fertilizer was 4:3:3. Phosphorus fertilizer was applied as a base fertilizer at a strength of 50 kg $P_2O_5^-$/ha using slow-release superphosphate. Potash was applied as a base fertilizer at a strength of 100 kg K_2O/ha in the form of potassium sulfide, with the ratio of base to spike fertilizer set at 1:1. Nitrogen and potassium fertilizers were mixed with water prior to application. During the rice flooding period, excluding the soaking, sunning, and pre-harvest periods, water level control was implemented. Irrigation was conducted at regular intervals, once a day, to maintain the maximum water levels of the four experimental systems at 1, 2, 3, and 4 cm, respectively. The remaining field management practices followed local conventional methods.

During the experimental period, water samples from the field and soil were collected at depths of 5 cm, 25 cm, and 45 cm to test for TN, NH_4^+-N, nitrite nitrogen (NO_2^--N), and NO_3^--N. The ultraviolet (UV) spectrophotometric method, using alkaline potassium persulfate, was employed for TN testing. The nano reagent photometric method was used for NH_4^+-N, while NO_2^--N testing was performed using the UV spectrophotometric method with naphthalene ethylenediamine hydrochloride. The UV spectrophotometric

method was also utilized for NO_3^--N testing. All analyses were conducted using a UV7504 spectrophotometer (SHHK, Shanghai, China) and a DRB200 reactor (Hach Company, Loveland, CO, USA). Soil temperature, redox potential, and soil moisture content were continuously monitored online throughout the experiment. Given that the application of basal fertilizer is a critical period for nitrogen loss and transformation in paddy fields, soil microbial samples were collected at depths of 5 cm, 25 cm, and 45 cm on the 5th day after the application of basal fertilizer. The sample numbering system is detailed in Table 2.

Table 2. Rules for numbering microbiological samples.

Water Level	1 cm	2 cm	3 cm	4 cm
Shallow soil (5 cm)	S1	S2	S3	S4
Medium soil (25 cm)	M1	M2	M3	M4
Deep soil (45 cm)	D1	D2	D3	D4

2.4. Microbial Sample Testing and Analysis

2.4.1. DNA Extraction

Total bacterial genomic DNA samples were extracted using the Fast DNA SPIN extraction kits (MP Biomedicals, Irvine, CA, USA) according to the manufacturer's instructions and stored at −20 °C until further analysis. The quantity and quality of the extracted DNA were assessed using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) for quantification and agarose gel electrophoresis for quality verification.

2.4.2. 16S rDNA Amplicon Pyrosequencing

PCR amplification of the bacterial 16S rRNA gene V3-V4 region was carried out using the forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and the reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Sample-specific 7 bp barcodes were incorporated into the primers for multiplex sequencing. The PCR reaction mixture contained 5 μL of Q5 reaction buffer (5×), 5 μL of Q5 High-Fidelity GC buffer (5×), 0.25 μL of Q5 High-Fidelity DNA Polymerase (5U/μL), 2 μL (2.5 mM) of dNTPs, 1 μL (10 μM) of each forward and reverse primer, 2 μL of the DNA template, and 8.75 μL of ddH_2O. The thermal cycling protocol consisted of an initial denaturation at 98 °C for 2 min, followed by 25 cycles of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. PCR amplicons were purified using Agencourt AMPure Beads (Beckman Coulter, Brea, CA, USA) and quantified with the PicoGreen dsDNA Assay Kit (Invitrogen, Waltham, MA, USA). After quantification, amplicons were pooled in equal amounts, and paired-end 2 × 300 bp sequencing was performed on the Illumina MiSeq platform using the MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

2.4.3. Sequence Analysis

The Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) pipeline was employed to process the sequencing data, as previously described [20]. In brief, raw sequencing reads with exact matches to the barcodes were assigned to their respective samples and identified as valid sequences. Low-quality sequences were filtered out based on the following criteria [21]: sequences with a length of less than 150 bp, sequences with average Phred scores below 20, sequences containing ambiguous bases, and sequences with mononucleotide repeats exceeding 8 bp. Paired-end reads were assembled using FLASH [22]. After chimera detection, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity using UCLUST [23]. A representative sequence from each OTU was selected using default parameters. OTU taxonomic classification was performed by BLAST searching the representative sequences against the Greengenes Database [24] using the best hit [25]. An OTU table was subse-

quently generated to record the abundance of each OTU in each sample, along with the taxonomy of these OTUs. OTUs representing less than 0.001% of total sequences across all samples were discarded. To minimize differences in sequencing depth across samples, an averaged, rounded, and rarefied OTU table was generated by averaging 100 evenly resampled OTU subsets, each subsampled at 90% of the minimum sequencing depth, for further analysis.

2.5. Bioinformatics and Statistical Analysis

In this study, redundancy analysis (RDA) was performed on the relative abundance matrix and environmental factors of microbial communities at the genus level using Canoco v5.02 software [26]. Non-redundant gene sequences were aligned with the KEGG database through the application of Kobas v3.0 software, and PICRUSt2 v2.5.2 software was used to predict the metabolic functions of microbial communities [27]. The nitrogen cycle pathways of the samples were analyzed and visualized using DiTing v0.9 software [28]. The results obtained from these analyses were subjected to statistical evaluation to ensure rigor and accuracy. Data were processed using Excel software, with plots generated using OriginLab v2023 and Tableau v2023 software. Statistical analysis methods were applied to produce box plots of soil water nitrogen levels and the stacked column chart of microbial species composition.

