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*Original Research*

# Response of Soil-Crop System and Adaptive Changes of Microbial Communities under Different Water Quality Irrigation

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## Abstract

This study used natural water (NW), reclaimed water from wastewater treatment plants (RW), Erhai Lake water (EH), and reclaimed water receiving water (RR) as irrigation sources. Through a 56-day irrigation experiment, the effects of different water quality irrigation on soil physicochemical properties, cabbage growth, and soil bacterial community structure were systematically evaluated. The results showed significant differences in water quality characteristics among the four types of water bodies. Furthermore, after irrigation, the RW and RR treatments led to significant accumulation of soil nitrate nitrogen and sodium salts. The RW and RR treatments promoted the growth of the aboveground biomass of cabbage but inhibited root development, while the NW treatment favored root construction and balanced growth. The diversity of soil bacterial communities was generally suppressed in the early stages of irrigation. By the end of the experiment, Chloroflexi and Acidobacteria had become the dominant phyla in all treated soils. This study reveals the positive role of reclaimed water irrigation in providing water and nutrients in the short term and in promoting aboveground crop growth, but it also highlights potential risks such as soil salt accumulation, nitrogen pollution, and restricted root development. This research provides a scientific basis for the short-term safe use of reclaimed water in agriculture.

**Keywords:** reclaimed water, irrigation, soil properties, biomass, microbial communities

## Introduction

At global and regional scales, the increasingly acute contradiction between water scarcity and food security demands has been driving the intensive development and recycling of various available water resources [1]. This challenge is particularly prominent in ecologically sensitive plateau lake basins, where the pressure to balance agricultural water use and lake ecosystem protection is immense [2]. Against this backdrop, the traditional model of relying solely on high-quality

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natural water sources has become unsustainable. Various potential water sources within the basin, including natural water bodies, lake water, treated wastewater from sewage treatment plants, and receiving water bodies for reclaimed water, are widely used for agricultural irrigation [3, 4]. Among these, reclaimed water, as a reliable reused water source, has become a key strategy for achieving sustainable water resource management in agricultural irrigation.

In recent years, research on reclaimed water irrigation has evolved from early feasibility assessments to an in-depth exploration of the multi-process impact mechanisms on soil, crops, and microbial systems [5]. Studies indicate that reclaimed water irrigation typically enhances crop yield, primarily due to its continuous supply of nutrients [6]. However, long-term or improper use may lead to increased soil electrical conductivity and sodium adsorption ratio, posing risks of secondary salinization [7], damage to soil structure, and inhibition of crop growth [8]. Simultaneously, high nutrient inputs may exacerbate water eutrophication through leaching or runoff, creating nonpoint source pollution concerns [9]. In terms of crop response, research has begun focusing on plants' adaptive strategies to water and nutrient conditions, particularly the trade-off relationship between aboveground and root biomass allocation, which is key to assessing long-term crop health and stress resistance [5]. In the field of microbial ecology, reclaimed water input has been shown to significantly alter the structure and diversity of soil bacterial communities [10], initially stimulating the growth of eutrophic bacterial groups, but the long-term succession trends and community resilience remain debated [11]. Although significant progress has been made, most studies focus on the long-term effects of single factors, lacking systematic comparisons of short-term comprehensive impacts across different water quality gradients, such as natural water bodies, treated reclaimed water, mixed receiving water bodies, and eutrophic lake water samples. There is also a notable gap in mechanistic analysis coupling dynamic soil physicochemical indicators, crop biomass allocation strategies, and microbial community responses.

The Erhai Lake Basin in Dali, China (25°41′N, 100°18′E) is an important agricultural production area and ecological conservation zone, characterized by the coexistence of highly intensive agricultural practices and ecological sensitivity [12]. The basin's agriculture primarily focuses on high-value vegetables (e.g., cabbage, garlic) and specialty cash crops (e.g., flue-cured tobacco, flowers), which are heavily dependent on and require large quantities of irrigation water [13]. Meanwhile, Erhai Lake, a key protected plateau freshwater lake in China, faces the core challenge of eutrophication control as a critical constraint for regional development [14]. Agricultural nonpoint source pollution is considered one of the primary contributors to eutrophication in Erhai Lake [15]. Consequently, water resource management in the basin has long been caught in a dilemma between the demand for increased agricultural production and the rigid constraints of lake protection. Against this backdrop, improving water use efficiency and optimizing irrigation water source structures have become key breakthroughs. This study examined four typical water sources – natural water (NW), reclaimed water from wastewater treatment plants (RW), Erhai Lake water (EH), and water bodies receiving reclaimed water (RR) – to evaluate the effects of water quality on soil physicochemical properties, cabbage growth, and soil bacterial community structure, as well as their coupling relationships. The research aims to clarify the short-term resource benefits and associated soil environmental risks of irrigation with different water qualities, reveal the biomass allocation strategies of crop growth and the ecological adaptability of microbial communities, and provide theoretical support for the sustainable use of diverse irrigation water qualities in plateau lake basins and other ecologically fragile regions.

