Influence of Different Irrigation Water Qualities and Irrigation Techniques on the Soil Attributes and Bacterial Community Structure

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Abstract: Rising freshwater scarcities pose a serious threat to agricultural production. Reclaimed water (RW) is increasingly utilized as one of the alternative resources for irrigation in agriculture. Microbial communities play crucial roles in the soil microenvironment and can be used as effective indicators to assess the ecological influence of RW irrigation in soil. However, there is a lack of research on the effects of RW with different irrigation techniques on soil attributes and microbial communities. The present experiment was conducted in China to investigate the effect of two kinds of water qualities (RW and clean water (CW)), two kinds of irrigation methods (full irrigation (FI) and alternate partial root-zone irrigation (APRI)), and two kinds of irrigation techniques (furrow irrigation (FUI) and subsurface drip irrigation (SDI)) on soil chemical properties, heavy metal concentrations, and bacterial community structure. The APRI treatments received 70% of the irrigation water volume of FI. The results revealed that electrical conductivity (EC), nitrate nitrogen (NO$_3^-$-N), and heavy metal (Cu, Cd, Pb, Zn) concentrations in soil irrigated with RW were significantly higher in comparison to the soil irrigated with CW. SDI significantly decreased the contents of TN by 4.88%, the EC by 13.78%, and the heavy metal Cd concentration by 13.14% in soils than that irrigated with FUI treatment. APRI significantly decreased the heavy metal Cu concentration in soils by 6.26% compared to FI treatment. Proteobacteria, Chloroflexi, Acidobacteria, and Gemmatimonadetes in soil irrigated with RW were more abundant than that irrigated with CW. The irrigation water quality, soil moisture content, heavy metal content, TN, and EC under various irrigation techniques and methods significantly affected the structure of soil bacterial communities. In conclusion, we highlight that the SDI-APRI treatment can be an efficient irrigation practice for reducing the EC, heavy metal pollution, and the security risks of soil irrigated by RW.

Keywords: reclaimed water; heavy metals; microbial community structure; alternate partial root-zone irrigation; subsurface drip irrigation

1. Introduction

The world’s freshwater supply is steadily decreasing due to the increase in population and water demand [1]. Available water resources are subjected to ever-increasing pressure due to increasing agricultural water demand for irrigated lands [2]. With increasing global conflicts over the shortage of water resources, reclaimed water (RW), as one of the essential water sources in agriculture, has received increasing attention [3–5]. Rational reuse of reclaimed water in irrigation will save huge amounts of freshwater resources [6]. However, the safety of RW for agricultural irrigation is an issue of concern. RW is obtained after sewage treatment through a series of regeneration processes. However, it still contains a
certain quantity of substances harmful to the environment, especially in the form of high levels of salts, organic residues, and heavy metals [7,8]. Accumulating numbers of these pollutants in the soil can be harmful in fields irrigated for a long time [9,10]. These can cause the soil environment to deteriorate, jeopardize agricultural sustainability, but also potentially threaten human health via the food chain [11]. Therefore, it is essential to identify optimized water-saving irrigation techniques to ensure safe and efficient agricultural production under RW irrigation.

RW irrigation with different irrigation techniques significantly affects the soil’s physical-chemical properties and microbial community characteristics. Alternate partial root-zone irrigation (APRI) has been shown to improve water use efficiency significantly and crop yield compared to traditional irrigation methods [12–14]. In addition, the study indicated that APRI could optimize soil environmental quality [15,16]. Alternate partial root-zone drip irrigation (ADI) has been found to promote tomato growth and fertilizer-nitrogen use efficiency [14]. The cycling and use of soil nutrients are closely associated with soil microbes [17]. Wang et al. [18] indicated that the soil bacterial community structure was markedly different under alternate partial root-zone drip irrigation compared to ground drip irrigation. Moreover, irrigation methods substantially affect the distribution of soil microbes.

The microbial community is an essential component of the soil and plays a vital role in maintaining the ecological functions of the soil; slight changes in soil attributes may affect the soil microbial community [19]. Maintaining the complexity and diversity of soil microbial communities is critical to maintaining soil fertility [20]. The soil microbial community structure changes will affect soil fertility and cause a comprehensive change in soil quality [19,21]. The contaminants and the nutrient amendment in RW can, directly and indirectly, influence soil fertility and alter the soil microbial community [22,23].

Relevant studies have been mainly performed from the perspective of irrigation water quality or irrigation technique as a single variable on the soil quality and microbial community changes [18,24]. There is a lack of a comprehensive list of the impacts of RW irrigation on soil characteristics, heavy metal concentration, and soil bacterial communities under various combinations of different irrigation methods and techniques. The relevant mechanism is still unclear. Thus, in this study, we used the micro-plot trials to assess the effects of RW and clean water (CW) irrigation on tomato (Solanum lycopersicum L.) soil’s environmental physicochemical properties and the bacterial community composition and structure under FUI or SDI systems with full irrigation (FI) or APRI. The aims of the current study were to (1) evaluate the impacts of soil physicochemical properties and heavy metal contamination on microbial community structure and composition with different irrigation practices, and (2) provide an appropriate irrigation management strategy and theoretical guidance under RW irrigation, to promote the safe utilization of RW for agricultural sustainability.

