Effects of different grades of rural reclaimed water irrigation and water levels on microbial community structure of rhizosphere soil in paddy field

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Abstract: The composition of soil microbial community structure and the diversity of microbial population could reflect the quality of soil environment to a certain extent. In this paper, three kinds of irrigation water sources and three kinds of water level regulations were set to investigate the effects of different sources of reclaimed water irrigation and the regulation of irrigation and drainage on species diversity and microbial diversity in paddy fields, aiming to clarify the microbial mechanisms under rural domestic reclaimed water irrigation. Through five-point method on soil samples in 0-40 cm layers, the soil characteristic was tested by 16S rDNA amplicon sequencing. Results showed that the maximum biodiversity is observed under primary treated water of domestic sewage R1 in soil layers of 0-20 cm and 20-40 cm. In the surface layer (0-20 cm) of soil, irrigation with rural reclaimed water can significantly increase the microbial diversity. The soil has the largest number of microbial communities and the richest biological communities under secondary treated water of domestic sewage R2, while it was the smallest under river water CK water source irrigation. The relative abundance of Proteobacteria is highest in 20-40 cm soil, and the relative abundance of Proteobacteria and Acidobacteria in 0-20 cm soil can be significantly increased under R1 and R2 water source irrigation conditions, while the abundance of Firmicutes can be reduced. The relative abundance of *Chloroflexi* in 20-40 cm soil layer can be significantly increased with R2, while reducing the relative abundance of Acidobacter. Moreover, in the 0-20 cm soil, both the Chao1 index and Ace index showed significant differences ($p<0.05$) between R1 and CK, and between R2 and CK. In the 20-40 cm soil, the Shannon index showed a significant difference ($p<0.05$) between R1 and R2, and also the Simpson index in this soil layer showed a significant difference ($p<0.05$) between R1 and CK treatments. Therefore, reclaimed water irrigation can significantly increase the biodiversity and community richness of the rice field root zone. Under the same water source conditions, the higher farmland water level could increase the difference in microbial diversity. This research clarifies the microbial action mechanisms in irrigation with rural reclaimed water, providing a theoretical basis for its application in agricultural fields.

Keywords: physiochemical properties, bacterial community, species diversity, species abundance, high-throughput sequencing
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1 Introduction

Microbes play an important role in the process of soil formation and material transformation, which can promote the process of soil nutrient cycling to a certain extent and reflect the status of soil nutrient cycling, and the size and distribution of its diversity and community structure are often regarded as key indicators for evaluation of soil fertility[1,2]. The soil microbial community is primarily affected by soil microenvironment factors such as pH, moisture, organic matter, nutrient content, and salinity. Among these, organic matter and pH are the predominant factors influencing the soil bacterial community[3]. As a source of irrigation water, the quality of reclaimed water directly influences the composition of soil microflora and consequently affects the bacterial community structure. It was demonstrated that reclaimed water irrigation shifted the soil microbial community structure and simulated the growth of microorganisms involved in the transformation of soil carbon and nitrogen[4]. It was found that reclaimed water irrigation increased the soil microbial population, which was predominantly composed of bacteria (over 90%), followed by actinomycetes and fungi[5]. The accumulation of nitrate in soil, resulting from reclaimed water irrigation, was identified as a key driver of the observed changes in the microbial community[6]. In addition, the rich nutrient elements in reclaimed water promote the growth and reproduction of rhizosphere microorganisms. This enhancement accelerates the cycling of nutrients within the root layer, which fosters a soil micro-ecosystem more favorable for both microbial activity and plant growth. This results in a mutually reinforcing interaction between the soil and its microbiota, ultimately improving the soil environment[7]. It showed that long-term reclaimed water irrigation could significantly improve soil microbial activity[8]. However, some studies have shown that the excessive accumulation of nutrients in soil from reclaimed water irrigation can also adversely affect the microbial community[9,10].

The impact of reclaimed water on soil microorganisms varies with its source. Specifically, there are certain concentrations of fungicides, antibiotics, and hormones in reclaimed water from domestic sewage, which have been shown to significantly increase the relative abundance of Chloroflexi and Nitrospirae following

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irrigation. The soil flora irrigated by reclaimed water from domestic sewage is mainly affected by total nitrogen (TN), total phosphorus (TP), and soil dissolved organic carbon (DOC)[11]. Exsiting research demonstrates that reclaimed water irrigation promotes the increase of *Bacillus*, in which TN and TP had significant effects on *Streptococcus*, *Aerococcus*, etc[12]. It is found that the abundance of soil amoeba increased and actinomycetes decreased after reclaimed water irrigation[13,14]. Multiple studies report increases in specific bacterial phyla such as Proteobacteria, Gemmatimonadetes, Bacteroidetes, Acidobacteria, and Planctomycetes, while decreases are observed in Firmicutes and Tectomicrobia[7,15]. It also shows that there are Proteobacteria, Bacteroides, Actinomycetes, and Firmicutes in the fruit and rhizosphere of crop plants under reclaimed water irrigation[16]. Compared with clean water irrigation, reclaimed water irrigation will increase the number of soil bacteria and actinomycetes, but has no significant impact on the number of soil fungi[17]. It indicates that the total bacterial abundance is increased by 16% after reclaimed water irrigation, but had no significant effect on the total archaea abundance (p>0.05)[18]. Huang et al.[19] reported that the richness, evenness, and diversity of rhizosphere bacterial communities decrease with the increase of disturbance intensity of reclaimed water. Research on long-term reclaimed water irrigation reveals complex microbial responses. Han et al.[20] reported that the total counts of bacteria, actinomycetes, and fungi in the soil were not significantly affected by the irrigation of reclaimed water with different water quality. In contrast, other research indicates that sewage irrigation can increase bacterial and fungal diversity and change the relative abundance of dominant genera[21]. A further study notes that the fundamental composition types of bacterial communities remain unchanged, but the abundance distribution of these types is altered[22]. These shifts are particularly evident in functional groups, such as a decrease in nitrifying bacteria but an increase in ammonia-oxidizing bacteria.

The reclaimed water irrigation leads to a change in soil habitat. On the one hand, the nutrients and microorganisms brought in by reclaimed water irrigation can promote the decomposition of crop residues, and change the soil biological environment by affecting the distribution of physical and chemical indicators. On the other hand, it provides energy sources for microorganisms, stimulates microbial activity, and then affects the number, species, and activity of soil microorganisms[23-25]. At present, the impact of domestic reclaimed water irrigation on soil habitat and microbial diversity under field conditions remains unclear. To address this knowledge gap, this study investigates the effects of different reclaimed water sources and irrigation and drainage management practices on physicochemical properties and microbial diversity of paddy soils. The findings are crucial to clarify the mechanism of microbial action under reclaimed water irrigation in rural areas.