## Materials and Methods

### Sampling of Irrigation Water for Testing

The natural water (NW) used in this study was collected from the rain garden on Yingxue Road, Shanghe Village, Dali City, China (100°12'48.1"E, 25°39'49.8"N). The project covers an area of approximately 23,313 m², with a water area of about 10,459 m². Its functions include stormwater regulation, water conservation, and rainwater purification. Erhai Lake water (EH) was collected from the shore of Erhai Lake in Xiahe Village, Dali City (100°12'28.8"E, 25°36'49.3"N). Erhai Lake is the seventh-largest freshwater lake in China and the second-largest plateau lake in Yunnan, serving as the ecological core of the Dali Basin. It has a watershed area of 2,565 km² and a water area of 249 km². The reclaimed water (RW) used in this study was collected from the sunken reclaimed water plant in Shuanglang Town, Dali, China (25°56′51.79″N, 100°11′24.46″E). The plant has a treatment capacity of $0.5\times10^4$ m³/d, covers an area of 10,247 m², serves an area of 3.03 km², and supports a population of 47,200. The treated reclaimed water is discharged to the Lihua Tan Reservoir in Shuanglang Town, Dali City. The recycled water reuse (RR) used in this study was collected from the post-treatment purification pond of Lihua Tan Reservoir (25°54'33.4"N, 100°11'17.8"E), with a water area of approximately 26,612 m². The reclaimed water, after purification in the reservoir, is used for agricultural irrigation and landscape greening in the surrounding areas.

### Experimental Design of Different Water Bodies for Agricultural Irrigation

The test soil was collected from the 1-30 cm cultivated layer in Xizhou Town, Dali City, Yunnan

Province. The collected soil was air-dried, crushed, and sieved (2 mm) for later use. The specifications of the test pots were 14 cm in upper diameter, 11 cm in lower diameter, and 10.8 cm in height. Each pot was filled with 1 kg of pre-treated soil. Compound fertilizer ($N:P_2O_5:K_2O$ = 1:1:1) was applied as a base fertilizer in all pots at a rate of 1 g/kg (the fertilization rate followed local farmers' standards). The test crop was cabbage. Before sowing, all treatments were irrigated with pure water to create moisture. The irrigation water quality included four treatments: reclaimed water from a wastewater treatment plant (RW), Erhai Lake water (EH), natural water (NW), and water from bodies receiving reclaimed water (RR), with four replicates for each treatment. The collected soil underwent a 56-day irrigation treatment. The experiment began on May 22, 2024, in an indoor laboratory with one cabbage seedling planted per pot. Different water sources were used for irrigation treatments. Initially, watering was performed every other day. Later, as the crop's water demand increased, the frequency was adjusted to daily watering, with the same amount of water applied each time.

## Determination of Physicochemical Indicators of Water Quality

On-site measurements of water samples were conducted using a portable multi-parameter water quality analyzer and a portable conductivity meter for temperature (T), pH, and dissolved oxygen (DO). Suspended solids (SS) were determined by the gravimetric method. Total nitrogen (TN) in water was measured using the alkaline potassium persulfate digestion-UV spectrophotometric method. Ammonia nitrogen ($NH_4^+$-N) was determined by the Nessler's reagent spectrophotometric method. Nitrate nitrogen ($NO_3^-$-N) and nitrite nitrogen ($NO_2^-$-N) were measured using spectrophotometry. Total phosphorus (TP) was analyzed by the ammonium molybdate spectrophotometric method. Total organic carbon (TOC) was measured using a total organic carbon analyzer. Chemical oxygen demand (COD) was determined by the rapid digestion method. All measurement methods followed the "Environmental Quality Standards for Surface Water" (GB 3838-2002) issued by the Ministry of Ecology and Environment of China. Sodium ions ($Na^+$), potassium ions ($K^+$), magnesium ions ($Mg^{2+}$), and calcium ions ($Ca^{2+}$) in water were measured using inductively coupled plasma optical emission spectrometry (ICP-OES).

## Determination of Soil Physicochemical Indicators

Soil samples were collected before the start of the experiment, on day 28 of the experiment, and after the experiment (i.e., day 56). The soil samples were air-dried, crushed, sieved (2 mm), and stored for later use. Referring to soil agrochemical analysis, soil moisture content was determined using the oven-drying method; a soil-water ratio of 1:5 was prepared for soil extract, and the electrical conductivity (EC) of the soil extract was measured using a conductivity meter; a soil-water ratio of 1:2.5 was prepared for soil extract, and the pH of the soil extract was measured using a pH meter; total nitrogen (TN) was determined using the Kjeldahl method; ammonium nitrogen ($NH_4^+$-N) and nitrate nitrogen ($NO_3^-$-N) were determined using the potassium chloride extraction-spectrophotometry method; total phosphorus (TP) was determined using the alkali fusion-molybdenum antimony anti-spectrophotometry method; available phosphorus (AP) was determined using the sodium bicarbonate-molybdenum antimony anti-spectrophotometry method; organic matter (OM) was determined using the loss-on-ignition method; available potassium (AK) was determined using the ammonium acetate extraction-atomic absorption spectrometry method; sodium ion ($Na^+$), potassium ion ($K^+$), magnesium ion ($Mg^{2+}$), and calcium ion ($Ca^{2+}$) in the soil were measured using the ammonium acetate extraction-inductively coupled plasma atomic emission spectroscopy (ICP-AES) method.

## Plant Growth Indicators

Crop yield and dry matter were measured using the gravimetric method. After harvesting the cabbage, the above-ground and underground parts were separated. Each part was rinsed with ultrapure water and air-dried, after which the fresh weight of the above-ground and underground parts of the cabbage was measured separately using a balance. The cabbage parts were first deactivated at 105ºC in a constant-temperature oven for 15 min, then dried at 75ºC to a constant weight. The dry weight of the above-ground and underground parts of the cabbage was calculated separately using a balance.