2. Materials and Methods

2.1. Experimental Design

The study was carried out in greenhouses at the Agriculture Water and Soil Environment Field Science Research Station, China (35°19′ N, 113°53′ E, elevation 73.2 m), in the continental monsoon climate area of the temperate zone. The annual mean air temperature of the site is 14.1 °C. The site has 588.8 mm of precipitation annually and 2398.8 h of sunlight per year, and a 210-day frost-free period. In the present study, we assessed the impact of two types of water qualities (RW and CW), two types of irrigation methods (FI and APRI), and two types of irrigation techniques (FUI and SDI) on soil heavy metal concentrations and bacterial community composition. There were eight treatments in total:

i. APRI with RW and SDI (RSA);
ii. FI with RW and SDI (RSF);
iii. APRI with RW and FUI (RFA);
iv. FI with RW and FUI (RFF);
v. APRI with CW and SDI (WSA);
vi. FI with CW and SDI (WSF);

vii. APRI with CW and FUI (WFA); and

viii. FI with CW and FUI (WFF).

The experiment was conducted as a randomized complete block design, and each treatment was replicated three times. The RW was obtained from the Camel Bay sewage treatment plants, a source of city urban sewage, after secondary treatment. The typical factors of RW met the National Standard for Farmland Irrigation Water Quality (GB5084-2005). The water quality results are shown in Table 1. The APRI treatments obtained 70% of the FI irrigation water volume. The basic physical and chemical properties of tested soil are shown in Table 2.

Table 1. Quality index of reclaimed water and tap water.

<table>
<thead>
<tr>
<th>Index</th>
<th>pH</th>
<th>Cd  /µg L⁻¹</th>
<th>Cu  /mg L⁻¹</th>
<th>Pb  /mg L⁻¹</th>
<th>Zn  /mg L⁻¹</th>
<th>CODMn /mg L⁻¹</th>
<th>TN  /mg L⁻¹</th>
<th>TP  /mg L⁻¹</th>
<th>EC /µS cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reclaimed water</td>
<td>7.84</td>
<td>0.0021</td>
<td>0.035</td>
<td>0.026</td>
<td>0.772</td>
<td>17.6</td>
<td>29.57</td>
<td>1.95</td>
<td>2.06</td>
</tr>
<tr>
<td>Clean water</td>
<td>7.32</td>
<td>0.0004</td>
<td>0.006</td>
<td>0.005</td>
<td>0.016</td>
<td>7.2</td>
<td>4.63</td>
<td>0.23</td>
<td>1.62</td>
</tr>
</tbody>
</table>

pH, water pH value; CODMn, permanganate index; TN, total nitrogen; TP, total phosphorus; EC, electrical conductivity. The same as below.

Table 2. Basic physicochemical properties of tested soil before the experiment.

<table>
<thead>
<tr>
<th>Soil Texture</th>
<th>Bulk Density /g cm⁻³</th>
<th>OM /g kg⁻¹</th>
<th>TP /g kg⁻¹</th>
<th>TN /g kg⁻¹</th>
<th>EC /µS cm⁻¹</th>
<th>NO₃⁻-N /mg kg⁻¹</th>
<th>pH</th>
<th>Pb /mg kg⁻¹</th>
<th>Cd /mg kg⁻¹</th>
<th>Cu /mg kg⁻¹</th>
<th>Zn /mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandy loam</td>
<td>1.40</td>
<td>19.9</td>
<td>1.70</td>
<td>0.89</td>
<td>657.05</td>
<td>68.2</td>
<td>8.81</td>
<td>12.08</td>
<td>0.126</td>
<td>25.94</td>
<td>90.08</td>
</tr>
</tbody>
</table>

OM, soil organic matter; NO₃⁻-N, nitrate nitrogen; pH, soil pH value. The same as below.

GBS-fushi 1 tomatoes (*Solanum lycopersicum* L.) were used as the test crops. Tomato seedlings were planted in the plots at a density of 4.5 × 10⁴ plants ha⁻¹, with a planting distance of 0.5 m and a row spacing of 1.2 m. Each tomato row had two surface drip lines, one on each side of the tomato row. The automated irrigation system was installed with a pump, control unit, and pipelines. Irrigations in all plots were carried out when about 40% of the available water in the control plot was consumed.

In the SDI treatments, drip irrigation lines were laid at the centers of crop rows and separated by a 0.7 m distance in the experimental FUI treatment plots. On the other hand, in the APRI treatment plots, each row of tomatoes had two lateral lines with a distance of 0.4 m between them and with a nested shape for the emitters. The distance between the treatments was 0.75 m.

2.2. Water, Soil, and Plant Measurement and Analysis

Water quality indices included nitrate nitrogen (NO₃⁻-N), ammonium nitrogen (NH₄⁺-N), total nitrogen (TN), and total phosphorus (TP), which were determined with a flow analyzer (Bran Luebbe AA3); permanganate index (chemical oxygen demand, CODMn), which was determined with a COD analyzer; and the pH and electrical conductivity (EC) in water samples, which were measured directly by a pH meter (PHS-1, Shanghai, China) and electrical conductivity meter (DDB-303A, Shanghai, China), respectively. Cu, Cd, Pb, and Zn concentrations were determined by microwave digestion atomic absorption spectrophotometry (AA-6300, Shimadzu, Kyoto, Japan).