2 Materials and methods

2.1 Experimental site

The field experiment was carried out at the Yongkang rural domestic wastewater reclamation and reuse base (28°48 ′N, 120°10E) in Jinhua City from May to October in 2020 and from May to October in 2021 (Figure 1). The study area is a low mountain and hilly area with a subtropical monsoon climate. The average annual rainfall is 1787 mm, the maximum annual precipitation is 2385.8 mm, and the minimum annual precipitation is 1119.9 mm. The multi-year average evaporation is 930.2 mm, the annual average temperature is 17.5°C, the annual average sunshine is 1909 h, the multi-year average wind speed is 2.8 m/s, and the frost-free period is 245 d. The reclaimed water was coming from a domestic sewage disposal station with design scale of 400 m^3/d. The treatment process adopted the secondary biological treatment process (primary treatment was conventional process and secondary treatment adopted improved A^{2}O process), and the effluent quality met the first-class B standard of the discharge standard of pollutants for municipal sewage treatment plant.

Figure 1 Experimental site

2.2 Experimental design

The rice variety is Jiayou Zhongke 13-1, and the soil in experimental site is sand or sand clay, with bulk density of 1.3-1.5 g/cm^3. The rice density was 10 plants/m^2. There were two fertilizations during growth period, with basal fertilizer of 200 kg/hm^2 compound fertilizer and 100 kg/hm^2 urea, and dressing was 250 hm^2 compound fertilizer. The physical and chemical properties of 0-40 cm soil layers are listed in Table 1.

Table 1 Chemical properties of soil

Soil depth	pH	EC/ mS·m^{-1}	Salinity/ g·kg^{-1}	TN/%	TP/%	Organic matter/ g·kg^{-1}	NH_4^+-N/ mg·kg^{-1}	NO_3^--N/ mg·kg^{-1}
0-20 cm	5.56	2.6	0.44	0.12	0.069	17.7	8.24	2.84
20-40 cm	5.88	2.9	0.27	0.09	0.032	14.8	5.75	2.69

Three kinds of irrigation water sources were used, namely primary treated water of domestic sewage (R1), secondary treated water of domestic sewage (R2), and river water (CK), respectively, and the water was pumped for irrigation through a simple submersible pump. The source of sewage mainly originates from local wastewater treatment plants and is primarily composed of rural domestic sewage. R1 was conventional sewage treatment process and R2 adopted improved A^{2}O process, suggesting that R2 is the wastewater derived from R1 after a certain degree of biological purification. The properties of the irrigation water during the experimental period are listed in Table 2. All indicators have reached the irrigation water quality standards[26]. Three kinds of water level regulations were also set up, and each treatment was repeated three times. Basically, W1, W2, and W3 represented low, medium, and high water levels. The field water level regulation was controlled strictly at each growth period. When the water level dropped to the low limit, water was supplemented immediately, while it was drained when heavy rain exceeded the upper limit of rain storage. The setting principle of field water level regulation was different from that of conventional rice water-saving irrigation. The field water level regulations are listed in Table 3.

Table 2 Physical and chemical properties of irrigation water

Water source	pH	COD/ mg·L^{-1}	LAS/ mg·L^{-1}	NH_4^+-N/ mg·L^{-1}	NO_3^--N/ mg·L^{-1}	TN/ mg·L^{-1}
R1	6.7-7.2	15-84	0.06-0.88	8.250-11.900	0.016-0.061	13.500-18.900
R2	6.9-7.2	10-59	0.00-0.16	3.520-11.900	0.010-6.250	8.200-15.100
CK	6.8-7.3	7-56	0.00-0.10	0.116-1.490	0.624-2.560	3.450-10.700

Note: COD and LAS represent the chemical oxygen demand and anionic surfactant, respectively.

Table 3 Standard of water level control in paddy field (mm)

Water control	Up and low limit	Turning green	Early tillering	Later tillering	Jointing booting	Heading flowering	Milkying
W1	Up limit of sewage	0	3-5 d exposing field	1-2 d exposing field	1-2 d exposing field	1-2 d exposing field	3-5 d exposing field
	Low limit of sewage	30	30	exposing field	40	40	30
	Up limit of sewage (rain) storage	50	70		80	80	60
W2	Up limit of sewage	0	10	10	10	10	10
	Low limit of sewage	30	50	exposing field	50	50	50
	Up limit of sewage (rain) storage	50	70		100	100	100
W3	Up limit of sewage	0	40	40	40	40	10
	Low limit of sewage	30	60	exposing field	60	60	60
	Up limit of sewage (rain) storage	50	100		150	150	100

Note: When field water layer was lower than low limit, the field needs irrigation; and drainage is needed when the water layer was higher than up limit. The rain storage limit is the highest water layer in paddy field when there was a rain event (at the bottom of the table).

2.3 Indicators and measurements

The typical microbial community structure of soil was determined by 16S rDNA amplicon sequencing before and after the experiment. Microbial molecular sequencing was done by Miseq sequencing platform.

The soil samples in 0-20 cm and 20-40 cm were taken respectively by five-point method before rice seeding and after the rice harvest. The pH was measured by the potentiometric method using a pH meter (FE28-Standard). Electrical conductivity (EC) was measured by the electrode method using a conductivity instrument (DDSJ-308F). Organic matter (OM) was measured by potassium dichromate-sulfuric acid solution method determination. Ammonium-nitrogen (NH_4^+-N) and nitrate-nitrogen (NO_3^--N) were measured by potassium chloride solution extraction-spectrophotometric method using a UV–visible spectrophotometer (UV-1800), and total nitrogen (TN) was determined by semi micro Kjeldahl method (K9860).

2.4 Statistical analysis

Data calculation and diagramming were completed by Excel 2013. The statistical analysis methods such as T-test, MetaStat, LEfSe, Anosim, and MRPP were used to test the significance of species composition and community difference. Statistical analysis and correlation analysis were completed by SPSS 20.