3. Results

3.1. Distribution of Nitrogen Concentration at Different Depths of Rice Paddy Soil and Water System under Different Field Water Level Conditions

In Figure 2, the distribution of NH_4^+-N concentrations at different soil depths (field surface water, 5, 25, and 45 cm) in the paddy field system under different water levels (1, 2, 3, and 4 cm) can be seen. The NH_4^+-N concentration in the surface water was significantly higher than at other depths, and the effect of water level on this concentration was particularly pronounced. The maximum NH_4^+-N concentration exhibited a decreasing trend as the water level increased from 1 cm to 4 cm. The highest concentration was observed at the 1 cm water level, with an average of 5.79 mg/L, and the peak concentration in one sample reached 12.19 mg/L. In contrast, the average NH_4^+-N concentrations at water levels of 2–4 cm were similar to each other, which was reduced by 49.6%, 51.1%, and 60.6%, respectively, as compared to the 1 cm water level. The effect of water level on NH_4^+-N concentration in shallow soil water (at a depth of 5 cm) was comparatively weaker. In the middle and deeper soil water layers, the NH_4^+-N concentration at the 1 cm water level was higher than at 2–4 cm. Overall, there was a significant decline in NH_4^+-N concentrations in the soil water as depth increased.

As shown in Figure 3, the NO3$^-$-N concentration in surface water was significantly higher than at other depths, and the water level had a pronounced effect on this concentration. The highest NO_3^--N concentration was 5.63 mg/L at a water level of 1 cm. Under water level conditions of 1 to 4 cm, the average concentrations were 1.60 mg/L, 1.34 mg/L, 0.67 mg/L, and 1.03 mg/L, respectively. Compared to the 1 cm water level, the average concentrations at 2 to 4 cm water levels decreased by 16.3%, 58.1%, and 35.6%, respectively. Although the NO_3^--N concentration at a depth of 5 cm was lower than that of the surface water, it still remained relatively elevated. Notably, the NO_3^--N concentration at the 1 cm water level at this depth was significantly lower than at higher water levels. At depths of 25–45 cm, the NO_3^--N concentration decreased substantially, although the differences between water levels were minimal, indicating that the effect of water level on NO_3^--N concentration at these depths was limited.

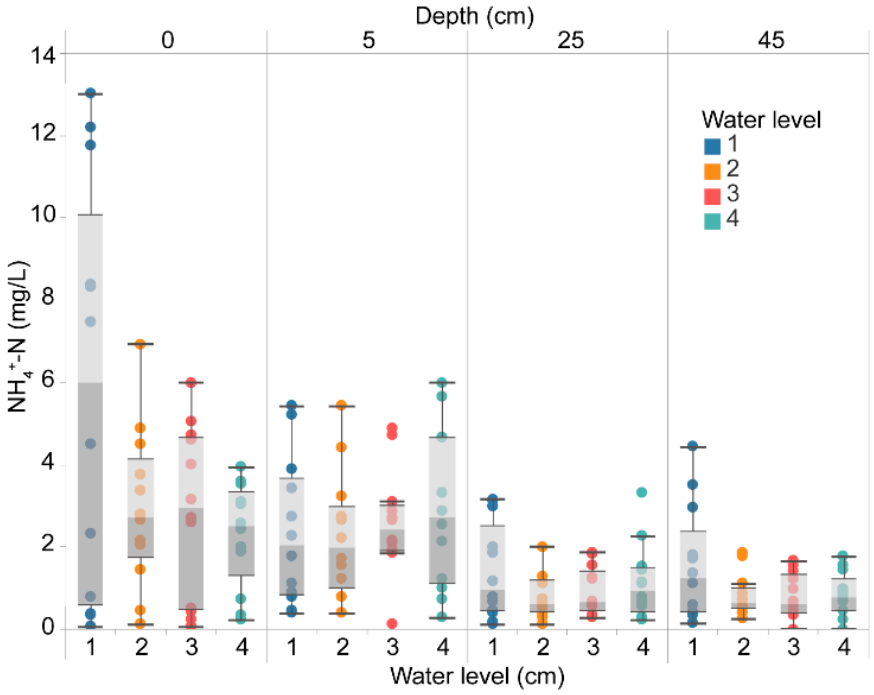


Figure 2. Distribution of NH_4^+-N concentration at different depths of paddy field system under different water level conditions.