## Microbial Community Composition Analysis

To analyze the microbial community diversity of different irrigation water and soil samples, water samples were filtered through 0.22 μm mixed cellulose ester membranes and stored at a -80ºC ultra-low temperature freezer until DNA extraction. Soil samples were collected using sterile metal scrapers and stored at a -80ºC ultra-low temperature freezer until DNA extraction. Initial soil samples (O) were collected as controls, along with reclaimed water from the wastewater treatment plant (RW1), Erhai Lake water (EH1), natural water (NW1), and reclaimed water receiving water (RR1) before the experiment began. On day 28 of the experiment, soil samples irrigated with reclaimed water from the wastewater treatment plant (RW2), Erhai Lake water (EH2), natural water (NW2), and reclaimed water receiving water (RR2) were collected. On day 56 of the experiment, soil samples irrigated with reclaimed water from the wastewater treatment plant (RW3), Erhai Lake water (EH3), natural water (NW3), and reclaimed water receiving water (RR3) were collected. Microbial DNA was extracted using the FastDNA® Spin Kit for Soil (FastDNA® Spin Kit for Soil, USA), and the extracted DNA was used for quality testing, amplification, purification, and sequencing. Universal primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used for PCR amplification of the bacterial 16S rRNA gene [16], and the amplified products were recovered and purified. Biological sequencing was performed on the Illumina MiSeq PE250 platform at Majorbio Biotechnology Co., Ltd. (Shanghai, China).

Using Python software (version 3.6.13), the raw data of each sample was obtained by demultiplexing based on barcodes, followed by the removal of barcodes and primers. The R1 and R2 sequence data were then assembled using FLASH software (version 1.2.11). The assembled Tags were quality-controlled using Fastp (version 0.23.1) to obtain Clean Tags. Chimeric sequences were filtered out using Cutadapt software (version 3.3), yielding valid data for subsequent analysis. To study the species composition of each sample, effective sequences from all samples were clustered into OTUs at 97% similarity. The resulting OTU sequences were then annotated for species identification. To obtain taxonomic information for each OTU, the RDP classifier Bayesian algorithm was used to cluster representative sequences at 97% similarity, and the Silva database (version 138.1) was employed for species annotation of OTU sequences. The alpha diversity indices of microbial communities were calculated using MOTHUR software (version 1.30.2). The raw data of this study have been deposited in the NCBI database (Project ID: PRJNA1314383).

## Results

### Physicochemical Indicators of Water Quality for Different Irrigation Water Sources

According to the water quality data analysis, the four types of water bodies exhibit distinct pollution gradients. Natural water (NW) shows excellent performance across all indicators, with particularly low concentrations of nitrogen, phosphorus, and organic matter. Its ionic composition is dominated by calcium and magnesium, consistent with typical freshwater characteristics. In stark contrast, reclaimed water from wastewater treatment plants (RW) faces core issues, including severe nitrogen pollution (total nitrogen as high as 15.12 mg/L, primarily nitrate nitrogen) and elevated phosphorus levels, alongside significant enrichment of potassium and sodium ions. This reflects the typical residual inorganic salts and nutrients in treated wastewater. Erhai Lake water (EH) displays a distinct "endogenous pollution" pattern. It has higher concentrations of dissolved oxygen, suspended solids, organic matter, and total phosphorus, indicating