3 Results and discussion

3.1 Change of physicochemical properties

The physicochemical properties of different rhizosphere soil samples are listed in Table 4. Soil pH exhibited distinct variations depending on the water source and water level control. Generally, the pH was higher in the 20-40 cm layer than in the 0-20 cm layer. The soil pH irrigated with reclaimed water was lower than that with river water. With the increase of water level control, the soil pH decreased gradually. Under reclaimed water irrigation, soil EC increases significantly, in which R1 and R2 were 55.1% and 56.2% higher than CK respectively in 0-20 cm soil layer, and they were 57.3% and 21% higher than CK in 20-40 cm soil layer. Therefore, reclaimed water irrigation enhances the dissociation and exchange performance of adsorbed soil ions, increasing the intensity of interaction with soil colloids, and thereby improves soil fertility. Moreover, compared with R2 water source, irrigation with R1 resulted in greater water penetration, reaching the 0-20 cm soil layer and consequently having a greater impact on soil EC of 20-40 cm soil layer. Under reclaimed water irrigation, soil organic matter increased significantly. In the 0-20 cm soil layer, R1 and R2 were 29.4% and 42.4% higher than CK respectively, and these increases were 42.7% and 56.2% higher than CK respectively in the 20-40 cm layer. The soil organic matter content increased progressively with higher field water level controls. In the 0-20 cm layer, the value under the W3 treatment was 50.4% and 17.9% higher than under W1 and W2, respectively. A similar trend was observed in the 20-40 cm layer, where the value for W3 was 50.9% and 43.6% greater than that for W1 and W2. Organic matter was one of the main sources of soil nutrients, which has a greater impact on the physical and chemical properties and biological characteristics of the soil; thus R2 irrigation is more conducive to increasing the organic matter.

Table 4 Physicochemical properties of different rhizosphere soil samples

Soil depth	Treatment	pH	EC/ $mS \cdot m^{-1}$	OM/ $g \cdot kg^{-1}$	NH_4^+-N/ $mg \cdot kg^{-1}$	NO_3^--N/ $mg \cdot kg^{-1}$	TN/%
0-20 cm	W1R1	6.48	5.2	9.19	5.73	1.87	0.145
	W2R1	5.77	4.4	20.20	6.61	3.48	0.107
	W3R1	5.30	7.3	26.50	8.40	1.84	0.150
	W1R2	5.49	6.7	16.60	5.38	2.12	0.107
	W2R2	5.70	5.2	20.90	5.73	1.80	0.100
	W3R2	5.62	5.1	24.00	7.60	4.28	0.174
	W1CK	6.13	3.2	16.70	5.39	3.28	0.090
	W2CK	5.69	4.8	13.10	2.44	3.28	0.075
	W3CK	6.01	2.9	13.40	1.95	2.19	0.131
20-40 cm	W1R1	6.43	5.4	7.29	4.82	1.58	0.045
	W2R1	6.17	2.8	13.19	3.03	1.88	0.044
	W3R1	5.52	4.4	21.70	5.51	1.14	0.127
	W1R2	6.08	2.0	15.20	4.79	1.44	0.026
	W2R2	5.80	5.5	14.40	3.98	1.46	0.088
	W3R2	6.24	2.2	16.57	4.31	2.42	0.140
	W1CK	6.38	1.5	10.62	6.40	1.12	0.038
	W2CK	6.12	3.9	7.22	2.16	1.61	0.035
	W3CK	5.93	2.6	11.70	2.54	2.63	0.078

Note: EC, OM, NH_4^+-N, NO_3^--N, TN: electric conductivity, organic matter, ammonium nitrogen, nitrate nitrogen, total nitrogen, respectively.

Reclaimed water irrigation (R1, R2) increased soil TN content relative to CK by 30% for both treatments in the 0-20 cm layer and by 40% and 60%, respectively, in the 20-40 cm layer, with the highest overall TN content found under high water level control. In 0-20 cm layer, the NH_4^+-N content under R1 and R2 is 2.12 and 1.91 times that of CK, respectively, and it is only 20.3% and 17.8% over CK in 20-40 cm layer. Consequently, the NH_4^+-N content in 0-

20 cm layer increases most significantly. However, the change of NO$_3^-$-N content is in the opposite direction, showing R1<R2<CK, indicating that reclaimed water irrigation could reduce soil NO$_3^-$-N, and also the NO$_3^-$-N content in 0-20 cm and 20-40 cm layers is the lowest under the low water level control.

3.2 Change of species diversity

In order to study the diversity of species under three different irrigation water sources and three water level regulations (W1, W2, W3), the effective tags from all soil samples were clustered into Operational Taxonomic Units (OTUs) at a 97% similarity threshold using UPARSE software. Then, the representative sequences of OTUs were annotated. The results of comprehensive statistics on OTU clustering and annotation for each soil sample are summarized in Table 5.

Table 5 Statistics of the number of OTUs and Tags

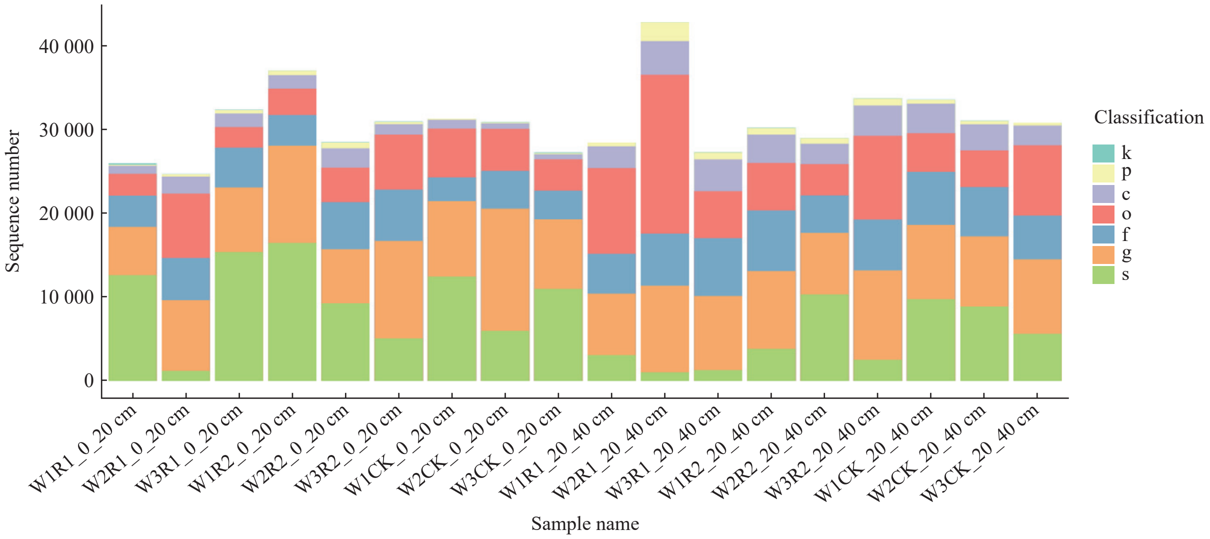
Soil depth	Treatment	Total tags	Non-pollution tags	Taxon tags	OTUs
0-20 cm	W1R1	38 762	38 080	25 953	1984
	W2R1	43 172	43 116	24 672	2354
	W3R1	45 134	45 101	32 359	2389
	W1R2	42 024	41 993	37 031	2484
	W2R2	43 095	43 031	28 480	2681
	W3R2	45 679	45 521	30 943	2682
	W1CK	43 457	43 383	31 268	1255
	W2CK	44 729	44 663	30 873	1705
	W3CK	39 990	39 734	27 263	1902
20-40 cm	W1R1	42 774	42 734	28 446	1775
	W2R1	50 321	50 310	42 797	1784
	W3R1	45 546	45 528	27 297	2652
	W1R2	44 407	44 364	30 206	3076
	W2R2	40 950	40 939	28 954	2302
	W3R2	42 654	42 592	33 704	2075
	W1CK	43 052	42 947	33 563	2429
	W2CK	44 253	44 166	31 047	2407
	W3CK	43 885	43 846	30 811	1978

