RESEARCH ARTICLE

# Nitrification of loamy and sandy soil under water irrigation with different oxygen content

Qingyong Bian[1, 2], Yanbo Fu[1, 2, *], Zhiguo Wang[1, *], Yaozu Feng[3], Wenlong Zhang[2]

[1]Institute of Soil Fertilizer and Agricultural Water Conservation, Xinjiang Academy of Agricultural Sciences, Urumqi, Xinjiang, China. [2]Baycheng Agricultural Experiment Station/National Soil Quality Aksu Observation and Experiment Station, Xinjiang Academy of Agricultural Sciences, Aksu, Xinjiang, China. [3]Scientific and Technological Achievement Transformation Center, Xinjiang Academy of Agricultural Sciences, Urumqi, Xinjiang, China. [4]Grass Land Science, College of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang, China.

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**Oxygenated water irrigation can increase soil ventilation to optimize the gas environment of crop roots, which not only promotes crop growth, but also has a certain impact on soil quality. In this indoor study, four kinds of water with different oxygen contents were used for loam and sandy soil irrigation including conventional water irrigation (CK), natural aeration (RD1), 33% oxygenated irrigation (RD2), and 90% oxygenated irrigation (RD3), respectively. The results showed that oxygen-enhanced irrigation significantly increased soil inorganic nitrogen content, ammonia nitrogen ($NH_4^+$-N) consumption, consumption rate, nitrate-nitrogen ($NO_3^-$-N) generation, and generation rate throughout the cultivation period. The inorganic nitrogen content in loam soil was always higher than that in sandy soil. Under different oxygen-enhanced treatments, the consumption of ammonium nitrogen was much lower than the generation of nitrate nitrogen. Oxygen-enhanced irrigation primarily influenced soil inorganic nitrogen migration and transformation through nitrification. Comprehensive cluster heatmap analysis showed that oxygen enhancement accelerated $NO_3^-$-N generation in loam soil, facilitated $NH_4^+$-N consumption in sandy soil. The results suggested that oxygen-enhanced irrigation effectively improved soil nitrification. This study provided a theoretical basis for the development of efficient water and fertilizer utilization techniques in agriculture.**

**Keywords:** indoor culture; oxygenated irrigation; loamy soil; sandy soil; nitrification.

[*]**Corresponding authors:** Yanbo Fu and Zhiguo Wang, Institute of Soil Fertilizer and Agricultural Water Conservation, Xinjiang Academy of Agricultural Sciences, Urumqi 830000, Xinjiang, China. Email: guotaiminan7@163.com (Fu Y), 985954632@qq.com (Wang Z).

## Introduction

In agricultural production, water, fertilizer, air, and heat are the four important factors responsible for the capacity of soil to provide various nutrients for crop growth. Inadequate soil aeration inevitably affects the physical and chemical properties of the soil, restricting normal growth of crops and ultimately reducing crop yield [1]. Oxygenated irrigation, a type of irrigation technology, involves addition of oxygen to the irrigation water. Delivery of oxygen directly to the crop root system leads to the optimization of inter-root gaseous environment, which promotes crop growth and in turn, crop production and income [2]. Oxygenated irrigation is reported to improve soil oxygen content, water and fertilizer use efficiency, and crop quality and efficiency. Zhang *et al*. reported that in rice, aerated irrigation significantly increased chlorophyll content in leaves, leaf area, and dry matter accumulation [3]. Aerated irrigation positively affected the growth, physiological indicators, yield, and irrigation water use efficiency in sweet pepper [4]. Positive effects of aerated irrigation are reported in wheat [5], vegetables grown in greenhouses [6], and other crops.

Soil oxygen content directly or indirectly affects the soil nitrogen cycle and effectively regulates the process of soil nitrogen transformation. $NO_3^-$-N and $NH_4^+$-N are the two forms of nitrogen in soil that can be directly used by plants and soil microorganisms, mainly *via* the nitrogen transformation activities of soil microorganisms. Therefore, it is extremely important to regulate the oxygen content in soil pores for obtaining high yield of crops and efficient use of nitrogen through optimizing oxygenated water input. Dissolved oxygen is an important factor affecting the release of nitrogen in the substrate. When dissolved oxygen concentration is greater than 7 mg/L and less than 1 mg/L, respectively, nitrogen is released mainly in the form of $NO_3^-$-N and $NH_4^+$-N [7]. $NO_3^-$-N is the main form of nitrogen transported from farmland to groundwater bodies. Controlling or slowing down the transformation of $NH_4^+$-N to $NO_3^-$-N helps to reduce the migration of soil nitrogen to water bodies and improves nitrogen use efficiency, therefore, it is one of the important countermeasures to reduce nitrogen leaching [8]. Cyclic aerated irrigation and pure-oxygen-aerated irrigation significantly improve soil respiration and soil aeration in the root zone [9, 10], and the improvement in soil microenvironment promotes crop root growth [11, 12]. The activity of anaerobic microorganisms in soil is suppressed after oxygen concentration increases, and the intensity of denitrification is decreased, which reduces the loss of gaseous nitrate nitrogen via denitrification and increases $NH_4^+$-N content in soil [13]. Oxygen distribution directly affects the microbial community in paddy fields, and increased dissolved oxygen content can significantly increase the abundance and activity of soil nitrifying bacteria [14].