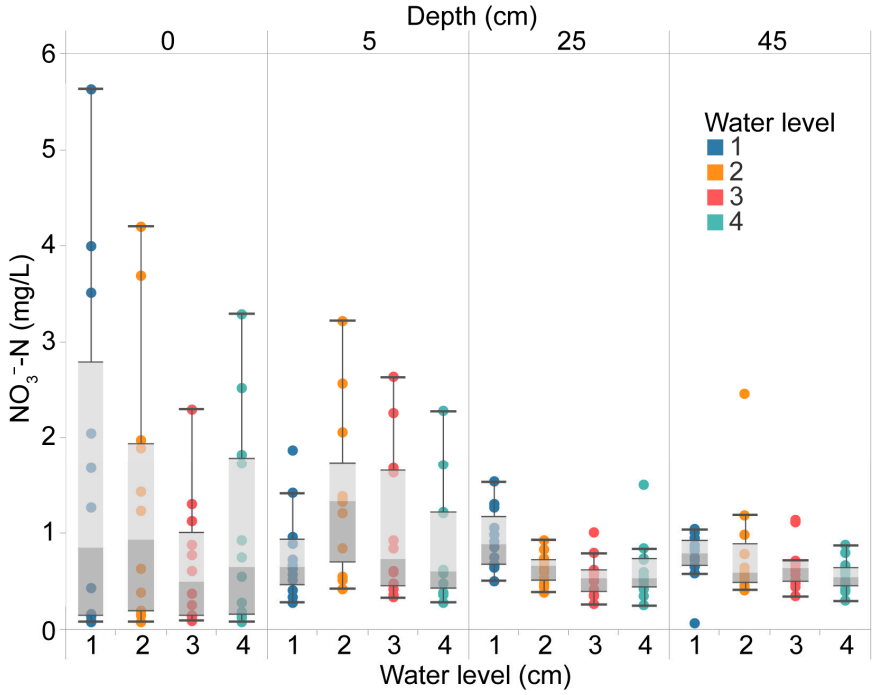


Figure 3. Distribution of NO_3^--N concentration at different depths in paddy system under different water level conditions.

As shown in Figure 4, the NO_2^--N concentration in field water exhibited a strong dependence on the water level. The 1 cm water level condition resulted in the highest NO_2^--N concentration, with a peak value of 3.85 mg/L and an average concentration of 0.82 mg/L, which was significantly higher than that observed under other water level conditions. Under water level conditions of 2 to 4 cm, the average concentration of NO_2^--N decreased by 79.3%, 80.5%, and 76.8%, respectively, compared to the 1 cm water level. However, this

pattern was not reflected in the soil water. At a depth of 5 cm, NO_2^--N concentrations were significantly lower compared to the field water, ranging from 0.1 to 0.5 mg/L across all water level conditions. While NO_2^--N concentrations remained elevated under the low water level condition (1 cm), they gradually decreased with increasing water level. In the middle and deeper soil water layers (25–45 cm), NO_2^--N concentrations remained at lower levels, between 0.01 and 0.07 mg/L across all water levels, with no significant effect of water level on NO_2^--N concentration. The effect of water level on NO_2^--N concentration was only evident in field surface water and shallow soil water.

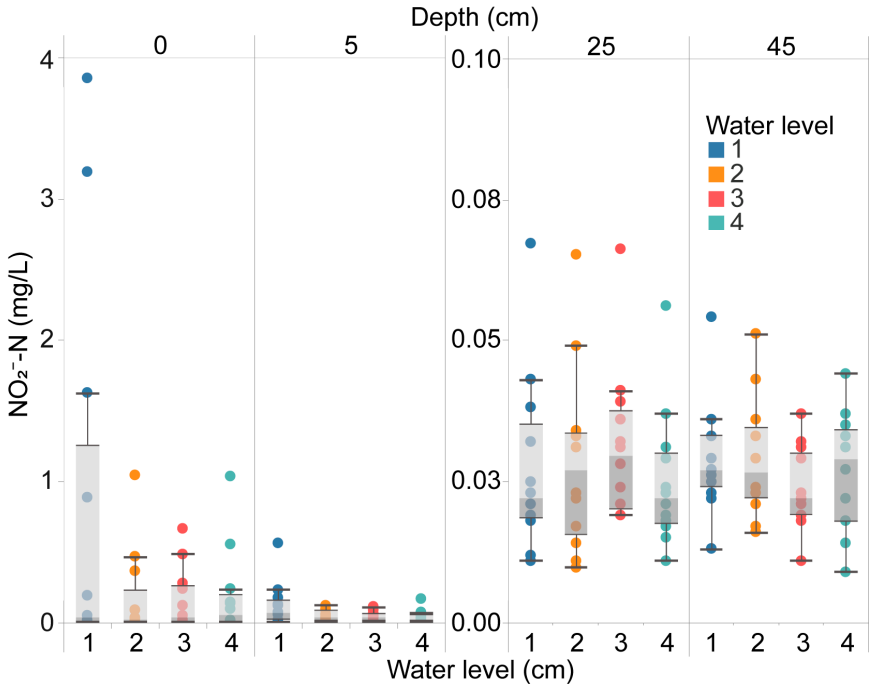


Figure 4. Distribution of NO_2^--N concentration at different depths in paddy field system under different water level conditions.

3.2. Distribution of Microbial Composition of Paddy Soil Under Different Field Water Level Conditions

Understanding the spatial distribution characteristics of the relative abundance of microorganisms at the phylum level can help to explore the differences in microbial community structure and diversity under different water level conditions. Based on the relative abundance analysis of microbial communities shown in Figure 5, the microbial community structure in paddy soils under different water levels differed significantly among soil depth layers (S, M, and D). Gammaproteobacteria and Alphaproteobacteria were the most dominant clades in all samples, especially in surface soils (S1–S4), where the relative abundance of Gammaproteobacteria accounted for over 60% of the total. This indicates that the aerobic conditions in surface soils were more conducive to the growth of these microorganisms. The relative abundance of anaerobic microorganisms, such as Deltaproteobacteria and Clostridia, increased in the medium and deep soils (M1–M4 and D1–D4) with increasing water levels, with a notable increase in the relative abundance of Deltaproteobacteria in deep soils, particularly at water levels of 1–3 cm. This suggests that the decrease in soil oxygen content, due to higher water levels, promoted the proliferation of anaerobic microorganisms. In addition, bacteria such as Actinobacteria and Verrucomicrobiae showed varying degrees of abundance changes in shallow and medium soils, although their proportion in deep soil remained relatively low.