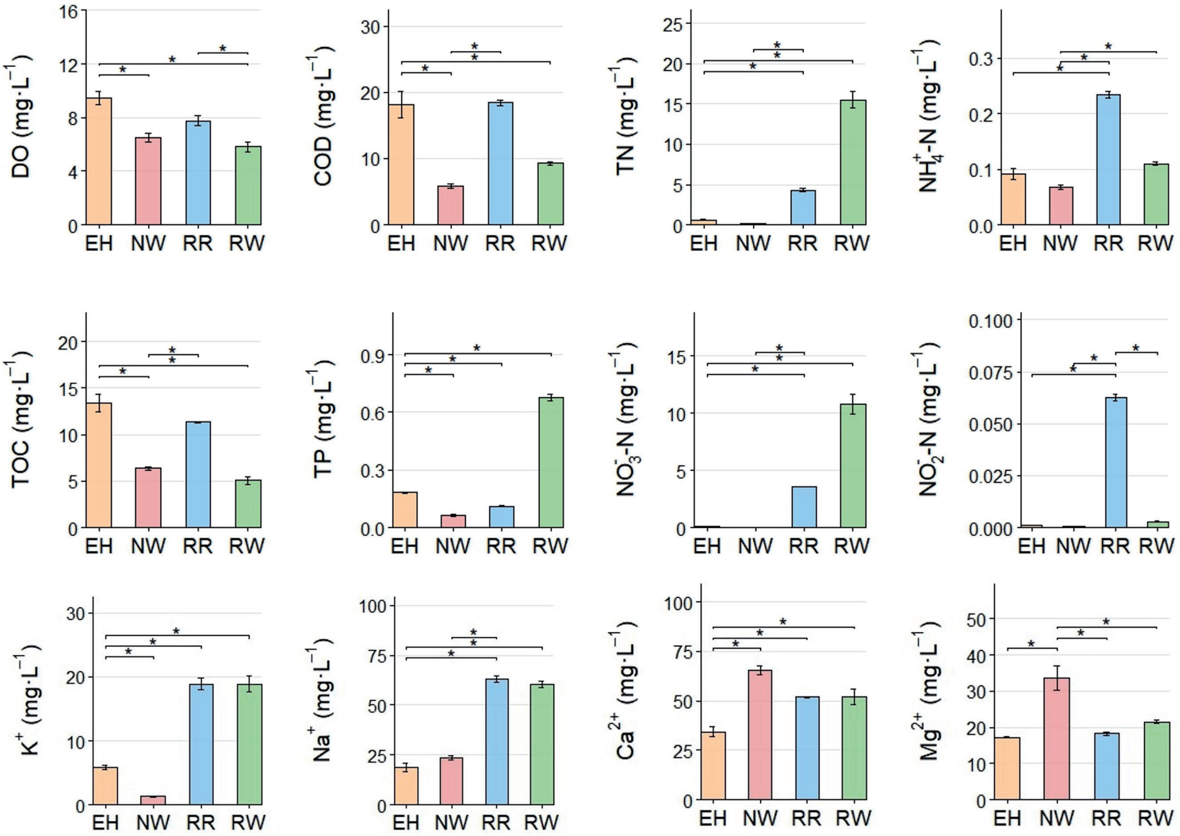

Fig. 1. Water quality analysis of different irrigation water sources. (One-way ANOVA; *$p<0.05$; **$p<0.01$.).

potential algal proliferation and eutrophication risks – a finding consistent with current research findings on Erhai Lake [17]. The receiving water body of reclaimed water (RR) clearly reflects the mixing and influence of reclaimed water discharge. Parameters like total nitrogen, nitrate nitrogen, and potassium/sodium ion concentrations are significantly higher than in natural water but lower than in reclaimed water, with abnormal accumulation of nitrite nitrogen. This may indicate active nitrogen transformation processes (nitrification-denitrification) in the water body (Fig. 1).

## The Impact of Reclaimed Water Irrigation on Soil Physicochemical Properties

Different water qualities for irrigation significantly affect soil nitrogen dynamics, with the input of reclaimed water (RW) and its receiving water (RR) leading to notable nitrate accumulation. By day 28 of the experiment, the soil nitrate content in the RW and RR groups surged to 102.6 mg/kg and 108.5 mg/kg, respectively, far exceeding that of Erhai Lake water (EH) at 34.9 mg/kg and natural water (NW) at 26.1 mg/kg. Although the levels decreased by day 56, they remained relatively high (RW 29.1 mg/kg, RR 11.5 mg/kg). This indicates the migration of high-concentration nitrate from reclaimed water into the soil.

The sodium ion ($Na^+$) content in the RW and RR groups reached 254 mg/kg and 257 mg/kg by day 56, significantly higher than the initial value (76 mg/kg) and other groups, showing a clear trend of sodium salt accumulation. Correspondingly, the soil moisture content in the RR group remained consistently high (reaching 45.9% by day 56), possibly due to the ion effect altering soil structure. Additionally, the RR group exhibited prominent calcium ion ($Ca^{2+}$) accumulation (reaching 4.1 g/kg by day 56). These data suggest that irrigation with reclaimed water and its receiving water introduces significant salt input (Table 1).

## Effects of Different Water Quality Irrigation on the Biomass of Cabbage

For the above-ground growth of cabbage, the reclaimed water treatment group showed a promoting effect. The fresh weight of above-ground parts was highest in the reclaimed water (RW) and its receiving water (RP) irrigation groups (26.73 g and 23.96 g, respectively), significantly higher than that of natural water (21.03 g) and Erhai Lake water (21.41 g). The dry weight of above-ground parts also followed the same trend: RW (2.18 g) > RP (2.09 g) > NP (1.76 g) > EH (1.75 g). This indicates that reclaimed water rich in nitrogen nutrients effectively promoted the vegetative growth of crop above-ground parts in the short term.

For below-ground (root) growth, natural water treatment was more conducive to root development. The fresh weight (2.18 g) and dry weight (0.87 g) of below-ground parts were the highest in crops irrigated with natural water (NW). In contrast, the root biomass under reclaimed water (RW) irrigation was the lowest (fresh weight 1.34 g, dry weight 0.37 g). The root

Table 1. Effects of reclaimed water irrigation on soil physicochemical properties.