The results of these previous studies indicate that oxygenated irrigation can change the oxygen environment in the soil root zone, effectively enhance crop yield, and promote soil nitrogen transformation. However, the underlying mechanisms are unclear. The aim of this study was to investigate the effect of oxygenated water input on inorganic nitrogen content in two soil types and on nitrification and reveal the intrinsic relationship between oxygenated water input and nitrogen transformation *via* indoor experiments. This study provided a theoretical basis and enhanced the understanding of the effect of oxygenated irrigation on soil nitrogen transformation.

## Materials and methods

### Soil sample collection and processing

The air-dried soil samples were taken from the National Ash Desert Soil Fertility and Fertilizer Effectiveness Monitoring Base of the Soil Fertilizer and Agricultural Water Conservation Research Institute, Xinjiang Academy of Agricultural Sciences, Anningqu Town, Urumqi City, Xinjiang, China during 2020 – 2022 at the location of 43°49'12''N and 87°34'45''E, which is in the middle reaches of the oasis zone on the northern slopes of the Tianshan Mountains in northwestern China with a mesothermal arid and semiarid desert climate. The basic physical and chemical properties of the soil samples were shown in Table 1.

### Soli treatments

The loamy and sandy soils were thoroughly mixed, air-dried, ground at room temperature to remove stubble, passed through a sieve with 2 mm aperture, and stored. The soil mass was measured, and the soil water content was adjusted to 40% of the field water-holding capacity by adding water conventionally (spraying the soil evenly with a spraying water bottle and adjusting the soil water content to 30% of the field water-holding capacity). The soils were then incubated in dark at 25°C for 7 days with stirring once every 3 days to activate the soil microorganisms. 8 treatments including conventional water irrigation for loam soil (RCK), natural air oxygenation and aeration with oxygen enrichment for loam soil (RH1), 30% oxygen enrichment with oxygen supply and aeration for loam soil (RH2), 90% oxygen enrichment with oxygen supply and aeration for loam soil (RH3), conventional water irrigation for sandy soil (SCK), natural air oxygenation and aeration with oxygen enrichment for sandy soil (SH1), 30% oxygen enrichment with oxygen supply and aeration for sandy soil (SH2), and 90% oxygen enrichment with oxygen supply and aeration for sandy soil (SH3) were performed at 3 repeats for each treatment. The soli sample aeration was done using B&W Micro-Nano Bubble Generation Device (Benzhou New Technology Promotion Limited Company, Beijing, China) to produce micro-nano bubble water with a pressure of 0.015 MPa and an air flow rate of 1.5 L/min. The oxygen was supplied using Yuyue YU300 Oxygen Generator (Jiangsu Yuyue Medical Equipment Co., Ltd, Yancheng, Jiangsu, China). Different oxygen-enriched irrigation waters were produced through aeration by adjusting the oxygen volume according to different oxygen concentrations supplied by the oxygen generator. Fresh soil samples that equivalented to 15 g of dry soil were weighed per portion (converted by taking the same pre-cultivated soil measured moisture content) and placed in 250 mL culture bottles. Oxygenated water with various concentrations of dissolved oxygen was added to the soils to make their field water-holding capacity up to 60%. After weighing the soil, culture bottles were sealed, and holes were punched. The bottles were incubated at 25°C and humidity (adjusted by gravimetric method) in dark. The field water-holding capacity of the soils was maintained up to 60% by replenishing water at 3-day intervals to make up for the evaporation loss of water, which was determined by weighing the samples for the difference between the initial weight and current weight as the weight of water to be added. Soil samples were collected at 0, 7, 14, 35, and 49 days after incubation to determine soil $NH_4^+$-N and $NO_3^-$-N contents.

**Table 1.** Basic physical and chemical properties of the test soil samples.

| | | Type of soil tested | |
|---|---|---|---|
| | | **Sandy soil** | **Loam** |
| Particle compositions (%) | Clay particles | 0.48 | 16.48 |
| | Mealy sand | 2 | 34 |
| | Sand grains | 97.52 | 49.52 |
| pH | | 9.1 | 8.05 |
| Soluble salt content (g/kg) | | 3.7 | 2.3 |
| Total nitrogen (g/kg) | | 0.13 | 0.63 |
| Total phosphorus (g/kg) | | 0.37 | 0.72 |
| Soil total potassium (g/kg) | | 21.3 | 18.87 |
| Hydrolyzable nitrogen (mg/kg) | | 50.4 | 123.6 |
| Available phosphorus (mg/kg) | | 49.3 | 17.5 |
| Rapidly available potassium (mg/kg) | | 65 | 195 |

**Note:** Soil particle size classification was based on the United States Department of Agriculture (USDA) soil classification standard. Clay particles were < 0.002 mm. Silt particles ranged from 0.002 to 0.02 mm. Sand particles ranged from 0.02 to 2 mm.