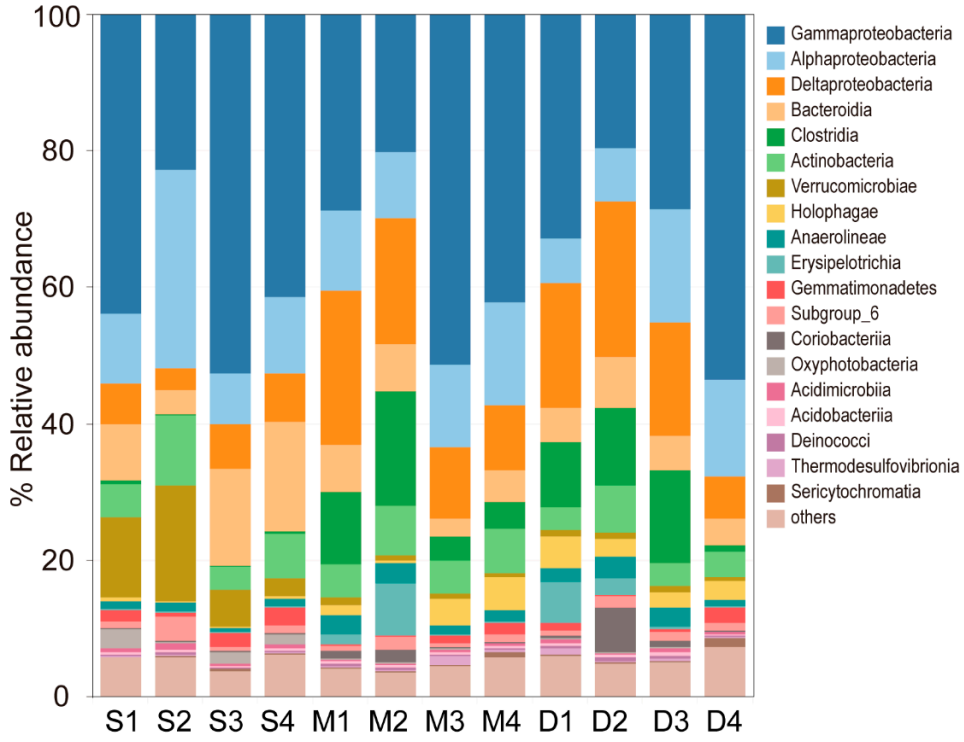


Figure 5. A histogram showing the relative abundance of microorganisms at the phylum level in paddy soil across different soil depths under different water levels (1, 2, 3, and 4 cm).

3.3. Microbial-Mediated Nitrogen Biochemical Responses Under Different Field Water Level Conditions

Building on the understanding of the overall differences in microbial community structure and diversity under different water level conditions, the functional generalization of microbial relative abundance at the genus level (as shown in Figure 6) helps to elucidate the mechanisms by which water level influences nitrogen-cycling processes at different soil depths. Nitrogen fixation, mineralization, ammonia oxidation, denitrification, and DNRA were the main nitrogen biogeochemical processes occurring in the paddy soil in this experiment. Among these, nitrogen-fixing functional bacteria were identified using the *nifH* gene, with *Geobacter* and *Azoarcus* as the primary functional genera in the system. Nitrogen fixation was low in the field water but increased in the soil, with overall similar levels of nitrogen fixation observed at 25 cm and 45 cm depths.

Mineralization was identified using the *argF* gene, and the main functional bacterium belonged to a genus of the family Moraxellaceae that has not yet been successfully isolated and cultured. Mineralization occurred mainly in the surface water. Ammonia oxidation was detected using the *AmoB* gene, with *Sphingomonas*, *Comamonas*, and *Nitrospira* as the main functional bacteria. Ammonia oxidation was active throughout the soil profile, with the highest activity occurring in the field surface water.

Denitrification was identified using the *nirS*, *nirK*, and *nosZ* genes, with *Azoarcus*, *Azospira*, and *Pseudomonas* as the dominant functional genera. DNRA was labeled using the *nrfA* gene, with *Anaeromyxobacter* and *Thermincola* as the main functional genera. Denitrification and DNRA activity increased significantly with deeper water levels, while activity was lower in the field water. The effect of water level on the relative abundance of mineralization, denitrification, and DNRA in field water followed the following trend: 1 cm > 4 cm > 3 cm > 2 cm. The effect of water level on the relative abundance of nitrogen-transforming microorganisms in soil water showed more variability.