The number of OTUs showed distinct trends across water sources and soil depths. In the 0-20 cm layer, it increased with the water level control under R1, R2, and CK. In contrast, in the 20-40 cm layer, the number of OTUs continued to increase with water level only under R1, while it gradually decreased under both R2 and CK. Generally speaking, the number of OTUs is the largest under R2 water source irrigation, and it is 18.3% and 31% higher than that under R1 and CK. The number of OTUs under CK water source irrigation is the smallest in the 0-20 cm soil layer, and it is the smallest under R1 water source irrigation in the 20-40 cm soil layer. It can be seen that rural domestic reclaimed water irrigation can significantly increase the microbial diversity in the surface (0-20 cm) soil layer.

3.3 Change of species abundance

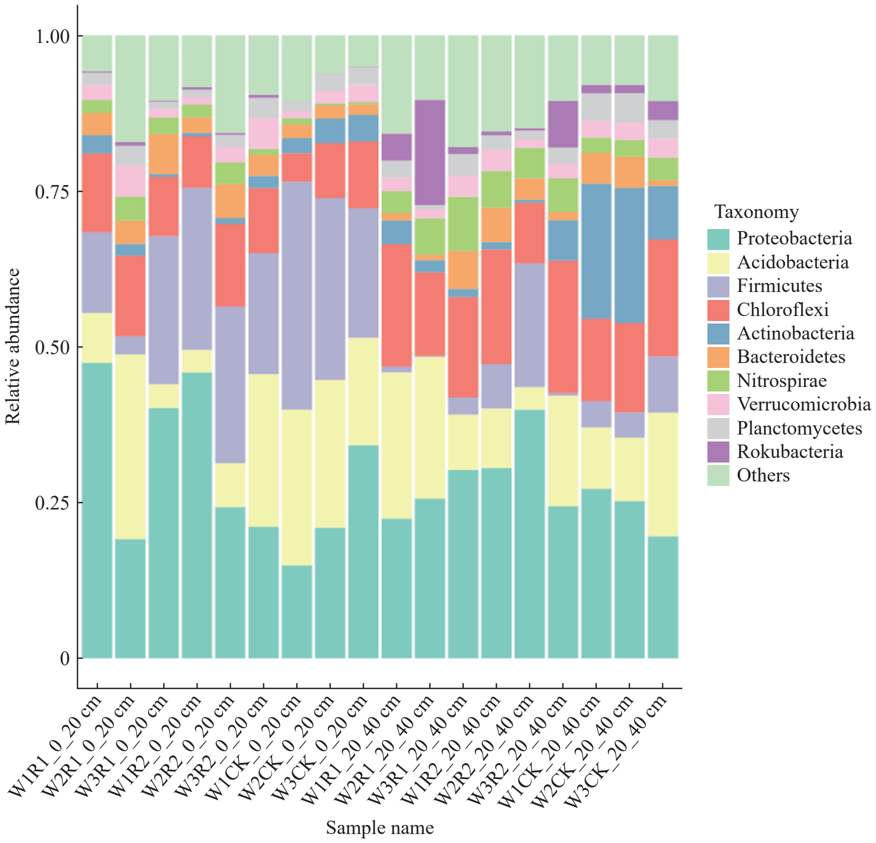
The sequence number of soil samples at each classification level is shown in Figure 2. Based on the species annotation results, the absolute abundance (both pre- and post-homogenization) and the relative abundance (post-homogenization) are quantified for each soil sample at all classification levels (Phylum, Class, Order, Family, Genus). A relative abundance histogram was generated for each sample by selecting the top 10 most abundant species at each classification level (Phylum, Class, Order, Family, Genus). This visualization facilitates the comparison of dominant species and their proportions across different classification levels.

Take the histogram of relative abundance of species at the phylum level as an example (Figure 3). In 0-20 cm soil layer, Proteobacteria was the dominant phylum. Under R1 and R2 irrigation, its average relative abundance accounted for 35.6% and 30.5% of the microbial community respectively. It was followed by Acidobacter (13.8%) and Firmicutes (23.5%). However, under CK irrigation, the relative abundance of Acidobacteria (21.9%) in 0-20 cm soil layer is lower than Proteobateria (23.4%) and Firmicutes (28.9%), indicating that reclaimed water irrigation (R1 and R2) can significantly increase the relative abundance of Proteobacteria and Acidobacteria in surface soil and reduce the abundance of Firmicutes. The relative abundance of Proteobacteria is the highest in 20-40 cm layer, and the other microbial flora is relatively small. Under R1 irrigation, Proteobacteria (26.1%) and Acidobacteria (18.4%) were the most abundant, and Chloroflexi had a relative abundance of 16.4%. Under R2 irrigation, Proteobacteria (31.6%) and Chloroflexi (16.5%) were dominant, while Acidobacteria decreased to 10.3%. Under CK irrigation, Proteobacteria (24.0%) and Actinobacteria (17.3%) were relatively higher, with Chloroflexi at 15.5%. Therefore, the secondary rural domestic reclaimed water (R2) irrigation can significantly increase the relative abundance of Chloroflexi in 20-40 cm layer and reduce the relative abundance of Acidobartia.



Note: k, p, c, o, f, g, s represent Kingdom, Phylum, Class, Order, Family, Genus, Species, respectively.