The surface layer (0–20 cm depth) soil samples in all plots were collected using a 3.8 cm diameter soil auger after the fresh tomatoes were harvested. Each sample consisted of 9 cores from the surface layer soil that were immediately mixed together in one composite sample representing one plot. Collected soil samples were homogenized and sieved (2.0 mm) to obtain a representative microbial community. The sieved samples were subsequently divided into two sub-samples. One sub-sample was stored at −20 °C for microbial analysis, while the other sub-sample was air-dried for the analysis of heavy
metals and soil properties [25] (Liu et al., 2018). All parameters (bulk density, soil water content (SWC), and pH, EC, OM, TN, Pb, Zn, Cu, Cd, and Cr contents) were determined by common methods used in practice. Soil pH was measured with a pH acidity meter (PHS-1, Shanghai, China) in deionized water at a soil-to-solution mass ratio of 1:2.5. Soil organic matter (SOM) content was determined according to Bao [26] (2000) by the oxidation volumetric method for the determination of potassium dichromate. The EC of soil was measured using a conductivity meter (DDB-303A, Shanghai, China) in extracts of soil pastes (1:5 soil-to-water ratios).

2.3. The Bacterial Communities in the Soils

The bacterial communities of the soils were determined by performing high-throughput 16S rRNA pyrosequencing on an Illumina MiSeq platform (Majorbio Company Technologies Co., Ltd., Shanghai, China). Total soil DNA was extracted from 500 mg of freeze-dried soil from each sample using a Power Soil DNA Isolation kit (MO BIO, Carlsbad, CA, USA). The V3–V4 hypervariable region was amplified by PCR using the universal forward 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and reverse 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers. Each sample’s sequences were optimized by removing low-quality reads, unrecognized reverse primers, and ambiguous base calls. The high-quality sequences that remained were clustered into operational taxonomic units (OTUs) using UCLUST at a 97% similarity level. A representative sequence from each OTU was classified phylogenetically and assigned a taxonomic identity using BLAST against the Ribosomal Database Project. All high-quality reads were classified taxonomically (phylum to genus) using the default settings of Quantitative Insights into Microbial Ecology (QIIME) [27]. The bacterial diversity and richness index was calculated based on 97% OTU similarity of obtained bacterial sequences. The structure and abundance of the bacterial community were analyzed using 16S rRNA sequencing.

2.4. Data and Statistical Analysis

The differences among treatment means were tested by analysis of variance (ANOVA), using SPSS 23.0 (SPSS Inc. Chicago, IL, USA). Duncan test was used to perform multiple comparative analyses among the treatments at the selected confidence level of 95%. Redundancy analyses (RDA) were conducted with CANOCO (Version 4.5, Microcomputer Power Company, Ithaca, NY, USA). Other statistical analyses were carried out using R version 2.8.1 (https://www.r-project.org, accessed on 22 December 2008).

3. Results

3.1. Soil Attributes

Three-factor analysis of variance showed that soil TN, TP, EC, NO$_3^-$-N, and SWC were significantly changed by the irrigation water type (Table 3). The TN and TP contents in RW-irrigated soil were significantly decreased by 7.23% and 12.5% compared to those of the CW-irrigated soil ($p < 0.01$), while EC, NO$_3^-$-N, and SWC significantly increased by 5.45%, 83.48%, and 18.7% in RW-irrigated soils in relative to CW-irrigated soils, respectively ($p < 0.05$). The irrigation water type had no significant effect on the soil pH and OM ($p > 0.05$). The irrigation techniques had a significant effect on soil TN, EC, and SWC ($p < 0.05$). SDI significantly decreased the contents of TN by 4.88% and the EC by 13.78% in the soils compared to FUI treatment. In addition, the interactions between irrigation water type and irrigation method significantly influenced soil TN, OM, and NO$_3^-$-N ($p < 0.05$). The interactions between irrigation water type and irrigation technique significantly influenced soil EC, TP, and NO$_3^-$-N. The interactions between irrigation methods and irrigation techniques significantly influenced soil EC, OM, pH, and NO$_3^-$-N (Table 3).
Table 3. Soil physicochemical properties in the root layer under different irrigation treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TN /g kg⁻¹</th>
<th>EC /µS cm⁻¹</th>
<th>OM /g kg⁻¹</th>
<th>pH</th>
<th>TP /g kg⁻¹</th>
<th>NO₃⁻-N /g kg⁻¹</th>
<th>SWC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Type</td>
<td>11.86 **</td>
<td>7.77 *</td>
<td>NS</td>
<td>NS</td>
<td>30.15 ***</td>
<td>33.82 ***</td>
<td>25.61 ***</td>
</tr>
<tr>
<td>RW</td>
<td>0.77 B</td>
<td>792.8 a</td>
<td>22.95 a</td>
<td>8.70 a</td>
<td>1.61 B</td>
<td>61.1 A</td>
<td>20.18 A</td>
</tr>
<tr>
<td>CW</td>
<td>0.83 A</td>
<td>751.8 b</td>
<td>22.89 a</td>
<td>8.74 a</td>
<td>1.84 A</td>
<td>33.3 B</td>
<td>17.00 B</td>
</tr>
<tr>
<td>Irrigation Methods</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>7.33 *</td>
<td>NS</td>
</tr>
<tr>
<td>FI</td>
<td>0.81 a</td>
<td>777.8 a</td>
<td>23.55 a</td>
<td>8.71 a</td>
<td>1.72 a</td>
<td>40.71 b</td>
<td>18.70 a</td>
</tr>
<tr>
<td>APRI</td>
<td>0.79 a</td>
<td>766.8 a</td>
<td>22.29 a</td>
<td>8.73 a</td>
<td>1.73 a</td>
<td>53.64 a</td>
<td>18.49 a</td>
</tr>
<tr>
<td>Irrigation Techniques</td>
<td>5.18 *</td>
<td>42.41 ***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>5.19 *</td>
</tr>
<tr>
<td>Water Type × Irrigation Methods</td>
<td>5.26 *</td>
<td>0.37</td>
<td>7.62 *</td>
<td>1.03</td>
<td>1.01</td>
<td>4.54 *</td>
<td>0.07</td>
</tr>
<tr>
<td>Water Type × Irrigation Techniques</td>
<td>3.85</td>
<td>5.14 *</td>
<td>3.00</td>
<td>0.14</td>
<td>7.50 *</td>
<td>44.24 ***</td>
<td>0.08</td>
</tr>
<tr>
<td>Irrigation Methods × Irrigation Techniques</td>
<td>0.18</td>
<td>5.99 *</td>
<td>9.47 **</td>
<td>6.64 *</td>
<td>3.89</td>
<td>47.32 ***</td>
<td>1.12</td>
</tr>
<tr>
<td>Water Type × Irrigation Methods × Irrigation Techniques</td>
<td>5.50 *</td>
<td>0.99</td>
<td>0.93</td>
<td>0.05</td>
<td>10.72 **</td>
<td>0.14</td>
<td>0.58</td>
</tr>
</tbody>
</table>