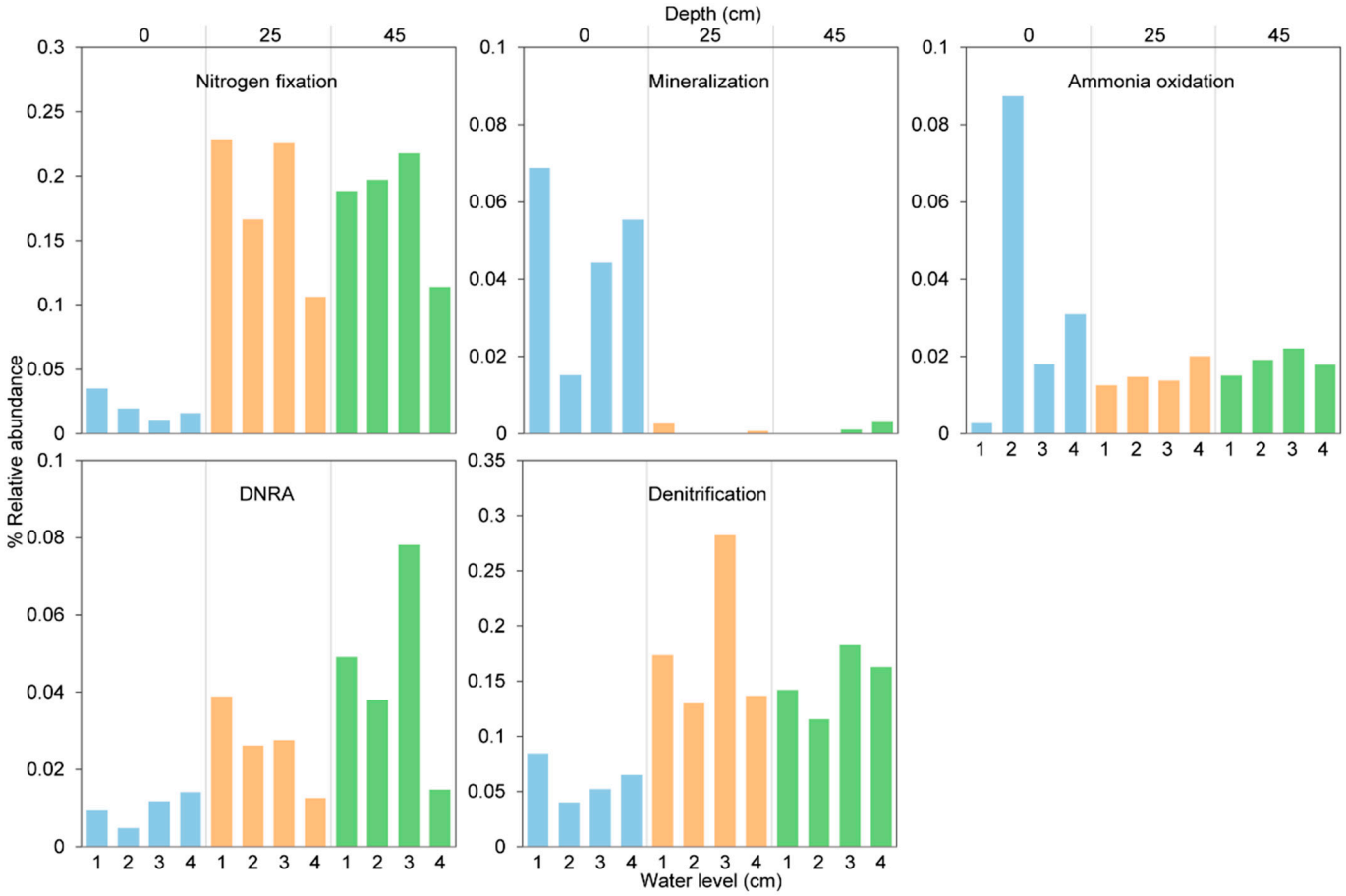


Figure 6. Relative abundance of nitrogen-converting functional microorganisms at different soil depths under different water level conditions.

The RDA method allows for the biogeochemical processes of nitrogen to be linked to environmental factors such as field water levels and helps to reveal the relationships between them. As shown in Figure 7, the first two RDA axes explained a total of 87.41% of the variance, with RDA1 accounting for 81.37%. On the first RDA axis, ORP was the most significant environmental factor, showing a positive correlation with nitrite and NH_4^+-N concentrations and a negative correlation with NO_3^--N concentration. Depth, water level (shown as WT in Figure 7), and soil water content were negatively correlated with nitrite N and ammonium N concentrations and positively correlated with NO_3^--N concentrations on the same axis.

Mineralization and ammonia oxidation were positively correlated with RDA1, indicating their association with environments containing higher concentrations of nitrogen compounds. In contrast, nitrogen fixation and DNRA were negatively correlated with ORP and nitrogen compound concentrations but positively correlated with the water level, soil water content, and depth along RDA1. RDA2, which explained 6.04% of the variance, showed that processes such as denitrification, nitrogen fixation, and DNRA are also influenced by factors such as depth and soil water content, although these relationships were less pronounced than those observed at RDA1.