Figure 2 Sequences number of soil sample at each classification level



Note: Others represents the sum of relative abundances of all gates except the 10 gates in the figure.

Figure 3 Histogram of relative abundance of species at the phylum level

3.4 Alpha diversity of soil microorganisms

The method of random sampling of sequences is used to construct a rarefaction curve based on the number of sequences extracted and the number or diversity of their corresponding species (OTUs). It can be seen from Figure 4 that the number of species observed in each sample increases with the amount of sequencing data. The observed species for W1R2 in 20-40 cm layer is highest, while it is lowest for W1CK in 0-20 cm soil. When the curve tends toward being flat, it indicates that the amount of sequencing data is reasonable, and more data will only produce a small number of new species. It shows that the sequencing results can objectively reflect the vast majority of bacterial community diversity information in each sample.

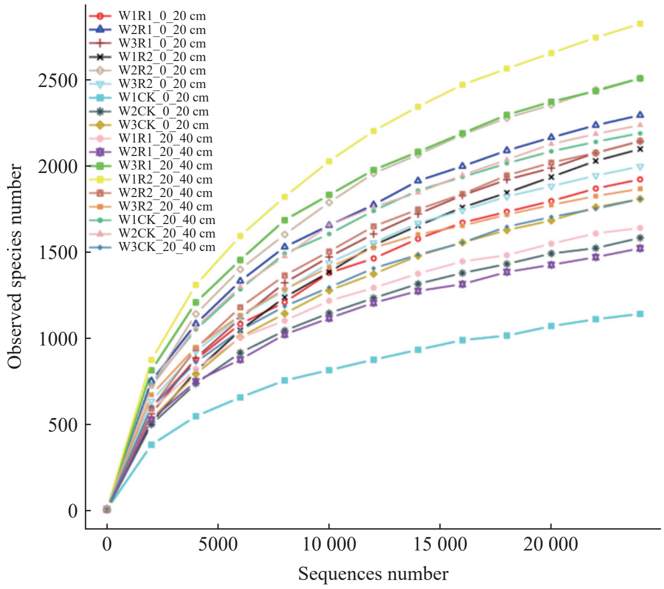


Figure 4 Rarefaction curves of bacterial communities

Alpha diversity is used to analyze the microbial community diversity in the soil sample. The diversity analysis of a single sample can reflect the richness and diversity of the microbial community. The alpha diversity analysis index of different samples at 97% consistency threshold was counted. Through four diversity indices (Shannon, Simpson, Ace, Chao1), it was analyzed whether there was diversity difference among the treatments, as shown in Figure 5. Shannon diversity index and Simpson diversity index can reflect the community diversity. The larger the index value is, the higher the community diversity is. Under R1, R2, and CK irrigation, the Shannon (Simpson) indices were 7.8 (0.96), 7.73 (0.96), and 7.01 (0.94) in the 0-20 cm layer, and 8.81 (0.99), 8.75 (0.98), and 8.44 (0.98) in the 20-40 cm layer, respectively. Therefore, in the 0-20 cm layer, soils irrigated with rural domestic sewage (R1, R2) exhibited higher biodiversity than the control (CK), with R1 supporting the highest level and CK the lowest.

The Chao1 and Ace indices are used to estimate community richness, reflecting the total number of species (OTUs) present in a community. Higher index values indicate greater species richness. In 0-20 cm soil, the Chao1 and Ace indices were highest under R2 (3042.25; 3065.93) and lowest under CK (2139.14; 2133.3). In 20-40 cm layer, the indices remained highest under R2 (2969.56; 2994.03). Consequently, the R2 irrigation fostered the greatest community richness, whereas the CK treatment led to the poorest richness in the surface layer.

The Chao1 and Ace indices in 0-20 cm layer showed significant difference between CK with R1 and R2 ($p<0.05$), and the other treatments have no significant differences ($p>0.05$). Shannon index in 20-40 cm layer is significantly different between

R1 and R2 water source irrigation ($p<0.05$), and Simpson index in this soil layer is significantly different between R1 and CK treatments ($p<0.05$).

To sum up, biodiversity under R1 in 20-40 cm layer is the largest, and it is the smallest in 0-20 cm layer under CK. The number of soil biological flora species under R2 in 0-20 cm layer is the largest, while it is the smallest under CK in 0-20 cm layer. Therefore, reclaimed water irrigation can significantly increase the biodiversity and community richness in the root zone of rice fields.