## Determination of inorganic nitrogen content

The content of soil inorganic nitrogen (ammonium nitrogen and nitrate nitrogen) was determined using continuous flow analyzer method and SmartChem® 200 Automated Discrete Analyzer (KPM Analytics, Westborough, MA, USA) after leaching with 2 mol/L KCl following the instructions of "LY/T 1228-2015 Determination of Nitrogen in Forest Soil" (https://www.chinesestandard.net/PDF/English.aspx/LYT1228-2015).

## Data analysis

Data was processed using Microsoft Office Excel 2010 software (Microsoft, Redmond, WA, USA). SPSS 19.0 (IBM, Armonk, New York, USA) was employed for analysis of variance (ANOVA) and the Duncan multiple range test was applied to compare means with $P < 0.05$ as significant difference. Graphs were generated using Origin 19.0 (https://www.originlab.com/). The heatmap clustering visualization was performed using R (4.3.1) (https://www.r-project.org/).

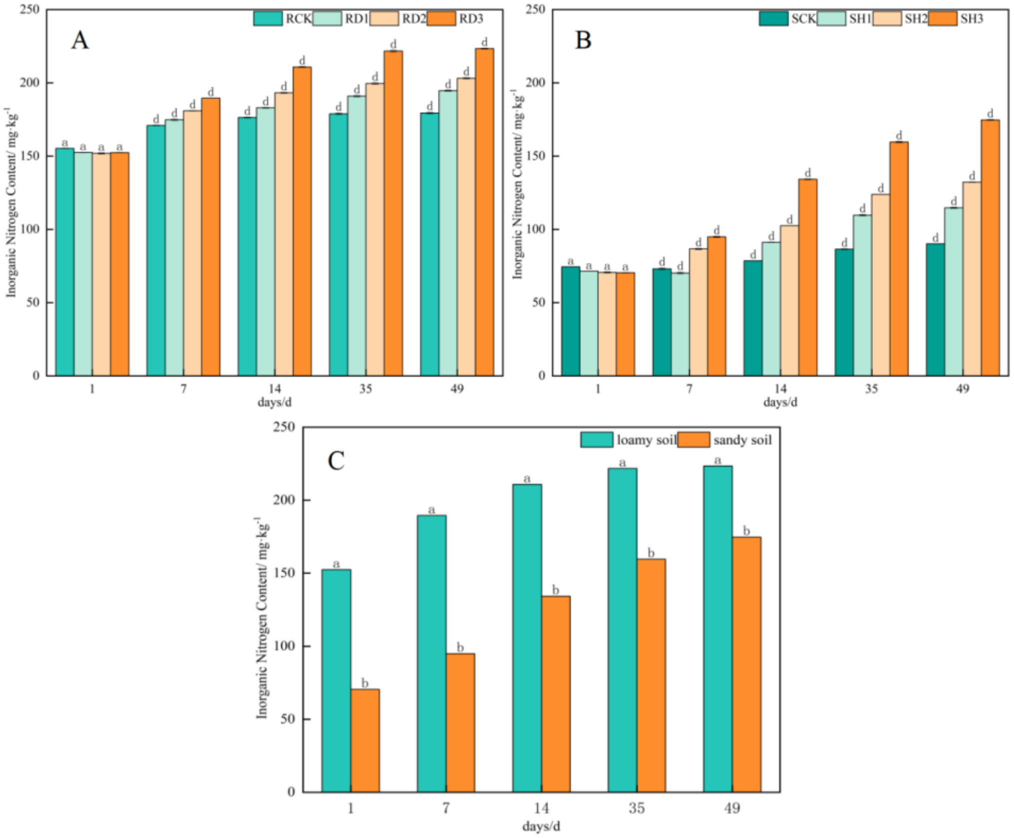

**Figure 1.** The impact of different aeration treatments on inorganic nitrogen in loamy soil (A) and sandy soil (B), as well as the influence of different soil types on inorganic nitrogen content (C).