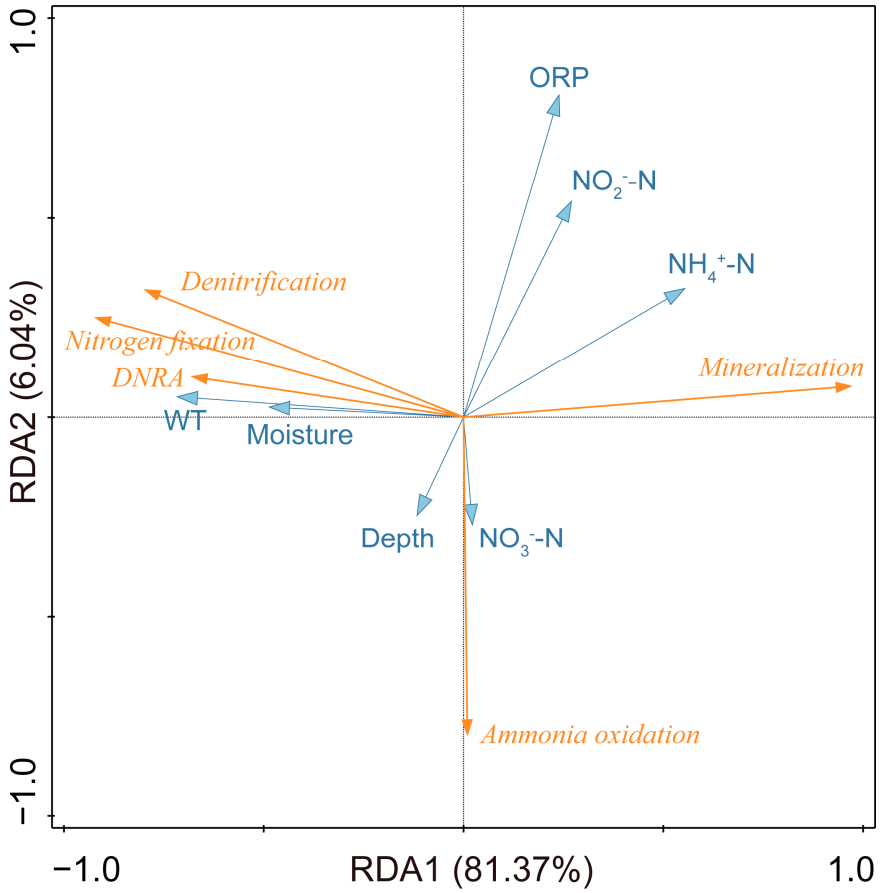


Figure 7. RDA of environmental factors and nitrogen biogeochemical processes.

3.4. Nitrogen-Cycling Pathways and Relative Abundance Analysis

Conducting nitrogen-cycling pathway and relative abundance analyses allows for a deeper understanding of the specific effects of field water levels on nitrogen-cycling processes within the system. As shown in Figure 8, T1, T2, T3, and T4 represent different field water levels. Nitrification, denitrification, DNRA, nitrogen fixation, and ANRA are the main biochemical processes involved in the nitrogen cycle of paddy fields in this experimental system. Among these, the nitrification pathway showed the highest relative abundance of NO_3^--N reductase, encoded by the *nxrAB* gene cluster. The relative abundance of nitrite reductase, encoded by *nirK* and *nirS* in the denitrification pathway, was significantly higher than that of N_2O reductase, encoded by *nosZ*.

Water level had a significant effect on the relative abundance of ammonia monooxygenase and hydroxylamine oxidase in the nitrification pathway. Hydroxylamine oxidase had a higher relative abundance under low water level irrigation conditions (1–2 cm), whereas ammonia monooxygenase had a higher relative abundance under high water level conditions (4 cm). In addition, water levels significantly influenced nitrogen fixation and the activity of *NT-6* and *nirA* gene-labeled ANRA, with both showing higher relative abundance under low water level irrigation conditions (1–2 cm).