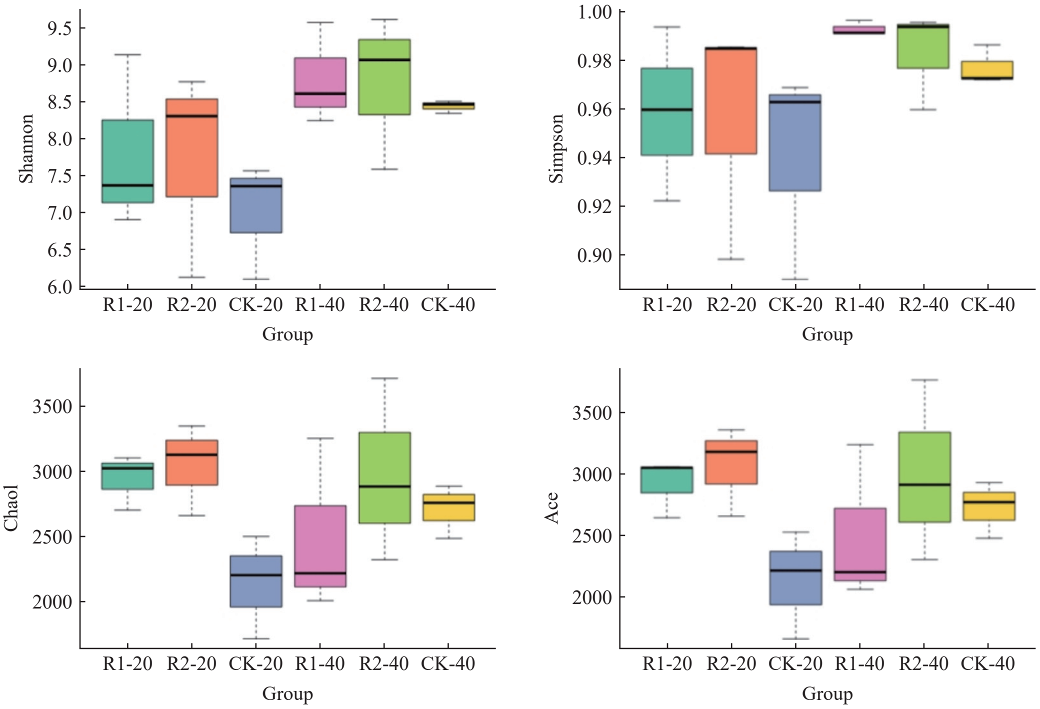


Figure 5 Analysis of differences between groups of alpha diversity index

3.5 Beta diversity of soil microorganisms

Beta diversity measures the differences in microbial community composition between samples. It is typically analyzed using principal component analysis (PCA), where a smaller distance between samples on the PCA plot indicates higher similarity in community structure. As shown in Figure 6, the higher the sample similarity, the more aggregated the performance in the image. The abscissa and ordinate represent the first principal component PC1 (18.91%) and the second principal component PC2 (12.37%) respectively, and the cumulative contribution rate of the two principal components is 31.28%. For both R1 and R2 water sources, significant differences emerged primarily under the high water level (W3) compared to the low and middle levels (W1, W2), where communities were more similar. This pattern was also observed in the 20-40 cm layer under CK irrigation. These results indicate that, under a given water source, low and middle water level controls had a minimal impact on beta diversity, whereas a higher water level significantly increased compositional differences.

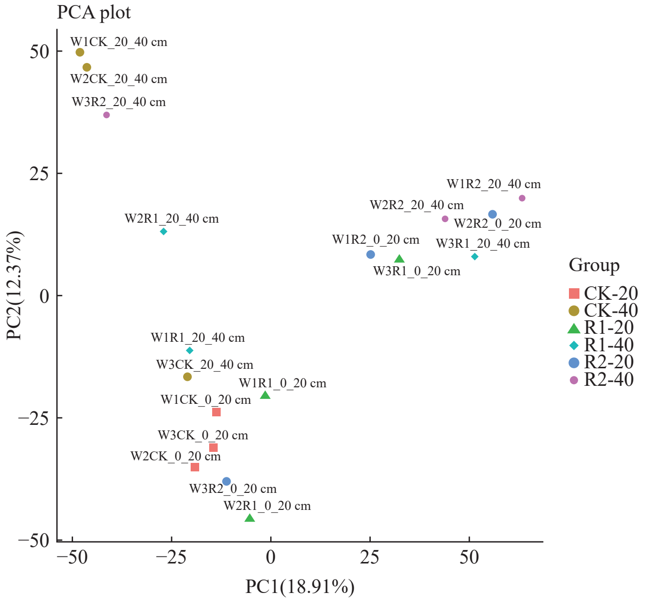


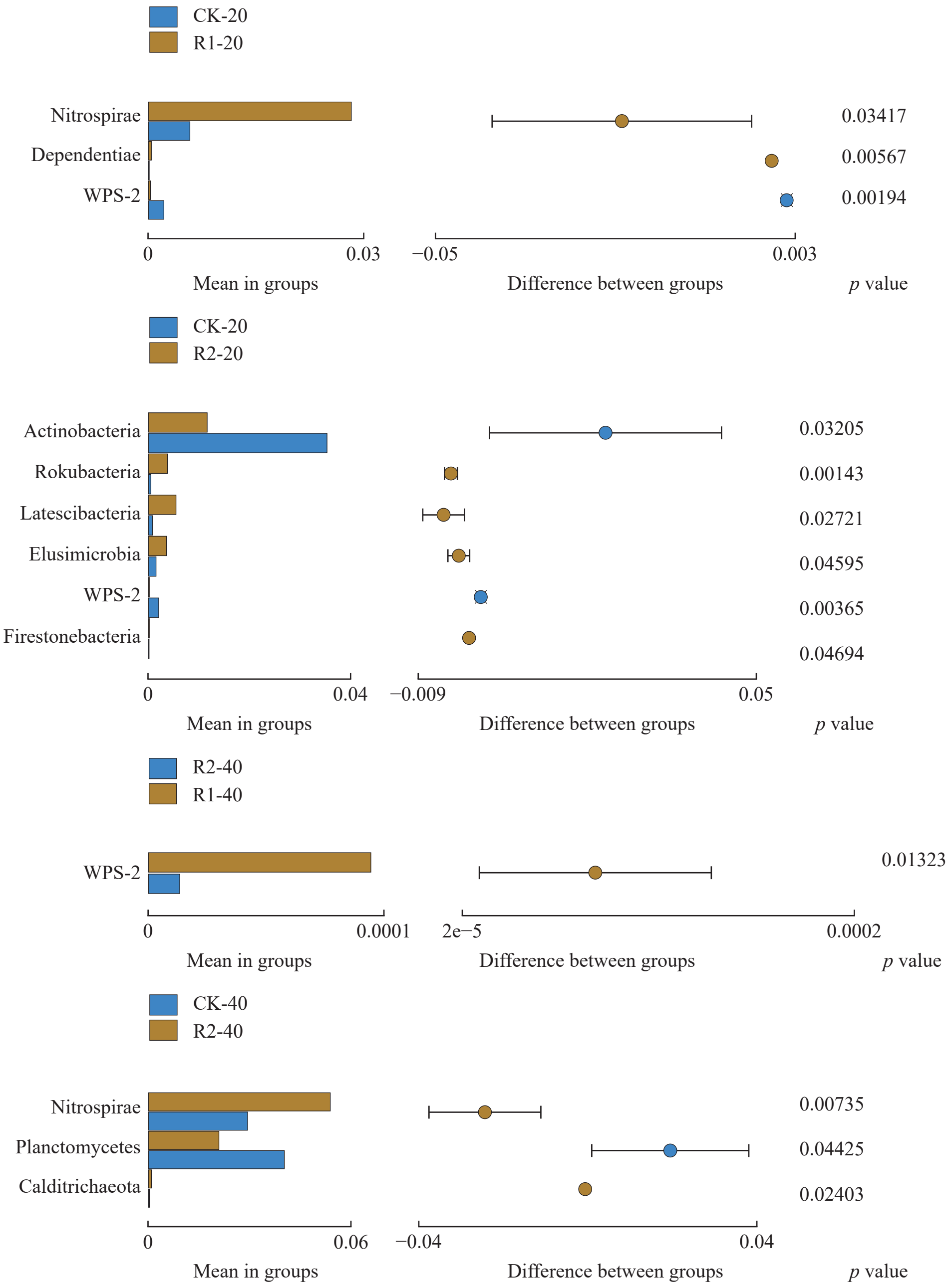
Figure 6 Analysis of PCA

3.6 Analysis of species difference between T-test groups

In order to find the difference of species among groups at each classification level (phylum, class, order, family, genus, species), T-test between groups is conducted to find the species with significant difference ($p\leq0.05$). Take the phylum level as an example. The significant difference after screening is shown in Figure 7. Significant differences in species composition were observed between the reclaimed water (R1, R2) and control (CK) groups in the 0-20 cm layer, with the R2-CK comparison showing more pronounced differences than R1-CK. In the 20-40 cm layer, significant differences were found between R1-R2 and R2-CK, and the number of differentially abundant species was greater for R2-CK than for R1-R2. The 0-20 cm soil layer under R2 irrigation exhibited higher species richness. This is likely because R2 water, sourced from R1 that had undergone specific biological treatment, contained a greater abundance of microorganisms. These introduced microbes proliferated in the surface soil, contributing to the increased diversity observed in the top layer.