| Sample | Control group | RW | | EH | | NW | | RR | |
|---|---|---|---|---|---|---|---|---|---|
| Time | d0 | d28 | d56 | d28 | d56 | d28 | d56 | d28 | d56 |
| MC | 36.9±1.2 | 40.3±4.8a | 39.1±7.5a | 38.2±1.1a | 38.3±2.8a | 38.5±3.1a | 37.9±5.7a | 46.3±4.1a | 45.9±3.7a |
| EC | 61±1 | 364±21a | 215±27a | 231±24b | 122±24c | 229±21b | 104±27c | 351±24a | 194±37b |
| pH | 7.0±0.01 | 6.8±0.04b | 7.1±0.13b | 6.9±0.06b | 7.4±0.17a | 7.0±0.06a | 7.4±0.08ab | 6.8±0.05b | 7.2±0.21ab |
| TN | 1.6±0.04 | 1.4±0.07b | 1.8±0.1a | 1.5±0.05ab | 1.7±0.04ab | 1.4±0.05ab | 1.7±0.2ab | 1.5±0.07a | 1.6±0.07b |
| TP | 1.6±0.06 | 1.7±0.08a | 2.6±0.11a | 1.7±0.07a | 2.7±0.08a | 1.6±0.06a | 2.6±0.10a | 1.7±0.13a | 2.6±0.03a |
| OM | 15.3±0.1 | 15.0±0.3a | 14.9±0.6a | 14.9±0.1a | 14.3±0.2a | 14.9±0.4a | 14.4±0.3a | 14.9±0.2a | 14.3±0.2a |
| AP | 24.4±2.8 | 26.5±3.7a | 26.2±2.3a | 25.2±3.5a | 24.7±3.5a | 27.4±2.1a | 25.1±2.1a | 26.6±2.3a | 27.5±2.6a |
| AK | 37.4±4 | 49.1±15ab | 64.5±23a | 77.3±24a | 43.4±30ab | 71.5±18a | 20.9±3b | 28.3±3b | 25.1±8ab |
| $NH_4^+$-N | 2.0±0.3 | 0.9±0.2ab | 7.8±1.2a | 0.6±0.4b | 6.7±1.6a | 1.4±0.2a | 8.9±1.8a | 1.2±0.6ab | 6.7±1.5a |
| $NO_3^-$-N | 0.7±0.1 | 102.6±12a | 29.1±13a | 34.9±8b | 10.8±3ab | 26.1±14b | 10.3±7b | 108.5±22a | 11.5±2ab |
| $K^+$ | 65.3±7 | 61.1±13a | 72.8±8a | 61.3±9a | 61.6±10a | 46.7±4a | 54.9±2b | 59.4±6a | 57.9±2b |
| $Na^+$ | 76±8 | 241±17a | 254±30a | 115±17b | 133±31b | 104±24b | 142±23b | 245±53a | 257±29a |
| $Ca^{2+}$ | 2.7±0.3 | 2.3±0.3ab | 2.8±0.3b | 2.4±0.2b | 3.4±0.3ab | 1.9±0.2ac | 1.9±0.2a | 4.1±0.7a | 4.1±0.4a |
| $Mg^{2+}$ | 256±24 | 499±78a | 383±39a | 442±90a | 384±48a | 404±32a | 453±84a | 468±68a | 436±35a |

Note: Moisture content (MC) and organic matter (OM) are in %, total nitrogen, total phosphorus, $NO_3^-$–N, and $Ca^{2+}$ are in g/kg, and the units for all other indicators are mg/kg.

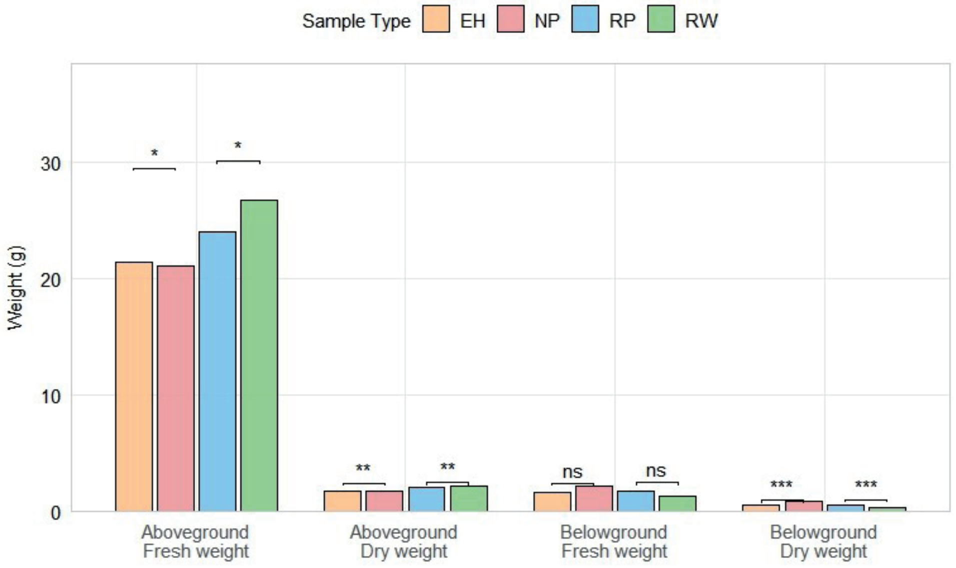

Fig. 2. Changes in biomass of cabbage under different water quality irrigation. (One-way ANOVA; *p<0.05; **p<0.01.).

biomass under receiving water (RP) irrigation (dry weight 0.53 g) was also lower than that under NW and EH treatments (Fig. 2). The RW/RR treatment group exhibited a combination of above-ground growth advantage and below-ground growth disadvantage. The NW treatment group showed a pattern of "robust above-ground growth and well-developed below-ground parts". The EH treatment group had all biomass indicators at intermediate levels, showing neither extreme promotion nor inhibition effects.