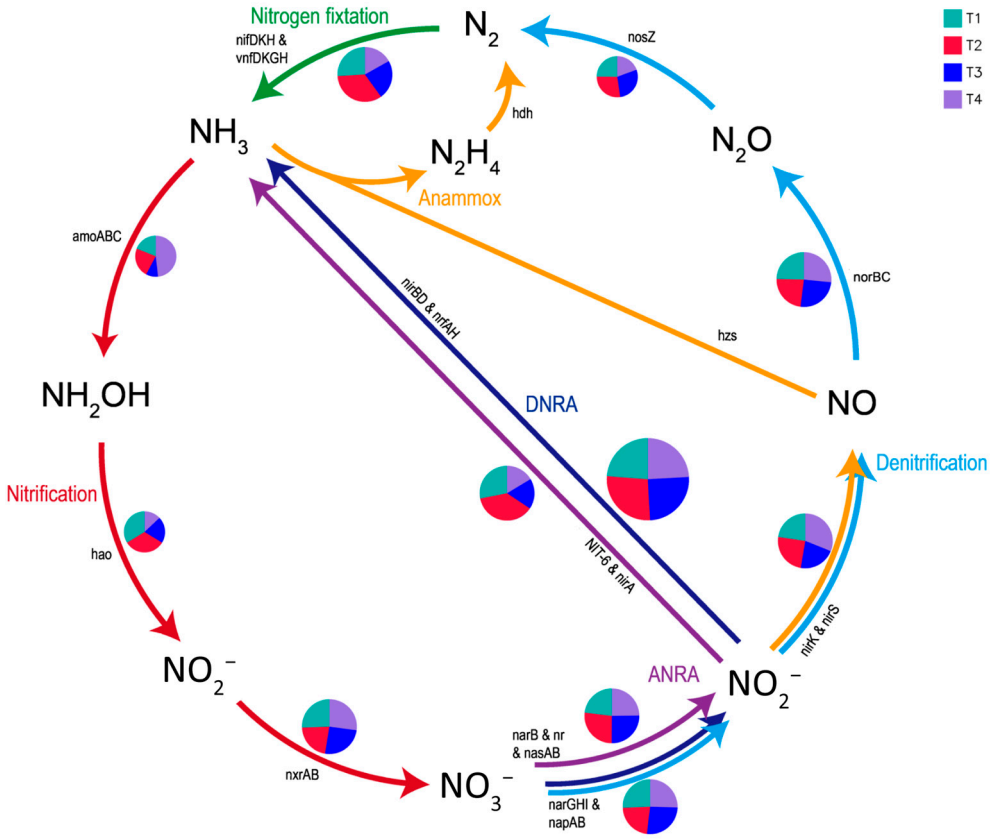


Figure 8. The relative abundance of nitrogen-cycling pathways. The size of the pie charts represents the total relative abundance of each pathway, while the pie chart segments depict the relative abundance of each nitrogen-cycling pathway in each sample. ANRA: assimilatory reduction of NO_3^--N to ammonium; Anammox: anaerobic ammonia oxidation.

4. Discussion

4.1. Effects of Water Levels on Nitrogen Transformation in Surface Soils

In urea-applied rice production, nitrogen transformation in the soil and water environments is mainly controlled by microbial processes, such as urea hydrolysis, nitrification, and denitrification, which are regulated by irrigation water levels and oxygen availability. In this study, NH_4^+-N and NO_3^--N concentrations in paddy soils gradually decreased with increasing water levels. On the one hand, a higher field water level represents a larger volume of irrigation water, and the dilution effect directly reduces the urea concentration in the field water [29]. The decrease in urea concentration limits urease activity, resulting in significantly lower NH_4^+-N concentrations in field water under 2–4 cm water level conditions compared to 1 cm (see Figure 2). Therefore, when using low water level irrigation methods, appropriately increasing the water level can help mitigate the potentially harmful effects of fertilizers on crops, reduce ammonia volatilization losses, and improve nutrient use efficiency [30]. As a cation, NH_4^+-N is strongly adsorbed by negatively charged soil particles, such as clay minerals and organic matter, resulting in its retention in the surface soil. Its transport rate is limited by factors such as soil texture, cation exchange capacity (CEC), and hydrodynamic conditions [31]. This leads to significant differences in the effect of water level on $NH4^+$-N concentrations between field water and surface soil water. In addition, $NH4^+$-N in soil is not converted to nitrate under anaerobic conditions, reducing the potential for nitrogen leaching losses from soil water [32].

Since nitrogen-fixing bacteria, such as *Geobacter* and *Azoarcus*, and DNRA bacteria, such as *Anaeromyxobacter* and *Thermincola*, are mainly distributed in the middle and deep soils (see Figure 6), NH_4^+-N in the middle and deep soil water is likely to originate

from biological nitrogen fixation and auto-nitrification, in agreement with the findings of Kaviraj et al. [33]. In contrast to NH_4^+-N, negatively charged NO_3^--N is not easily adsorbed by clay minerals or organic matter and therefore tends to move deeper into the soil with water flow [31]. As a result, NO_3^--N in the medium and deep soils mainly migrates from the shallow soils and is subject to the delayed effect of the water level at the field surface. As shown in Figure 3, the NO_3^--N concentrations at a depth of 5 cm under the 1 cm water level were significantly lower than those under the 2–4 cm water levels, whereas NO_3^--N concentrations at depths of 25 cm and 45 cm were significantly higher than those under the 2–4 cm water levels, suggesting that excessively low irrigation water levels may lead to NO_3^--N leaching and increased nitrogen losses [34]. It is important to note that excessively high NH_4^+-N concentrations in the topsoil can also reduce the activity of nitrifying bacteria [35], resulting in low NO_3^--N concentrations in surface soil water. Nitrite, as an intermediate product of nitrification, denitrification, and ANRA, showed only a transient increase in concentration in field surface water, similar to the results of previous studies [36]. Its concentration was closely related to the amount of fertilizer applied and was also influenced by the dilution effect of the irrigation water level.

4.2. Influence of Water Levels on Microbial Communities in Surface Soils

In the surface soil, the relative abundance of aerobic microorganisms, such as Gammaproteobacteria and Alphaproteobacteria, increased significantly under low water level conditions, as shown in Figure 5. This indicates that better atmospheric oxygen availability under low water level conditions favors the growth and reproduction of these microorganisms [37]. Gammaproteobacteria are widely involved in the nitrogen cycle, including both nitrification and denitrification processes, and are commonly found in ammonia-oxidizing bacteria such as *Nitrosomonas* [38]. In contrast, the Alphaproteobacteria include several nitrogen-fixing bacteria, such as Rhizobium, which, as demonstrated by Wang et al., showed to be most diverse in the 10–20 cm soil layer, with diversity decreasing significantly with increasing soil depth [39]. Conversely, the relative abundance of anaerobic microorganisms involved in denitrification, such as Deltaproteobacteria, increased significantly at higher water level conditions (3–4 cm). This further confirms that higher water levels enhance the activity of anaerobic microorganisms by promoting an anaerobic environment in the soil [40]. Bacteroidia, although not directly involved in the nitrogen cycle, contribute by decomposing complex organic compounds, thereby providing substrates for other nitrogen-cycling microorganisms [7]. Verrucomicrobia, one of the most important anaerobic bacteria in paddy soils, showed a higher relative abundance under low water level conditions, which contrasts with the findings of Chin et al. [41]. This discrepancy may be influenced by factors such as carbon availability and interactions with rice plants.