## Results

### Analysis of soil inorganic nitrogen ($NH_4^+$-N and $NO_3^-$-N) dynamics

The dynamics of inorganic nitrogen in each treatment were shown in Figure 1. The results showed that the inorganic nitrogen content increased with time. On the 1st day of incubation, the inorganic nitrogen content of the soil samples treated with conventional water was significantly higher than that of the soils treated with oxygenated irrigation ($P < 0.05$), while, on the 7th, 14th, 35th, and 49th days of incubation, the inorganic nitrogen content of soils with oxygenation treatments was significantly higher than that of soils with conventional water treatment ($P < 0.05$). Furthermore, inorganic nitrogen content significantly increased with the increase in dissolved oxygen concentration ($P < 0.05$). On the 49th day, inorganic nitrogen content increased by 17.9, 27.1, and 46.8 mg/kg in RH3, RH2, and RH1 samples, respectively, compared with that in RCK sample (Figure 1A), and increased by 27.5, 45.9, and 88.6 mg/kg in SH3, SH2, and SH1 samples, respectively, compared with that in SCK sample (Figure 1B). The results indicated that oxygenation could significantly increase the total content of inorganic nitrogen in soil, and higher the oxygenation, higher the inorganic nitrogen content. The inorganic nitrogen contents in two different soil types were illustrated in Figure 1C. Taking the 90% aeration treatment as an example, inorganic nitrogen content of loamy soil was significantly higher than that of sandy soil during the incubation period under oxygenation treatments ($P < 0.05$), which suggested that loamy soil was more favorable for the generation of inorganic nitrogen. Similar patterns were observed in the other treatments.

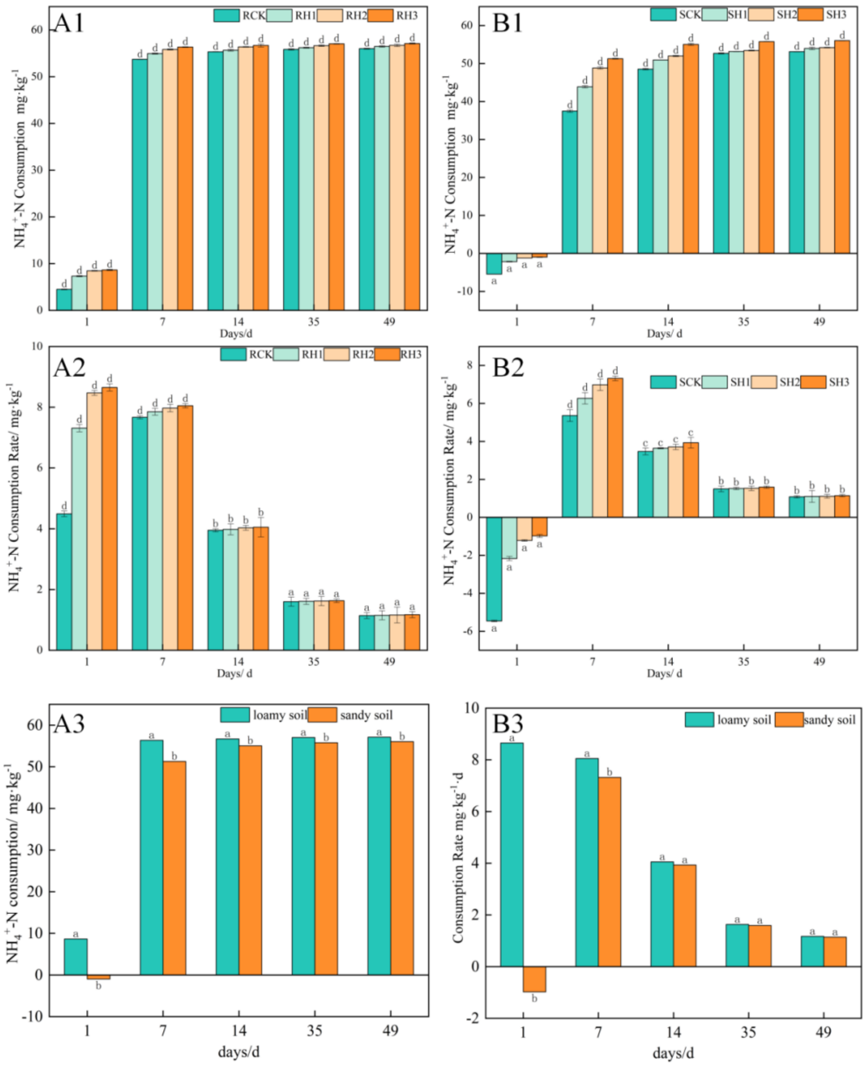

**Figure 2.** The impact of different aeration treatments on loamy soil $NH_4^+$-N consumption (A1), $NH_4^+$-N consumption rate (A2), sandy soil $NH_4^+$-N consumption (B1), $NH_4^+$-N consumption rate (B2), as well as the influence of different soil types on loamy soil $NH_4^+$-N consumption (A3), $NH_4^+$-N consumption rate (B3).