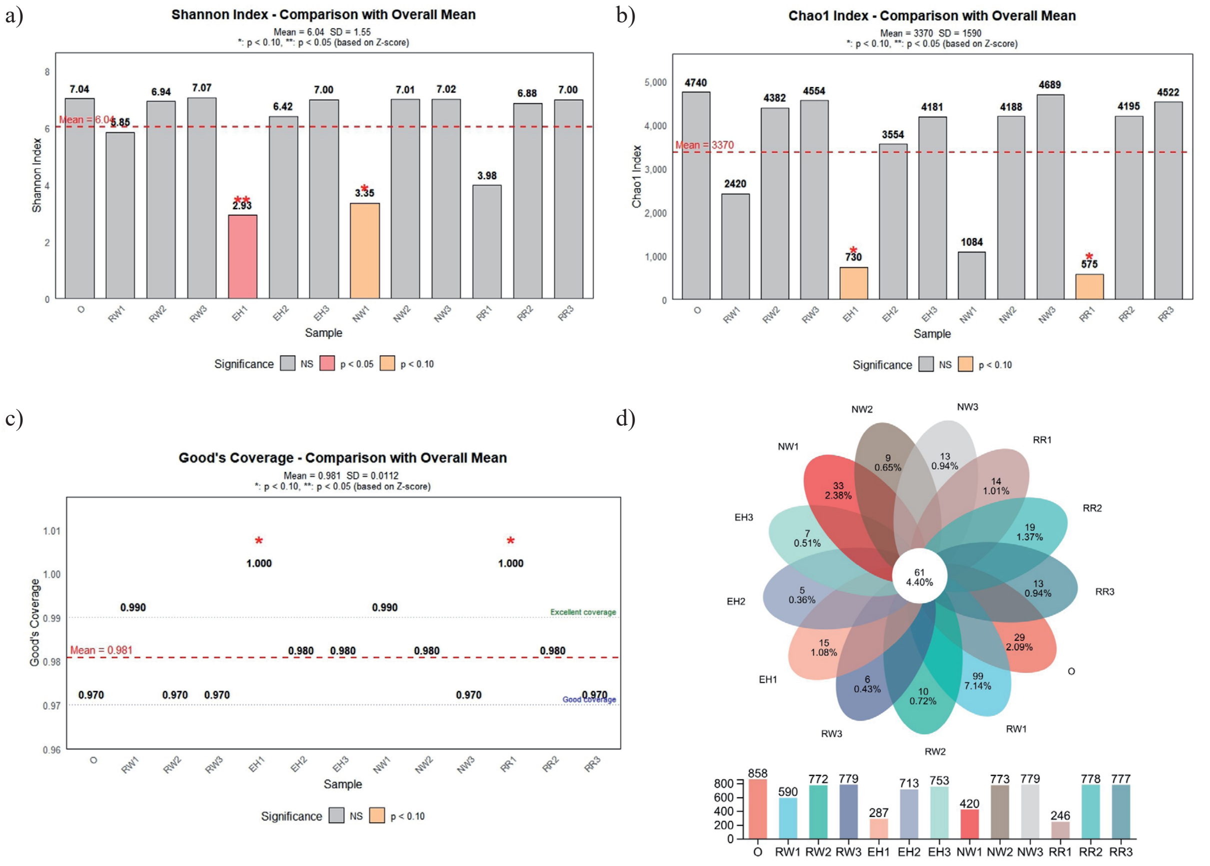

Fig. 3. Microbial community status after irrigation with different water qualities. a) Shannon index trend. b) Chao1 index trend. c) Good's coverage index trend. d) Genus-level microbial abundance trend.

## Effects of Different Water Samples on Bacterial Communities

The soil samples from reclaimed water treatment (RW) maintained relatively high microbial diversity at all three time points (RW1, RW2, RW3), without significant fluctuations. The patterns of change in Erhai Lake water and natural water treatment (EH and NW) were similar, both showing a trend of "sharp decline initially, followed by rapid recovery". The change pattern of reclaimed water receiving water treatment (RR) fell between RW and EH/NW, with lower diversity at the initial stage (RR1) but a faster recovery rate compared to EH1 and NW1. By day 56 (RR3), it had reached a very high level (Fig. 3a) and 3b)).

A total of 61 microbial genera were identified across all 13 samples. However, each treatment group harbored a considerable number of unique species at different time points. Notably, RW1 exhibited an exceptionally high count of unique genera, reaching 99, far exceeding any other sample. The microbial specificity of the reclaimed water treatment (RW) showed significant temporal variation, with the number of unique genera sharply decreasing from 99 in RW1 to just 6 in RW3. The natural water treatment (NW) and Erhai water treatment (EH) displayed similar patterns, with a higher number of unique genera in the initial stages (NW1 : 33, EH1 : 15). Over time, the count of unique genera decreased and stabilized. The reclaimed water receiving water treatment (RR) maintained a relatively even distribution of unique genera across time points (RR1 : 14, RR2 : 19, RR3 : 13), without the extreme peak observed in RW1, indicating a milder and more consistent selective pressure from water quality (Fig. 3d)).

Before irrigation began, the microbial structures of water source samples (RW1, EH1, NP1, RP1) exhibited differences from the original soil (O). Among them, the reclaimed water sample (RW1) was overwhelmingly dominated by a single phylum, Patescibacteria (relative