3.7 Correlation between bacterial community and soil physiochemical properties at phylum level

The correlation between bacterial community (OTUs, Shannon, and Chao1) and soil physiochemical properties at phylum level is listed in Table 6. The bacterial community structure correlated negatively with pH but positively with OM and NH_4^+-N. For specific nutrients, the number of OTUs showed a positive



Note: Each bar in the figure represents the mean value of species with significant difference in abundance between groups.

Figure 7 Species abundance difference between T-test groups

Table 6 Correlation between bacterial community and soil physiochemical properties

Treatment	Soil depth/cm	pH	EC/ $mS \cdot m^{-1}$	OM/ $g \cdot kg^{-1}$	NH_4^+-N/ $mg \cdot kg^{-1}$	NO_3^--N/ $mg \cdot kg^{-1}$	TN/%
OTUs	0-20	−0.5735	0.6066	0.5915	0.5392	−0.1108	0.4710
	20-40	−0.3822	−0.2261	0.4091	0.3716	−0.5456	−0.0979
Shannon	0-20	−0.0610	−0.1281	0.2581	0.1463	0.2587	0.1134
	20-40	−0.1374	−0.3311	0.5052	0.4221	−0.1687	0.1858
Chao1	0-20	−0.4583	0.6195	0.4051	0.4268	−0.4313	0.2536
	20-40	−0.4816	−0.0645	0.3864	0.3109	−0.5353	−0.0899

correlation with EC and TN in the 0-20 cm layer, but this relationship turned negative in the 20-40 cm layer; OTUs also correlated negatively with NO_3^--N across layers. In terms of diversity, the Shannon index correlated positively with NO_3^--N and TN but negatively with EC in the topsoil (0-20 cm). The Chao1 richness estimator correlated positively with EC and TN in the 0-20 cm layer, and with EC, NO_3^--N, and TN in the 20-40 cm layer, while exhibiting a negative correlation with NO_3^--N in the topsoil.

Previous studies have found that with domestic sewage irrigation, the total salt content was significantly negatively correlated with the content of TN, TP, and OM in soil ($p<0.01$), and the number of OTUs significantly increased as the salinity of irrigation water increases ($p<0.05$)[27]. However, in this study, the OTUs number was higher under R2 rather than R1. Another study has found that with rural mixed wastewater irrigation treatment, the contents of soil alkali-hydrolyzable nitrogen increased, while soil pH, the concentrations of organic matter, and total nitrogen were decreased[21]. However, Liu et al.[28] found that with aquaculture wastewater irrigation, it can increase the content of soil nutrients such as organic matter, total nitrogen, and available potassium, which was consistent with the results in this study. The diversity indices (Shannon, Ace, and Chao1) of bacteria and fungi increased in soil according to Liu's study, but the Simpson index decreased, which was opposite to the results in this study. Compared with the control group, the sewage treatment increased the relative abundance of Actinomycetes in soil, and decreased the relative abundance of Acidobacteria[29].

4 Conclusions

From the above results and discussions, the following conclusions can be drawn.

Compared to river water irrigation, reclaimed water irrigation significantly reduced soil pH while increasing electrical conductivity (EC) and organic matter content. These changes indicate that reclaimed water irrigation can enhance soil fertility. Between the two reclaimed water types tested, R2 irrigation demonstrated more favorable outcomes than R1.

1) The soil irrigated with R2 reclaimed water exhibited the highest number of OTUs, which was 18.3% and 31% greater than that under R1 and CK, respectively. Reclaimed water irrigation resulted in higher Shannon and Simpson diversity indices compared to CK, indicating enhanced microbial community diversity, particularly under the R1 water source. Similarly, the Chao1 and Ace indices were elevated under reclaimed water irrigation, reflecting greater community richness, with the most pronounced effect observed under R2.

2) Bacterial community composition showed a significant negative correlation with soil pH, but was positively correlated with organic matter (OM) and NH_4^+-N content. In the 0-20 cm layer, OTU richness was positively correlated with EC and TN, whereas in the 20-40 cm layer, it correlated negatively with these factors as well as with NO_3^--N. The Shannon index was positively correlated with NO_3^--N and TN in the 0-20 cm layer, but negatively correlated with EC. Similarly, the Chao1 index showed a positive correlation with EC and TN in the 0-20 cm layer. In the 20-40 cm layer, it was positively correlated with EC, NO_3^--N, and TN, but negatively correlated with NO_3^--N.

Novelty statement:

1. Soil pH under reclaimed water irrigation is lower than that under river water irrigation, while under reclaimed water irrigation, soil EC and organic matter increase significantly, and also the contents of TN and NH_4^+-N are higher than CK.

2. Reclaimed water irrigation can significantly increase the biodiversity and community richness of the rice field root zone.

3. Under the same water source conditions, the regulation of medium and low water levels has a relatively small impact on the variation of soil microbial Beta diversity, while higher farmland water levels increase the difference in microbial diversity.

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[References]

[1] Sun B, Wang X Y, Lyu X H. The historical venation in research on microbial mechanisms of soil nutrient cycling in the past 60 years—Based on bibliometric analysis and big data visualization. Journal of Plant Nutrition and Fertilizers, 2017; 23(6): 1590–1601. (in Chinese)

[2] Wang H Y, Xu M G, Ma X, Duan Y H. Research advances of microorganism and ammonia oxidizing bacteria under long-term fertilization in Chinese typical cropland. Soil and Fertilizer Sciences in China, 2018(2): 1–12. (in Chinese)

[3] Lu H F, Qi X B, Li P, Qiao D M, Shafeeq-u-R, Bai F F, et al. Effects of Bacillus subtilis and Saccharomyces cerevisiae inoculation on soil bacterial community and rice yield under combined irrigation with reclaimed and fresh water. Int J Agric & Biol Eng, 2022; 15(3): 33–46.