(1) RW, reclaimed water; CW, clean water; FI, full irrigation; APRI, alternate partial root-zone irrigation; FUI, furrow irrigation; SDI, subsurface drip irrigation. (2) SWC, soil water content. The different lowercase letters in the same column represent the significant difference at p < 0.05 among the treatments, and the different capital letters in the same column represent the significant difference at p < 0.01 among the treatments. * means the significance at 0.05 (p = 0.05) level, ** means the significance at 0.01 (p = 0.01) level, *** means the significance at 0.001 (p = 0.001) level, NS means no significance at 0.05 (p = 0.05) level. the same as below.

3.2. Soil Heavy Metal

The irrigation water type significantly changed soil heavy metal concentrations (Table 4). RW irrigation significantly increased the soil’s Cd, Pb, Zn, and Cu contents by 9.62%, 10.91%, 3.21%, and 7.59%, respectively, compared with CW irrigation (p < 0.05). The irrigation technique significantly changed the soil Cd and Pb contents. SDI significantly decreased the Cd content by 13.14%, and Pb by 3.79% in the soil compared with soils under FUI treatment (p < 0.05). Otherwise, APRI significantly decreased the heavy metal Cu concentration in soils by 6.26% compared to FI treatment. The interactions between irrigation water type and irrigation technique had a significant influence on the concentrations of Cd and Cu in soil (p < 0.001). The interactions between irrigation method and irrigation technique significantly influenced the concentrations of Cd, Pb, and Cu in the surface layer soil (p < 0.01).

Table 4. Heavy metal properties in the surface soil under different irrigation treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cd /mg kg⁻¹</th>
<th>Pb /mg kg⁻¹</th>
<th>Zn /mg kg⁻¹</th>
<th>Cu /mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Type</td>
<td>41.04 ***</td>
<td>42.41 ***</td>
<td>4.60 *</td>
<td>5.64 *</td>
</tr>
<tr>
<td>RW</td>
<td>1.71 A</td>
<td>16.88 a</td>
<td>95.95 a</td>
<td>26.64 a</td>
</tr>
<tr>
<td>CW</td>
<td>1.56 B</td>
<td>15.22 b</td>
<td>92.97 b</td>
<td>24.76 b</td>
</tr>
<tr>
<td>Irrigation Methods</td>
<td>1.47</td>
<td>0.29</td>
<td>NS</td>
<td>4.37 *</td>
</tr>
<tr>
<td>FI</td>
<td>0.162 a</td>
<td>15.98 a</td>
<td>95.13 a</td>
<td>26.53 a</td>
</tr>
<tr>
<td>APRI</td>
<td>0.165 a</td>
<td>16.12 a</td>
<td>93.79 a</td>
<td>24.87 b</td>
</tr>
<tr>
<td>Irrigation Techniques</td>
<td>102.82 ***</td>
<td>5.90 *</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>FUI</td>
<td>0.175 A</td>
<td>16.36 a</td>
<td>93.67 a</td>
<td>25.79 a</td>
</tr>
<tr>
<td>SDI</td>
<td>0.152 B</td>
<td>15.74 b</td>
<td>95.25 a</td>
<td>25.61 a</td>
</tr>
<tr>
<td>Water Type × Irrigation Methods</td>
<td>0.05</td>
<td>6.97 *</td>
<td>0.86</td>
<td>1.72</td>
</tr>
</tbody>
</table>
3.3. Composition and Diversity of Soil Bacterial Communities