### Alterations in soil $NH_4^+$-N

The dynamics of $NH_4^+$-N consumption under each treatment were shown in Figure 2. On the 1st day of incubation, $NH_4^+$-N consumption was less in loamy soil, whereas it was increased in sandy soil. However, during 7 - 49 days, the consumption of $NH_4^+$-N tended to be stabilized in each treatment. During the whole incubation period, overall $NH_4^+$-N consumption in the samples with aeration treatments was significantly higher than that in the corresponding samples with conventional water treatment ($P < 0.05$). $NH_4^+$-N consumption increased with the increase in the oxygenation concentration. $NH_4^+$-N consumption significantly varied among the aeration treatments for each soil ($P < 0.05$). On the 49th day, total $NH_4^+$-N consumption was higher by 0.83%, 1.25%, and 1.96%, respectively, in RH3, RH2, and RH1 treatments than that in RCK treatment (Figure 2A1). It was higher by 1.57%, 2.01%, and 5.46%, respectively, in SH3, SH2, and SH1 treatments than that in SCK treatment (Figure 2B1). Overall, soil $NH_4^+$-N consumption rate was higher during 1 - 7 days than that during 14 - 49 days. During 1 - 7 days, for each soil type, the $NH_4^+$-N consumption rate of each treatment was

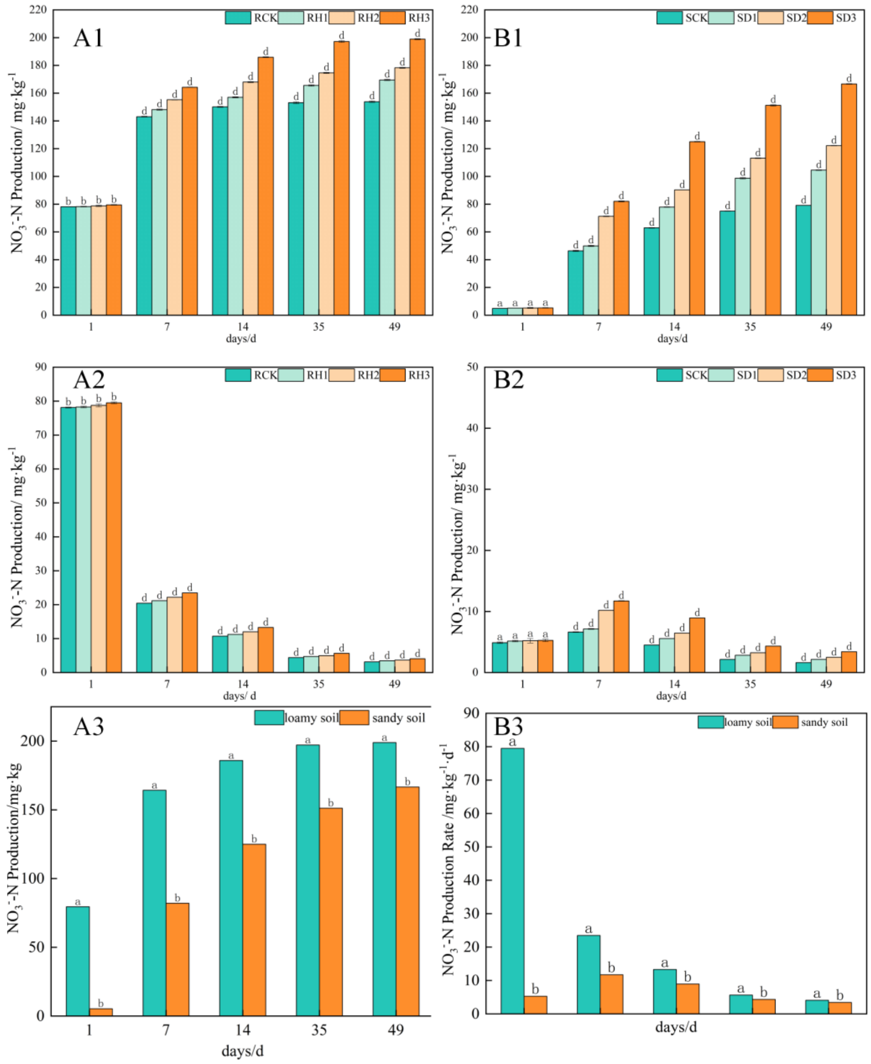

**Figure 3.** The impact of different aeration treatments on loamy soil $NO_3^-$-N production (A1), $NO_3^-$-N production rate (A2), sandy soil $NO_3^-$-N production (B1), $NO_3^-$-N production rate (B2), as well as the influence of different soil types on loamy soil $NO_3^-$-N production (A3), $NO_3^-$-N production rate (B3).

significantly different ($P < 0.05$). The $NH_4^+$-N consumption rate of the oxygenation treatments was higher than that of the corresponding conventional water treatment, and it increased with the increase in the oxygen concentration ($P < 0.05$). During 14 - 49 days, the differences in soil $NH_4^+$-N consumption rate among treatments for each soil were not significant ($P > 0.05$) (Figures 2A2 and 2B2). $NH_4^+$-N consumption was significantly higher in loamy soil than that in sandy soil ($P < 0.05$). The $NH_4^+$-N consumption rate was significantly higher in loamy soil than that in sandy soil during 1 - 7 days ($P < 0.05$) and was higher in loamy soil but not significantly different from that in sandy soil during 14 - 49 days ($P > 0.05$) (Figures 2A3 and 2B3).