[4] Guo W, Qi X B, Li P, Li Z Y, Zhou Y, Xiao Y T. Impact of reclaimed water irrigation and nitrogen fertilization on soil bacterial community structure. Acta Scientiae Circumstantiae, 2017; 37(1): 280–287. (in Chinese)

[5] Li J, Ma H X, Zheng E F. Influence of irrigation with reclaimed water on rhizosphere soil properties and leaf physiology of different landscape plants. Research of Soil and Water Conservation, 2017; 24(4): 70–76. (in Chinese)

[6] Turlapati S A, Minocha R, Bhiravarasa P S, Tisa L S, Thoms W K, Minocha S C. Chronic N-amended soils exhibit an altered bacterial community structure in Harvard Forest, MA, USA. FEMS Microbiology Ecology, 2013; 83(2): 478–493.

[7] Becerra-Castro C, Lopes A N R, Vaz-Moreira I, Silva E F, Manaia C M, Nunes O C. Wastewater reuse in irrigation: A microbiological perspective on implications in soil fertility and human and environmental health. Environmental International, 2015; 75(4): 117–135.

[8] Cirelli G L, Consolia S, Licciardello F, Aiello R, Giuffrida F, Leonardi C. Treated municipal wastewater reuse in vegetable production. Agricultural Water Management, 2012; 104: 163–170.

[9] Bastida F, Torres I F, Romero-Trigueros C, Baldrian P, Vetrovsky T, Bayona J M, et al. Combined effects of reduced irrigation and water quality on the soil microbial community of a citrus orchard under semi-arid conditions. Soil Biology and Biochemistry, 2017; 104: 226–237.

[10] Ramirez K S, Craine J M, Fierer N. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology, 2012; 18(6): 1918–1927.

[11] Xu J, Wu L S, Chen W P, Jiang P P, Chang A C S. Pharmaceuticals and personal care products (PPCPs), and endocrine disrupting compounds (EDCs) in runoff from a potato fifield irrigatedwith treated wastewater in Southern California. Journal of Health Science, 2019; 55: 306–310.

[12] Gong X, Wang J H, Guan J F, Yang X C, Chen D C. Impact of reclaimed water irrigation on soil chemical properties and culturable microorganisms. Environmental Science, 2014; 35(9): 3572–3579. (in Chinese)

[13] Gao Y, Lin S S, Li T Y, Hu Z. Effects of reclaimed water reuse on soil physiochemical properties and soil bacteria. Journal of Northwest Normal University (Natural Science), 2019; 55(4): 100–106. (in Chinese)

[14] Zolti A, Green S J, Ben Mordechay E, Hadar Y, Minz D. Root microbiome response to treated wastewater irrigation. Science of the Total Environment, 2019; 655: 889–907.

[15] Wang Y, Cheng D H, Tan W B, Yu H, Xi B D, Jiang Y H, et al. Different responses of soil microbial community structure to irrigation with treated wastewater from domestic and industrial sources. Environmental Science, 2020; 41(9): 4253–5261. (in Chinese)

[16] Cui B J, Gao F, Hu C, Li Z Y, Fan X Y, Cui E P. Effect of different reclaimed water irrigation methods on bacterial community diversity and pathogen abundance in the soil-pepper ecosystem. Environmental Science, 2019; 40(11): 5151–5163. (in Chinese)

[17] Jiao Z H, Huang Z B, Li Y, Wang W P, Yan B, Peng L C, et al. The effect of reclaimed water irrigation on soil performance and the microorganism. Journal of Agro-Environment Science, 2010; 29(2): 319–323. (in Chinese)

[18] Wu W Y, Ma M, Hu Y Q, Yu W C, Liu H L, Bao Z. The fate and impacts of pharmaceuticals and personal care products and microbes in agricultural soils with long term irrigation with reclaimed water. Agricultural Water Management, 2021; 251: 106862.

[19] Huang X R, Xiong W, Liu W, Guo X Y. Effect of reclaimed water effluent on bacterial community structure in the *Typha angustifolia* L. rhizosphere soil of urbanized riverside wetland, China. Journal of Environmental Sciences, 2017; 55(5): 58–68. (in Chinese)

[20] Han L B, Zhou L B, Gan Y P, Xie X M. Effects of reclaimed water irrigation on soil microorganism of turf. Journal of Beijing Forestry University, 2006; 28(S1): 73–77. (in Chinese)

[21] Liu H, Fan D F, Huang Y D, Wu Y G, Hu L N. Effects of rural mixed wastewater irrigation on soil nutrient content, enzyme activity, and microbial diversity. Chinese Journal of Ecology, 2019; 38(8): 2426–2432. (in Chinese)

[22] Zhang C Y, Wang Y J, Xu D L, Wang T X. Effects of sewage irrigation on quantity and distribution of microorganisms in soils. Ecology and Environmental Sciences, 2014; 3: 490–495. (in Chinese)

[23] Hubner U, Miehe U, Jekel M. Optimized removal of dissolved organic carbon and trace organic contaminants during combined ozonation and artificial groundwater recharge. Water Research, 2012; 46(18): 6059–6068.

[24] Zhou Y, Li P, Qi X B, Hu C, Guo W. Influence of nitrogen rate on nitrogen release pattern in soil irrigated with reclaimed wastewater. Acta Science Circumstance, 2016; 36: 1369–1374. (in Chinese)

[25] Wang Z, Li J S, Li Y. F. Using reclaimed water for agricultural and landscape irrigation in China: A review. Irrigation and Drainage, 2017; 66: 672–686.

[26] GB5084–2021. GAQSIQ (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China). Standards for Irrigation Water Quality, 2021. (in Chinese)

[27] Zhang D D, Zheng X Q, Li H Y, Chen P Z, Zhang G T, Kong H. Effects of rural domestic sewage irrigation with different salinity on soil microorganisms. Research of Environmental Sciences, 2022; 35(8): 1873–1884. (in Chinese)

[28] Liu Y, Cui E P, Li Z Y, Du Z J, Gao F, Fan X Y. Effects of irrigating biochar-and pectin-amended soil with treated municipal wastewater and swine wastewater on soil salinity and sodicity. Journal of Irrigation and Drainage, 2018; 37(6): 16–23.

[29] Wu X F, He Y, Huang Z P, Zhang D D, Zheng H Y, Ding J. Effects of different treatment levels of sewage on bacterial community structure and enzyme activity. Journal of Agro-Environment Science, 2020; 39(9): 2026–2035. (in Chinese)