Across eight treatments, a total of 1,142,215 high-quality bacterial sequences were obtained, which were classified into 3693 OTUs. They belong to 41 phyla, 91 classes, 196 orders, 371 families, and 726 genera. The predominant phyla of bacterial communities were: Proteobacteria (28.0–35.1%), Actinobacteria (14.2–16.4%), Chloroflexi (11.4–14.7%), Acidobacteria (9.8–15.3%), Firmicutes (8.5–12.0%), Bacteroidetes (4.9–6.4%), which accounted for more than 80% of the total bacterial sequences in the eight treatments (Figure 1). Proteobacteria was the dominant phylum in soils with different irrigation treatments. The abundances of Proteobacteria, Chloroflexi, and Acidobacteria in soil irrigated with RW increased by 6.4%, 3.5%, and 11.8%, respectively, compared to soil irrigated with CW irrigation. The abundances of Actinobacteria, Firmicutes, and Bacteroidetes in soil irrigated with RW decreased by 7.4%, 14.2%, and 49.1% compared to soil irrigated with CW irrigation. With the same irrigation method, the abundance of Proteobacteria in soil with APRI decreased by 5.8% compared to the soil under FI treatment, whereas the abundance of Chloroflexi, Acidobacteria, and Firmicutes in soil with APRI increased by 10.5%, 5.8%, 6.7%, respectively, compared with FI treatment. Using the same irrigation technology, the abundance of Firmicutes and Bacteroidetes in soil with SDI increased by 14.7% and 6.2%, respectively, compared to FUI treatment, and the abundance of Chloroflexi with SDI decreased by 6.1% compared to FUI treatment.

![Community barplot analysis](image)

**Figure 1.** The effect of APRI with reclaimed water on soil microbial community at phylum level.
As shown in Table 5, the ACE and Good’s coverage were significantly changed by the irrigation technique \((p < 0.05)\). SDI significantly increased the ACE, but decreased the Good’s coverage compared with FUI treatment. The interactions between irrigation method and irrigation technique significantly influenced the richness and diversity indexes, ACE, Good’s coverage, and Simpson indices of the bacterial community. The highest bacterial Shannon diversity and PD diversity were observed in RFA, while the Simpson diversity of RFA was the lowest, and that of WFF was the highest, which indicated that RFA significantly increased the diversity of the bacterial community \((p < 0.05)\). The ACE and Chao of WSF were the highest, and those of RFF were the lowest, which indicated that RFF significantly reduced the richness of the bacterial community \((p < 0.05)\). The values of the Good’s coverage estimators were in the range of 0.9738 and 0.9756 at a 97% similarity cutoff, which suggested that the current numbers of the sequence reads were sufficient to capture the diversity of the soil bacterial community.

### Table 5. Bacterial community diversity indices in different samples.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Richness</th>
<th>Diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sobs</td>
<td>Cha1</td>
</tr>
<tr>
<td>RSA</td>
<td>2511 ± 45 a</td>
<td>3123 ± 24 ab</td>
</tr>
<tr>
<td>RSF</td>
<td>2503 ± 14 ab</td>
<td>3106 ± 14 ab</td>
</tr>
<tr>
<td>RFA</td>
<td>2512 ± 21 a</td>
<td>3089 ± 24 ab</td>
</tr>
<tr>
<td>RFF</td>
<td>2477 ± 45 ab</td>
<td>3017 ± 54 a</td>
</tr>
<tr>
<td>WSA</td>
<td>2445 ± 32 b</td>
<td>3063 ± 78 bc</td>
</tr>
<tr>
<td>WFF</td>
<td>2513 ± 9 a</td>
<td>3153 ± 21 a</td>
</tr>
<tr>
<td>WFA</td>
<td>2520 ± 48 a</td>
<td>3151 ± 16 a</td>
</tr>
<tr>
<td>WFF</td>
<td>2465 ± 18 ab</td>
<td>3061 ± 15 bc</td>
</tr>
</tbody>
</table>

Significance based on three-way ANOVA \((F \text{ value})\)

- Water Type: 0.869, 2.306, 3.293, 2.780, 0.850, 0.850, 3.743, 0.259
- Irrigation Methods: 0.225, 2.148, 1.525, 0.182, 0.258, 0.081, 2.167
- Irrigation Techniques: 0.005, 4.151, 0.208, 0.066, 6.692 *, 8.149 *, 0.926
- Water Type × Irrigation Methods: 1.112, 2.135, 0.312, 0.144, 3.743, 2.097, 0.042
- Water Type × Irrigation Techniques: 0.958, 3.830, 0.301, 0.107, 3.283, 2.505, 0.134
- Water Type × Irrigation Methods × Irrigation Techniques: 3.284, 4.221, 0.095, 0.885, 7.838 *, 4.172, 0.303

RSA, APRI with RW and SDI; RSF, FI with RW and SDI; RFA, APRI with RW and FUI; RFF, FI with RW and FUI; WSA, APRI with CW and SDI; WSF, FI with CW and SDI; WFA, APRI with CW and FUI; WFF, FI with CW and FUI. The same as below. The different lowercase letters in the same column represent the significant difference at \(p < 0.05\) among the treatments. * means the significance at 0.05 \((p = 0.05)\) level, ** means the significance at 0.01 \((p = 0.01)\) level.