### Alterations in soil nitrate nitrogen ($NO_3^-$-N)

The alterations in nitrate nitrogen ($NO_3^-$-N) content in each treatment were shown in Figure 3. On the 1st day, sandy soil exhibited less $NO_3^-$-N production. However, no significant difference in $NO_3^-$-N production was observed between the two soil types. During 7 - 49 days, $NO_3^-$-N production tended to be stabilized in each treatment. During the whole incubation period,

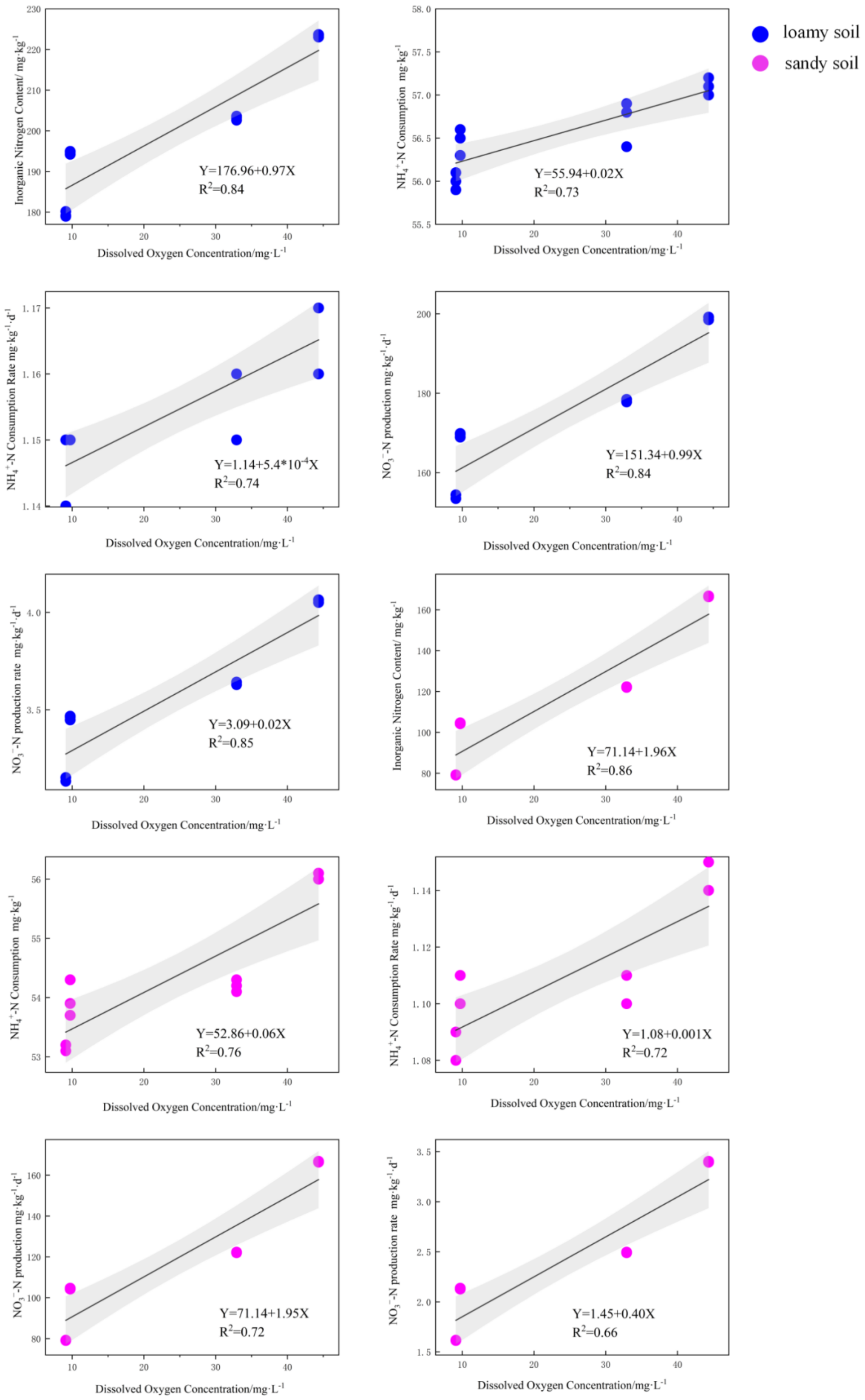

**Figure 4.** Correlation analysis of oxygen concentration with other indicators.

for both soils, overall $NO_3^-$-N generation was significantly higher in the samples with oxygenation treatments than in those with conventional water treatment ($P < 0.05$). $NO_3^-$-N generation significantly increased with the increase in the concentration of oxygen ($P < 0.05$). On the 49th day, $NO_3^-$-N generation was higher by 10.19%, 15.88%, and 29.33% in RH3, RH2, and RH1 treatments, respectively, than that in RCK treatment. It was higher by 16.51%, 27.97%, and 56.86% in SH3, SH2, and SH1 treatments, respectively, than that in SCK treatment (Figures 3A1 and 3B1). In loamy soil, the $NO_3^-$-N generation rate was higher on the 1st day than that during 7 - 49 days. However, sandy soil exhibited less variability in terms of $NO_3^-$-N generation rate throughout the incubation period. Overall, for both soils, the $NO_3^-$-N generation rate was significantly different among treatments with aerated treatments exhibiting higher $NO_3^-$-N generation rate than conventional water treatment ($P < 0.05$). Moreover, it significantly increased with the increase in oxygen concentration ($P < 0.05$) (Figures 3A2 and 3B2). During the incubation period, loamy soil exhibited significantly higher $NO_3^-$-N generation rate than that in sandy soil ($P < 0.05$) (Figures 3A3 and 3B3).