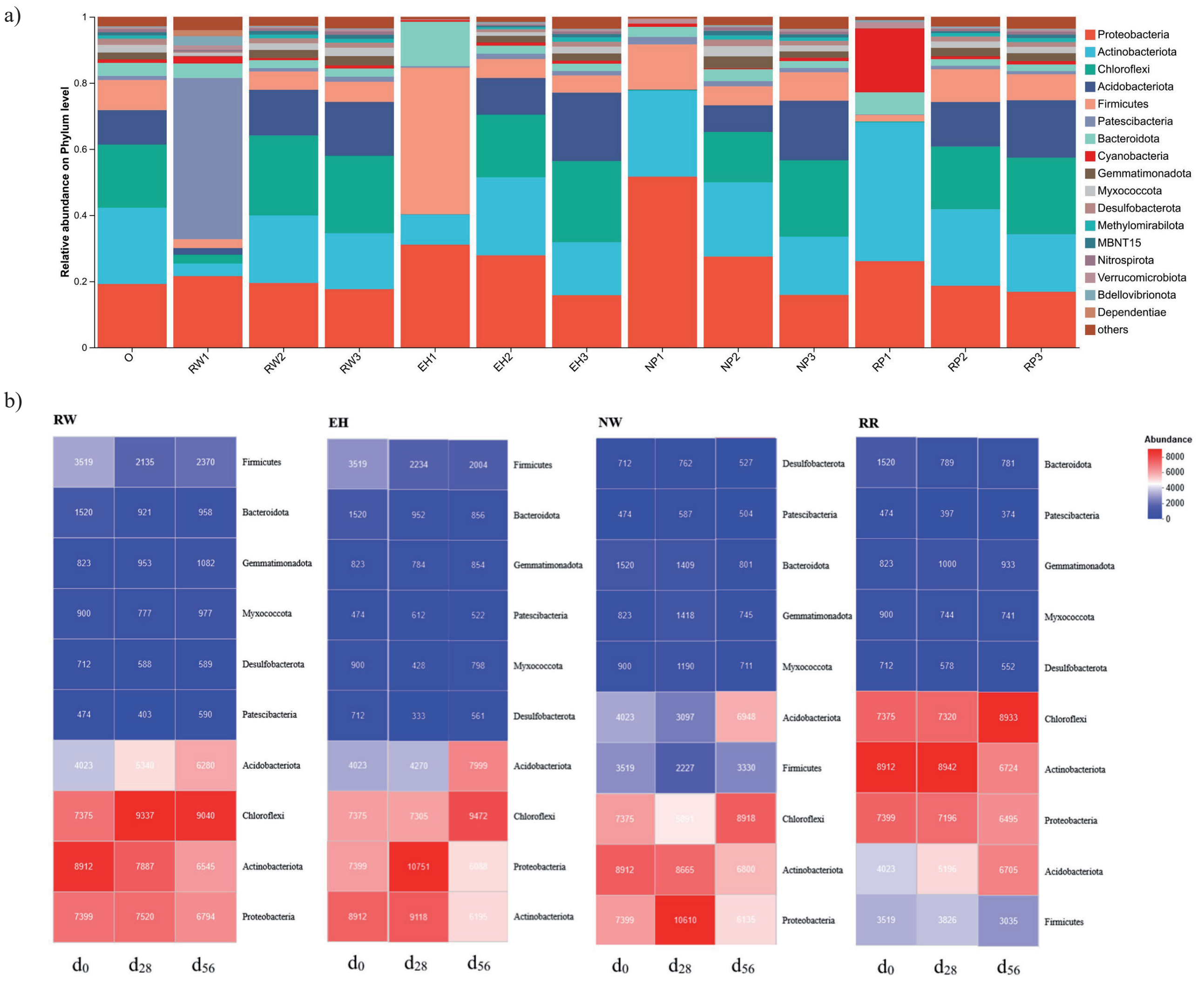

Fig. 4. Dominant microbial species and their abundance after irrigation with different water qualities. a) Dominant bacterial genera at the genus level after irrigation with different water qualities. b) Abundance of dominant bacterial species after irrigation with different water qualities.

abundance 48.8%), which was related to the high nutrient concentration and specific microenvironment in the reclaimed water. After 28 and 56 days of irrigation, the soil microbial communities demonstrated significant self-regulation and recovery capabilities. In particular, the reclaimed water treatment (RW) showed rapid succession from the abnormal state of RW1 to a more common structure dominated jointly by Chloroflexi, Acidobacteriota, and Proteobacteria in RW2 and RW3. By the end of the experiment (day 56), the community compositions of all treatment groups (RW3, EH3, NP3, RP3) displayed high similarity, with Chloroflexi, Acidobacteriota, Proteobacteria, and Actinobacteriota as the main components, indicating that soil microbial communities under different irrigation water sources may ultimately converge toward functionally similar structures (Fig. 4a)).

In soils irrigated with four different water qualities, the trends of bacterial phyla remained consistent throughout the experimental period. Specifically, the relative abundances of Chloroflexi and Acidobacteriota significantly increased, while other bacterial phyla, such as Actinobacteriota and Proteobacteria, decreased over time. In soils irrigated with reclaimed water (RW), Chloroflexi increased from 19.1% to 23.4%, and Acidobacteriota rose from 10.4% to 16.3%. Meanwhile, Actinobacteriota declined from 23.1% to 17.0%, and Proteobacteria decreased from 19.2% to 17.9%. In soils irrigated with Erhai Lake water (EH), Chloroflexi increased from 19.1% to 24.5%, and Acidobacteriota rose from 10.4% to 20.7%. Conversely, Actinobacteriota dropped from 23.1% to 16.0%, and Proteobacteria fell from 19.2% to 15.8%. In soils irrigated with natural water (NW), Chloroflexi increased from 19.1% to 23.1%, and Acidobacteriota rose from 10.4% to 18.0%. Meanwhile, Actinobacteriota decreased from 23.1% to 17.6%, and Proteobacteria declined from 19.2% to 15.9%. In soils irrigated with reclaimed water receiving water (RR), Chloroflexi increased from 19.1% to 23.1%, and Acidobacteriota rose from 10.4% to 17.4%. In contrast, Actinobacteriota dropped from 23.1% to 17.4%, and Proteobacteria decreased from 19.2% to 16.8% (Fig. 4b)).