### 3.4. Taxonomic Composition of Soil Bacterial Communities and Correlations between the Microbial Community and Soil Properties

NMDs based on the Bray–Curtis similarity was performed to analyze the separation patterns between the microbial community compositions of the samples under different irrigation treatments (Figure 2). The NMDs stress co-efficient of bacterial communities was 0.172. The results of NMDs indicated that the variation in soil bacterial community composition was significantly regulated by irrigation water type and irrigation technique using the PERMANOVA method. NMDs ordination clearly distinguished between communities in RW-irrigated and CW-irrigated soil samples.
RSA, APRI with RW and SDI; RSF, FI with RW and SDI; RFA, APRI with RW and FUI; RFF, FI with RW and FUI; WSA, APRI with CW and SDI; WSF, FI with CW and SDI; WFA, APRI with CW and FUI; WFF, FI with CW and FUI. The same as below. The different lowercase letters in the same column represent the significant difference at $p < 0.05$ among the treatments. * means the significance at 0.05 ($p = 0.05$) level, ** means the significance at 0.01 ($p = 0.01$) level.

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Furthermore, bacterial community variation from the phylum to genus levels in different treatments was identified by LEfSe analysis (LDA score > 3.0, $p < 0.05$). There were statistically significant differences in the bacterial taxa and the biological relevance of the species in soils irrigated with RW and CW (Figure 3a). More specifically, there was a significant enrichment of Pseudomonas (from order to genus), Sphingomonas (from order to genus), and Blastocatellaceae_Subgroup_4 (from order to genus) in RW-irrigated soils. Pseudomonas is a common human and plant pathogen and ammonifying bacterium found in soils. Compared to the soils treated with RW, six groups of bacteria were most strongly associated with CW irrigation, namely, cyanobacteria, Actinobacteria, Bacillales, Bacillus, Subsectionlll, and Phormidium. Conversely, Frenk et al. [22] found that the relative abundance of Sphingomonas increased and the relative abundance of Bacillales decreased when fresh water was used for irrigation, compared to the soils treated with RW.

Under different irrigation techniques, five groups were most strongly associated with FUI, namely Rhodospirillales, MSB_1E8_f_norank (from family to genus), Gemmatimonadaeae_f_norank (from phylum to genus), Micrococcaceae (family), and TK10_c_norank (from class to genus). In contrast, twelve groups were most strongly associated with SDI, namely Firmicutes, Xanthomonadaceae, Acidimicrobiales, norank_f_OM1_clade (from family to genus), Cytophagaceae (from class to family), Bacillus (from class to genus), norank_f_JTB255_marine_benthic_group (from family to genus), Pontibacter, Cytophagaceae, norank_c_KD4-96 (from class to genus), Myxococcales, and Bacillaceae_f_unclassified (Figure 3b). Under various irrigation methods, two groups were most strongly associated with FI, namely Rhodospirillales, and norank_f_MSB (from family to genus), whereas Verrucomicrobia (the phylum) were more abundant in APRI soils (Figure 3c). The results of LEfSe indicated that irrigation water type and irrigation technique have a greater impact on bacterial community composition than irrigation method. This may be because the irrigation water type and irrigation technology had a more significant influence on soil physicochemical properties, which further affected the bacterial community composition.
Figure 2. The soil bacterial community structure changes with different irrigation water qualities, methods, and techniques. Non-metric multidimensional scaling (NMDS) based on the Bray–Curtis of OTU clustered by 97% similarity of the bacterial community.

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Figure 3. Linear discriminant analysis Effect Size (LEfSe) results of bacterial taxa in soils irrigated with different water types (a), irrigation technologies (b), and methods (c). The linear discriminant analysis (LDA) scores derived from LEfSe analysis, showing the biomarker taxa (LDA score of >3.0 and a significance of \( p < 0.05 \) determined by the non-parametric factorial Kruskal–Wallis (KW) sum-rank test). R and C represent reclaimed water-irrigated and clean water-irrigated soil samples, respectively. F and S represent furrow-irrigated and subsurface drip-irrigated soil samples, respectively. AL and FL represent alternate partial root-zone irrigated and Full-irrigated soil samples, respectively.
To clarify the effect of RW with different irrigation technologies on bacteria related to soil nutrients, our study deeply analyzed the correlations among environmental factors and microbial abundance at the genus level (Figure 4). The abundance of *Lysobacter* was negatively correlated with TN, TP, and AK \( (p < 0.05) \), but *norank_f_Gemmatimonadetes* was positively correlated with TN, TP, and AK \( (p < 0.05) \), H16, *norank_c_Acidobacteria*, *norank_o_JG30-KF_CM45*, and RB41 were positively correlated with OM, H16 and *norank_f_Nitrosomonadaceae* were positively correlated with pH \( (p < 0.05) \), *Rhizobiales_o_unclassified* was significantly negatively correlated with EC \( (p < 0.01) \), *norank_f_OM1_clade*, and *norank_f_Gemmatimonadaceae* were significantly positively correlated with EC and SWC \( (p < 0.01) \), and *Bacillus* and *norank_f_JTB255_marine_benthic_group* were significantly negatively correlated with EC and SWC \( (p < 0.01) \), and NO\(_3^−-N\) was significantly negatively correlated with *Bacillus* and *norank_c_Gemmatimonadetes*, and significantly positively correlated with *norank_o_JG30-KF-CM45* \( (p < 0.01) \). *Sphingomonas* was significantly positively correlated with Zn, Cd, and Pb \( (p < 0.05) \), and *Rhizobiales_o_unclassified* was negatively correlated with Pb and Cu \( (p < 0.05) \). Cd was negatively correlated with *norank_c_Gemmatimonadetes*, and positively correlated with *norank_c_KD4-96*, *Streptomyces*, and *Sphingomonas* \( (p < 0.05) \); Pb was negatively correlated with *Bacillus*, *norank_f_OM1_clade*, and *norank_f_JTB255_marine_benthic_group*, and positively correlated with *Pseudomonas*, *Sphingomonas* and *norank_f_Gemmatimonadaceae*; *norank_o_JG30-KF_CM45* and *norank_f_Gemmatimonadaceae* were positively correlated with Cu; *Sphingomonas*, *Acidobacteria_c_norank*, and *norank_o_JG30-KF-CM45* were positively correlated with Zn.