**Correlation analysis**

Oxygen concentration, as the most important factor, is related with the contents of inorganic nitrogen, ammonium nitrogen, and nitrate nitrogen. Dissolved oxygen concentration exhibited a linear relationship with other indicators (Figure 4). For the correlation between dissolved oxygen concentration and inorganic nitrogen content, ammonium nitrogen content, ammonium nitrogen consumption rate, nitrate nitrogen generation, and nitrate nitrogen generation rate, $R^2$ was 0.84, 0.73, 0.74, 0.84, and 0.85 in loamy soil and 0.86, 0.76, 0.72, 0.72, 0.72, and 0.66 in sandy soil, respectively. A good fitting relationship existed between dissolved oxygen concentration and other indicators in both soils, which indicated that dissolved oxygen concentration could effectively reflect the indicators related to soil inorganic nitrogen and played a significant role in regulating the transformation of soil inorganic nitrogen.

**Comprehensive evaluation**

In the context of diverse soil types, following a 49-day cultivation with oxygenation treatment, the inorganic nitrogen indicators underwent cluster heatmap analysis (Figure 5). The color variations in the heatmap provided an intuitive representation of the data's magnitude and differences. In loam conditions, the four treatments were segregated into two groups including RCK and RD3 clustered together, while RD2 and RD3 were in closer proximity, forming a distinct cluster (Figure 5A). Notably, the RD3 treatment exhibited significant performance in inorganic nitrogen content, $NO_3^-$-N production, and $NO_3^-$-N production rate. RD2 demonstrated superior performance in $NH_4^+$-N consumption and consumption rate, while RD1 exhibited higher $NH_4^+$-N consumption. The results suggested that the overall elevation in oxygen concentration facilitated the transformation of inorganic nitrogen in loam, particularly by expediting $NO_3^-$-N production and promoting nitrification. In sandy soil conditions, the four treatments were segregated into two groups including SCK, SD3, and SD1 clustered together, while SD2 stood apart, forming an independent cluster (Figure 5B). Specifically, SCK, SD3, and SD1 treatments demonstrated superior performance in $NH_4^+$-N consumption, which suggested that the heightened oxygen concentration facilitated the conversion of inorganic nitrogen in sandy soil, particularly by expediting $NH_4^+$-N consumption and promoting nitrification.

## Discussion

**Effect of oxygenated irrigation on soil inorganic nitrogen content**

Soil inorganic nitrogen is the main form of nitrogen taken up by crops. Soil inorganic nitrogen content directly determines the availability of nitrogen supply to crops [15, 16]. According to the research results, under indoor cultivation conditions, oxygenated irrigation

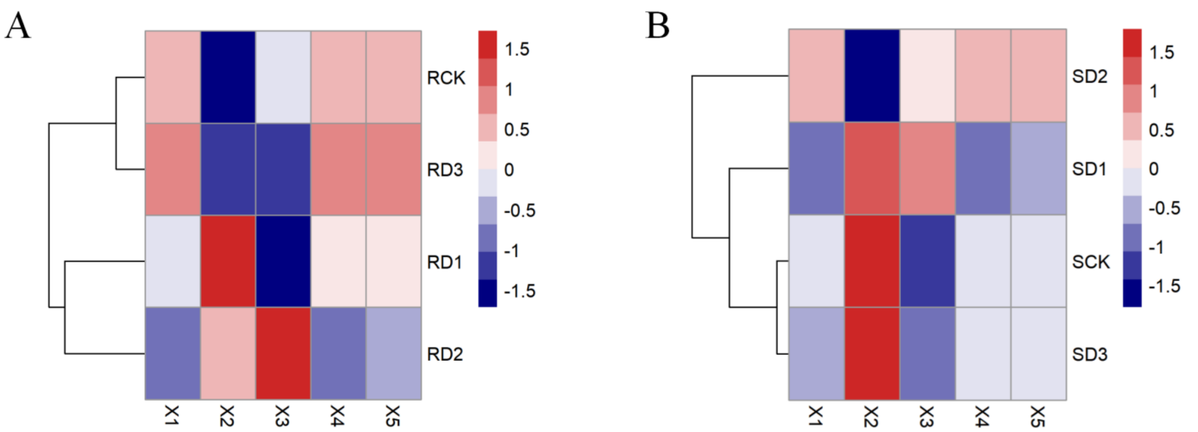

**Figure 5.** Inorganic nitrogen indicators clustering heatmap in different aeration treatments (A: loamy soil. B: sandy soil). X1: inorganic nitrogen content. X2: $NH_4^+$-N consumption. X3: $NH_4^+$-N consumption rate. X4: $NO_3^-$-N production. X5: $NO_3^-$-N production rate.