Overall, the water level directly alters the composition and distribution of microbial communities in the topsoil by modifying oxygen transport conditions, thereby influencing the biogeochemical pathways of nitrogen transformation in the region. The relative abundance of nitrifying and denitrifying microorganisms, such as *Nitrospira* and *Azospira*, increased significantly under low water level conditions, further explaining the enhanced nitrogen transformation through nitrification and denitrification pathways [42]. In contrast, the relatively anaerobic environment under high water level conditions promotes denitrification and DNRA processes in surface soils, resulting in greater nitrogen loss to the atmosphere via these pathways [43].

4.3. Impact of Water Levels on Nitrogen Cycling in Middle and Deep Soils

In addition to its significant effects on surface soils, water level management has a profound effect on nitrogen cycling in the middle and deep soils of paddy fields. The relative abundance statistics of nitrogen-cycling functional microorganisms at the genus level (see Figure 6) showed a significant negative correlation between the relative abundance of DNRA bacteria in the middle soil and the water level of the field. This was mainly due to the downward infiltration of NO_3^--N, generated by the nitrification of NH_4^+-N in the

surface layer, into the middle layer. This conclusion is consistent with the experimental results on NO_3^--N concentration in soil water and is in agreement with the findings of Li et al. [44]. Among these microorganisms, *Anaeromyxobacter* bacteria are commonly found in rice soils and river network sediments [45]. In addition to being a key component of the DNRA community, they also act as iron-reducing bacteria that enhance nitrogen fixation via iron oxides [46], thus participating in both nitrogen and iron cycling. *Thermincola* bacteria promote the DNRA process through sulfide solubilization, functioning in Fe–sulfur environments and contributing to nitrogen oxide metabolism via various regulatory factors [47]. Friedl et al. observed different results during the rewetting of tropical pasture soils, where wet soils promoted DNRA under anaerobic conditions [48], suggesting differences in the degree of reduction between wet pasture soils and chronically flooded paddy field soils. However, Figure 8 shows that the DNRA cycle was less affected by water level, indicating that not all genera involved in DNRA are affected by the same factors.

In addition to water level and substrate concentration, DNRA activity has been shown to be closely related to soil sulfide and total organic carbon content [44]. While the irrigation water level did not significantly affect the relative abundance of denitrifying bacteria such as *Azoarcus*, *Azospira*, and Pseudomonas in the middle and deep soils, DNRA's reduction of NO_3^--N in this zone contributed to lower N_2O emissions [49]. Since NH_4^+-N is a product of DNRA, the presence of ammonia-oxidizing bacteria such as *Sphingomonas*, *Comamonas*, and *Nitrospira* was also observed in the middle and deep soils. The water level indirectly influenced this process, confirming the findings of Wang et al. [50].

In summary, irrigation water levels have different degrees and mechanisms of influence on the nitrogen cycle, as well as the distribution and community structure of related microorganisms in surface water, surface soil, and deep soil environments. Through rational water level management, the use efficiency of nitrogen fertilizer can be improved, greenhouse gas emissions from rice production can be effectively reduced, and the sustainable development of agricultural production can be promoted.

4.4. Limitations and Future Directions

Although this study revealed the effects of different field water level management strategies on nitrogen transformation and microbial communities, several limitations remain. First, the experiment was conducted under controlled laboratory conditions, whereas more complex factors, including rainfall and soil type, may influence the real paddy field environment. For example, the rainfall factor was excluded in this study to allow for a comparison of differences between stabilized irrigation at different water levels. However, the results require verification under real conditions to ensure the study's applicability and reliability. Secondly, this research focused primarily on the effect of field water levels on nitrogen cycling. Future studies should further explore the interactive effects of different water levels combined with other farm management practices (e.g., fertilizer type and fertilization frequency).

5. Conclusions

The irrigation water level has a significant impact on nitrogen concentrations, microbial composition, and nitrogen biochemical processes in paddy fields. Under low water levels, NH_4^+-N, NO_3^--N, and NO_2^--N concentrations in surface water are higher, with average concentrations decreasing as soil depth and water level increase. Microbial-mediated nitrogen transformation processes (such as mineralization, ammonia oxidation, denitrification, and DNRA) vary under different water level conditions. In surface water, the relative abundances of mineralization, denitrification, and DNRA are significantly influenced by water levels, showing a trend of 1 cm > 4 cm > 3 cm > 2 cm. The water level primarily affects nitrogen cycling in shallow soil through dilution and changes in soil redox conditions, while in deeper soil, it indirectly influences nitrogen cycling by affecting nitrification activity, nitrate infiltration, and DNRA processes.

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