![Spearman Correlation Heatmap](image)

**Figure 4.** The heatmap analysis between soil environmental factors and the relative abundance of soil microbes (genus level) under different irrigation treatments. The correlation analysis was Spearman correlation. * means the correlation is significant at 0.05 level. ** means the correlation is significant at 0.01 level. *** means the correlation is significant at 0.001 level.

RDA was used to quantify the correlation between microbial community and environmental factors (Figure 5). The dots with different colors or shapes in the figure represent...
the sample groups under different treatments. The arrow length reflects the impact of environmental factors on microbial community structure. The results showed that the heavy metals Pb, TP, AK, EC, and SWC had significant effects ($p < 0.05$) on the changes in the soil bacterial community structure, indicating that these environmental factors were the key factors affecting the microbial community. The AK and TP are closely related to Firmicutes and Actinobacteria, and the heavy metals Pb, EC, and SWC are closely related to Acidobacteria and Chloroflexi.

![Redundancy analysis of bacterial communities and environmental factors.](image)

**Figure 5.** Redundancy analysis of bacterial communities and environmental factors.

4. Discussion

4.1. Soil Physicochemical Properties and Heavy Metal Characteristics

For agro-ecosystems, RW is not only a source of water and fertilizer, but also a pollution source. In this study, RW irrigation significantly increased the soil EC and NO$_3^-$-N content, which was similar to the findings of Bastida et al. [24] and Wu et al. [28]. This is attributed to higher salt concentrations and nitrogen contents in RW. EC in the surface soil was significantly lower under SDI than under FUI. In comparison to FUI, the infiltration of irrigation water was more uniform under SDI, and soil salinity migrated to the deep layer continuously with water movement. Thus, salt in the surface soil easily seeps down to the deeper layers under SDI, creating an excellent hydro-saline environment for crop growth in the surface soil. Therefore, SDI treatment can significantly reduce the salinization risk in the surface layer soil under RW irrigation [29]. RW irrigation also reduced the TN, and TP content of the surface soil in comparison to the CW irrigation treatment, which could be explained directly by the greater fertility effect of RW irrigation [30]. On the contrary, some studies showed that soil fertility increased under RW irrigation [31]. In this study, tomato plants absorb large amounts of nutrients for vegetative growth and reproductive development. The rich nutrients in RW had a beneficial effect on the growth of their roots and fruits, reducing the nutrient content in the surface soil. Gu et al. [32] indicated that long-term RW irrigation significantly increases SOM content and may improve soil quality. However, there is no significant increase in SOM content under RW irrigation in our study, and that may be because the irrigation water resource was different.

Heavy metal pollution can have potential and cumulative effects, posing a significant threat to the soil environment and human health. Heavy metals, EC, and other nutrient
elements dissolved in RW can accumulate in the soil, which is mainly dependent on the quality of RW, irrigation techniques, and the duration of RW irrigation [33,34]. The knowledge of heavy metal concentrations in the soil is one of the most important critical issues for correctly assessing the risk of RW irrigation. In the present study, heavy metal (Cd, Pb, Zn, and Cu) concentrations in the soil treated with RW in drip irrigation/root zone irrigation patterns were higher than in the soil treated with CW, although the contents of heavy metals in the soil were lower than the national standard (Table 4). This indicated the residual effect of RW irrigation: given the relatively higher content of heavy metal ions in RW, more of such ions entered the soil irrigated with RW than that irrigated with CW, thereby elevating the soil’s heavy metal contents [35]. The study of Khaskhoussy et al. [36] indicated that RW irrigation significantly increased the Cu, Cd, and Ni contents in soils compared with CW irrigation. Regardless of FI or APRI, the Cd and Pb contents in the surface soil were significantly lower under SDI compared with FUI treatments, indicating that SDI treatment can significantly reduce the risk of Cd and Pb pollution in soils irrigated with RW.