improved the soil's inorganic nitrogen content, which increased with the increase in dissolved oxygen concentration. This is consistent with a previous study [17]. Soil aeration plays a very important role in regulating soil nitrogen migration and transformation and even the nitrogen cycling process in the whole ecosystem. The irrigation–evapotranspiration process in farmland soil always keeps the soil environment in alternating wet and dry states. Application of excessive water to soil disperses soil oxygen, causing water and air imbalance in soil and reducing soil aeration, which inhibits nutrient uptake by the crop root system, affecting its growth and reducing crop yield [18-20]. Inorganic nitrogen generation in soil involves the participation of soil microorganisms. Oxygenated water indirectly affects nitrogen mineralization and aerobic microbial activity by regulating the input concentration and diffusion of oxygen in soil. The more the oxygen content, the more the microbial fixation of inorganic nitrogen [21, 22]. In this study, inorganic nitrogen content was significantly higher in loamy soil than that in sandy soil under various treatments ($P < 0.05$). Moreover, with the increase in dissolved oxygen concentration, the inorganic nitrogen content increased to a higher extent in loamy soil than in sandy soil, which could be because of following reasons. Loamy soil usually has higher nutrient retention capacity, higher content of clay particles, and more fine pores, which can adsorb and retain more inorganic nitrogen compounds. However, sandy soil has larger pore structure, and water and nutrients are more easily removed from the soil. This may result in lower inorganic nitrogen content in sandy soil. In addition, soil organic nitrogen content is closely positively correlated with the organic matter content. The content of organic nitrogen in soil and its chemical form determines the amount of nitrogen that can be mineralized by soil, rate of mineralization, and soil's ability to supply nitrogen [23]. Research has shown that sandy soil contains less organic matter (approximately 2.16 g/kg), which, in turn, affects the transformation of soil nitrogen.

### Effect of oxygenated irrigation on soil nitrification

Researcher proposed the process of soil nitrification, that was, the process of oxidizing ammonium nitrogen into nitrate nitrogen in soil under the action of nitrifying microorganisms [24]. In this process, soil nitrate nitrogen was transformed into ammonium nitrogen by microorganisms conducting nitrification. The results indicated that, during the incubation period, ammonium nitrogen consumption was accompanied by the generation of a large amount of nitrate nitrogen, and ammonium nitrogen consumption under various oxygenation treatments was much lower than nitrate nitrogen generation. With the increase in oxygenation concentration, nitrate nitrogen content increased. Therefore, it was concluded that the impact of oxygenated irrigation on the migration and transformation of soil inorganic nitrogen is mainly based on nitrification. In this study, the rates of $NH_4$+-N consumption and $NO_3^-$-N production were higher in the initial days than that in the later days in loamy soil. This may be because of the initial nitrogen mineralization process, which converts the nitrogen released from the decomposition of soil organic matter into $NH_4^+$-N in loamy soil. At the initial stage, the decomposition of organic matter in soil occurred more actively, resulting in higher rates of $NH_4^+$-N consumption and production. As time advanced, the rate of organic matter decomposition gradually slowed down, resulting in a decrease in the rate of $NH_4^+$-N consumption and $NO_3^-$-N production. This was observed in sandy soil as well. However, the rates were significantly lower than those in loamy soil ($P < 0.05$). In this study, $NH_4^+$-N consumption rate and $NO_3^-$-N generation rate was promoted as dissolved oxygen concentration increased. Many studies have reported that water input with suitable oxygen levels can significantly increase the number of soil microorganisms and improve the activity of microorganisms oxidizing ammonia [25, 26]. Changing the soil water : air ratio and effectively increasing soil aeration and soil redox potential can effectively improve the soil inter-root oxygen environment and promote uptake of inorganic N by crops [27, 28]. High correlation existed between oxygen concentration and inorganic nitrogen content, ammonium nitrogen consumption, ammonium nitrogen consumption rate, nitrate nitrogen production, and nitrate nitrogen production ($R^2 > 0.7$). The results were consistent with many previous studies [29, 30]. This study suggested that irrigation with various oxygen concentrations was beneficial to improve soil nitrogen migration and transformation. Irrigation with 90% oxygen exhibited the best effect. Crop roots absorb soil nitrogen more easily in nitrate nitrogen form. Oxygenated irrigation could accelerate nitrification and conversion of ammonium nitrogen to nitrate nitrogen, improve the supply of nitrate nitrogen in soil, and promote the nitrogen use and absorption by roots. The results of this study are important since only a few studies are available on the transformation and migration of soil inorganic nitrogen under oxygenated irrigation. Future studies should explore the mechanism of soil nitrogen transformation by oxygenated irrigation in detail by assessing the intrinsic mechanisms involving microbial function and community structure.

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