## Discussion

Water quality varies across different water samples, which directly determines the ecological effects of soil after irrigation. NW, as a natural high-quality water source, serves as a background reference for comparison. EH exhibits characteristics of endogenous eutrophication (high organic matter, phosphorus, and suspended solids), which has a relatively mild impact on soil properties under irrigation. This is related to the input characteristics of algae-derived organic matter in Erhai Lake [18]. The high nitrogen, phosphorus, and salinity (especially $Na^+$ and $K^+$) in RW are the direct material sources of nitrate nitrogen and salt accumulation in soil, indicating the dual nature of reclaimed water as both a water-fertilizer resource and a potential salinization risk [19]. The water quality of RR clearly reflects the dilution-transformation-mixing effects of reclaimed water discharge, with its main indicators falling between RW and NW. However, the abnormal accumulation of nitrite nitrogen reveals active and potentially incomplete nitrification in the receiving water body. This dynamic nitrogen transformation process adds a new dimension to the risk assessment of irrigation water quality [20]. Therefore, irrigation water quality is not only the basis for water source classification but also a key prerequisite for predicting soil and crop response trajectories.

RW and RR irrigation led to significant accumulation of soil nitrate nitrogen and sodium salts. The substantial presence of nitrate nitrogen in the soil, while serving as a readily available nitrogen source for crops in the short term [21], significantly increases the risk of nitrogen loss through leaching or runoff. The continuous input of sodium ions ($Na^+$) poses an even more severe long-term threat. $Na^+$ disrupts soil aggregate structure through exchange reactions, leading to soil compaction and reduced water permeability [22], which may be one of the reasons for the persistently high soil moisture content in the RR treatment group. Although no extreme salinization was observed during the 56-day experiment, the clear accumulation trend warns of a high risk of secondary salinization induced by long-term direct irrigation. Additionally, the simultaneous accumulation of calcium ions ($Ca^{2+}$) in the RR treatment may reflect the sodium-calcium ion exchange process, further supporting the alteration of base ion balance [23]. Therefore, the nutrient benefits of reclaimed water irrigation must be preconditioned by strict monitoring of soil salinity and nitrogen levels.

The growth response of cabbage to irrigation with different water qualities exhibits a classic resource allocation trade-off [24]. The aboveground biomass of the RW and RR treatment groups was significantly higher than that of the NW treatment (Fig. 2), directly benefiting from the abundant water and nitrogen nutrient supply in the water source. When nitrogen is sufficient, plants tend to increase aboveground biomass to capture more light energy [25]. However, this promotion comes at the cost of reduced investment in belowground parts. The root biomass under RW and RR treatments was the lowest, forming an unbalanced configuration of strong aboveground and weak belowground growth. This indicates that under non-limiting resource (especially nitrogen) conditions, plants adopt a luxury consumption strategy, reducing the cost investment in root expansion [26]. In contrast, the relatively balanced water and nutrient supply under the NW treatment prompted plants to adopt a more robust strategy, developing a more extensive root system to enhance their resource acquisition ability and stress resistance in variable environments [27]. This difference in root configuration suggests that crops relying on reclaimed water irrigation may exhibit greater vulnerability when facing drought stress.

The response of soil bacterial communities to irrigation disturbance demonstrates ecological resilience [28]. Although microbial diversity initially declined in all treatments (except RW) during the early irrigation phase (likely due to experimental disturbance), by days 28 and 56, the α-diversity indices of all treatment groups had recovered to levels similar to those of the initial soil (Fig. 3a) and 3b)). More importantly, the evolution of community structure (alpha-diversity indices) highlights strong environmental selection and convergence effects [29]. Before irrigation, the community structures of different treatments showed significant differences, particularly with the RW1 sample being unusually dominated by Patescibacteria, likely due to the explosive growth of specific oligotrophic bacteria under high nutrient input [30]. However, as irrigation progressed, strong soil environmental filtering dominated community assembly [31]. By the end of the experiment (day 56), the microbial community structures of all treatments exhibited high similarity, with Chloroflexi and Acidobacteriota emerging as stable dominant phyla (Fig. 4). These two phyla are typically associated with soil organic matter decomposition and adaptation to oligotrophic environments [32, 33]. This convergence phenomenon suggests that, while irrigation water quality is an important short-term disturbance factor, the soil's inherent physicochemical properties may be a stronger driver of the ultimate microbial community structure. The reduction in unique genera in the RW treatment from 99 to 6 (Fig. 3d)) is a direct manifestation of this succession and convergence process.

## Conclusions

This study systematically investigated the comprehensive effects of irrigation with four typical water sources - natural water (NW) from plateau lake basins, reclaimed water from wastewater treatment plants (RW), Erhai Lake water (EH), and its receiving water (RR) - on soil physicochemical properties, crop growth, and microbial communities. NW demonstrated excellent water quality indicators and maintained stable soil conditions after irrigation. RW, rich in nitrogen, phosphorus, sodium, and potassium ions, showed significant advantages in integrated water-fertilization in the short term, effectively promoting the aboveground biomass growth of cabbage, while also causing significant accumulation of soil nitrate nitrogen and sodium salts. RR water quality fell between NW/RW, with its unique nitrite nitrogen accumulation phenomenon suggesting complex nitrogen transformation processes in water environments after reclaimed water discharge. EH displayed endogenous eutrophication characteristics. For crops, while reclaimed water irrigation promoted aboveground growth, it significantly inhibited root development, creating an unbalanced growth pattern of "strong aboveground, weak underground", which may compromise long-term crop stress resistance and stability. However, soil bacterial communities demonstrated strong ecological resilience, with all treatment groups recovering to highly similar structures dominated by Chloroflexi and Acidobacteria by the end of the experiment. In conclusion, reclaimed water irrigation presents both resource benefits and ecological risks in the short term, requiring refined management strategies for safe utilization to achieve a win-win scenario of efficient water resource use and watershed ecological security.

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## Conflict of Interest

The authors declare no conflict of interest.

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