4.2. Influences of Soil Environmental Parameters on Bacterial Community Structure and Composition

RW irrigation changed the growth of functional microorganisms related to the soil carbon and nitrogen cycle. This study found that Proteobacteria was the most prominent bacteria. The abundance of Proteobacteria in soil irrigated with RW was higher than that under CW irrigation. Using the same irrigation method, APRI reduced the abundance of Proteobacteria in soils compared with FI. In agreement with the results of our study, Li et al. [19] indicated that the relative abundance of Proteobacteria increased with an increase in the amount of irrigation water. As a dominant flora, Proteobacteria is highly susceptible to environmental disturbance and water-limiting stress, which has various growth habits in a polluted environment [27,37]. The changes in Proteobacteria’s response to RW irrigation may be because this phylum exhibits complex lifestyles and is capable of using all kinds of organic substances. Actinobacteria, the second dominant bacteria, was different from Proteobacteria, which was more abundant in soil irrigated with CW than that under RW irrigation (Figure 1). This was similar to other studies [22,24]. The abundances of Chloroflexi, Acidobacteria, and Gemmatimonadetes in soil irrigated with RW were higher than those in CW-irrigated soil. Chloroflexi is capable of fixing inorganic CO$_2$ and anaerobic photosynthesis [38]. Acidobacteria is closely associated with the metabolism of SOM [39], and Gemmatimonadetes had a strong function in denitrification [40]. These functional microorganisms enriched in RW irrigated soil can directly characterize soil nutrient transformation and microbial environmental adaptability. The relative abundances of Actinobacteria, Firmicutes, and Bacteroidetes in soil irrigated with RW were lower than those in CW-irrigated soil. Bacteroidetes play an important role in degrading cellulose in soils [41]. Firmicutes and Bacteroidetes are fast-growing opportunistic organisms that might benefit from environmental disturbances by taking over niches commonly occupied by other bacterial taxa. The impacts of water quality and the amount of irrigation water on these taxonomic groups can have consequences for ecosystem functionality [24].

The composition and structure of soil microorganisms can reflect the state of soil fertility [42]. Soil physicochemical properties and microbes mutually affect and promote each other through interactions. The changes in microbial characteristics result from multiple factors, for instance, soil physicochemical properties and heavy metals [43]. Different irrigation practices may change the attributes of soil, which are closely related to the structure of soil microbial communities. In this study, the changes in the bacterial community structure and composition were clearly affected by soil heavy metal concentrations and soil physicochemical properties (Figures 4 and 5). There was a significant correlation between soil microbes and environmental factors under different irrigation treatments. Our study found that NO$_3^-$-N, TP, AK, EC, and SWC were the most important factors affecting the
microbial community structure in RW-irrigated soils (Figure 5). This indicated that the abundant nutrient element could promote the growth of some specific bacteria.

Nutrients are the basis for soil microbial survival, and were necessary for the survival of microorganisms [27]. TN, TP, and AK were significantly correlated with Gemmatimonadetes_c_norank, and EC was significantly negatively correlated with Rhizobiales_o_ unclassified, Bacillus, OM1_clade_f_norank, and JTB255_marine_benthic_group_f_norank, which showed that the different irrigation practices mainly regulate the composition of bacterial communities by affecting soil nutrient and salinity. These results highlight the connections between the composition of the soil microbial community and soil attributes. Many studies revealed that pH significantly affected the diversity and composition of the microbial community [44,45]. In this study, H16 and Nitrosomonadaceae_f_norank were positively correlated with pH. However, Li et al. [46] indicated that there was no obvious difference in microbial composition in the RW-irrigated soil and groundwater-irrigated soils with a 40-year history.

Soil moisture influences microbial properties and function [47]. The amount of water is an essential factor influencing microbial activities and community diversity [27]. Water dissolves different kinds of nutrient elements, and sufficient soil water indicates an efficient nutrient supply [19]. Our results revealed that SWC was a fundamental environmental variable for structuring bacterial communities, as observed by the RDA of phylogenetic distances. In this study, OM1_clade_f_norank and Gemmatimonadaceae_f_norank had a significant positive correlation with SWC (Figure 4). The study of Wu et al. [48] indicated that SWC was the second most important factor affecting the composition of microbial communities.

Heavy metal pollution can significantly alter microbial community structure [43]. Due to their high sensitivity to metal-induced stress, microbial properties are commonly used as indicators of metal pollution [49]. Previous studies have shown that heavy metal pollution adversely affects the microbial community structure [50]. In this study, the soil microorganisms both positively and negatively correlated with heavy metal concentrations (Figure 5). This indicated that heavy metals inhibited the growth of soil microorganisms, while some microorganisms can survive in plight [51]. The strong correlations between the heavy metal contents of soil and bacterial communities indicated that soil heavy metal pollution is a significant factor affecting the development of microbes [52].

5. Conclusions

This study evaluated the influence of RW irrigation with varying irrigation techniques and methods on soil attributes and bacterial community structure in northwest China. Our results suggest that RW irrigation significantly increased the EC, NO$_3^-$-N, and heavy metal (Cd, Pb, Zn, and Cu) concentrations in plow layer soil compared to CW irrigation treatment. SDI significantly reduces the risk of soil salinization and Pb and Cd pollution in soils compared with FUI treatment. Compared to FI, APRI decreased the Cu concentration, but had no significant effect on the Cd, Pb, and Zn concentrations. The environmental factors were closely related to the changes in microbial communities. The bacterial community composition was significantly changed by the irrigation water type and irrigation technique. The major factors affecting the structure of microbial communities in the soil were the irrigation water type, irrigation technique, heavy metal content, pH, SWC, TP, AK, AN, and EC. Therefore, SDI-APRI treatment is a reasonable irrigation model for reducing the environmental pollution caused by RW irrigation. Otherwise, the potential ecological risk of RW irrigation over a long period of time should not be ignored.

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