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Changes of Microbial Diversity in Rhizosphere of Different Cadmium-Gradients Soil under Irrigation with Reclaimed Water

Jiaxin Cui [1,2], Ping Li [3], Xuebin Qi [1,4,*], Shafeeq Ur Rahman [5,6] and Zulin Zhang [7,8]

1   Farmland Irrigation Research Institute, Chinese Academy of Agricultural Sciences, Xinxiang 453002, China;
    cjx89jess@163.com
2   Graduate School of Chinese Academy of Agricultural Sciences, Beijing 100081, China
3   Water Environment Factor Risk Assessment Laboratory of Agricultural Products Quality and Safety,
    Ministry of Agriculture and Rural Affairs, Xinxiang 453002, China; liping05@caas.cn
4   Agricultural Water Soil Environmental Field Research Station of Xinxiang, Chinese Academy of
    Agricultural Sciences, Xinxiang 453002, China
5   MOE Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences,
    Peking University, Beijing 100871, China; malikshafeeq1559@gmail.com
6   School of Environment and Civil Engineering, Dongguan University of Technology,
    Dongguan 523015, China
7   The James Hutton Institute, Craigiebuckler, Aberdeen AB15 8QH, UK; zulin.zhang@hutton.ac.uk
8   China-UK Water and Soil Resources Sustainable Utilization Joint Research Centre, Xinxiang 453002, China
*   Correspondence: qxb6301@sina.cn

Abstract: Water scarcity and the uneven distribution of water resources in China have resulted in water shortages for agricultural irrigation in arid and semi-arid areas. Reclaimed water used for agricultural irrigation has become an effective solution in the context of the global water shortage. In order to improve soil productivity and solve the shortage of water resources, we carried out reclaimed water irrigation experiments on polluted soil. Compared with full irrigation treatments, the EC value of reclaimed water under deficit irrigation treatments decreased by 2.89–42.90%, and the content of organic matter increased by 6.31–12.10%. The proportion of *Acidobacteria* community in soils with different cadmium concentration gradients irrigated with reclaimed water ranged from 13.6% to 30.5%, its relative abundance decreased with the increase of soil cadmium concentration. In particular, the relative abundance of *Pseudomonas* pathogens in deficit irrigation treatments was lower than that of the full irrigation treatments. RDA analysis showed that the environmental factors that played a leading role in the change of microbial community structure were organic matter and pH. Furthermore, the metabolic function potential of the rhizosphere soil bacterial community in deficit irrigation treatments was higher than that of full irrigation treatments with reclaimed water. This study proved that reclaimed water irrigation for cadmium contaminated soil did not aggravate the pollution level and promoted the soil ecological environment with better microbial community diversity.

Keywords: cadmium pollution; reclaimed water; microbial diversity; relative abundance; metabolic pathway

1. Introduction

As a key factor for human survival, water resources have always been the top priority of researchers. The Synthesis Report shows that soil and water are complementary systems that have reached the limits of their functionality. The shortage of water resources endangers global food security and sustainable development and 3.2 billion people in agricultural areas [1]. Furthermore, the water resources per capita in China are just 2257 m³,

which is about 1/4 of the world's water resources per capita. According to internationally recognized standards, water resources per capita in China belong to the level of mild water shortage, which is approaching the level of moderate water shortage. Agriculture is the largest consumer of water resources in China. According to the 2020 China Water Resources Bulletin, the total national water dissipation was 581.29 billion m^3, and agricultural water resources were 361.24 billion m^3, accounting for 62.1% of national water consumption. China's irrigation area and water consumption ranked first in the world, but the shortage of irrigation water for agriculture can lead to a series of problems such as stability of food security[2]. The shortage of agricultural irrigation water will become an increasingly prominent issue. So, how do we alleviate the shortage of water resources for agriculture? In addition to overcoming different irrigation techniques, opening up non-traditional water sources can be considered for this initiative. The national government issued a guiding report on the recommendation on the use of reclaimed water in agriculture. Increasing the amount of reclaimed water has been widely accepted all over the world. For example, reclaimed water replaces some clean water as irrigation water sources, which is in line with scientific and sustainable development.

Reclaimed water can be beneficially used after urban sewage has been properly treated to meet certain water quality requirements and certain functional requirements[3]. This is important to ensure the path of sustainable development. Many other countries have already begun to develop and utilize reclaimed water on a large scale, becoming the world's recognized "second water source for cities" [4]. According to the National Water Resources Bulletin statistics, the utilization of recycled water in China was 1.090 billion m^3 in 2005, 7.35 billion m^3 in 2018, and 8.73 billion m^3 in 2019, showing a steady growth trend. From 2005 to 2018, the proportion of total water supply increased by 1.03% every year[5]. In addition to being used in urban production and living, the most optimal configuration of recycled water is in agricultural irrigation water, which can reduce the impact of other non-traditional water sources on the environment and balance other economic benefits[6,7]. The development of reclaimed water based on agricultural irrigation water sources has broad application prospects [8–10]).

In most arid or semi-arid regions, there are already proven technologies for agricultural production activities with reclaimed water irrigation[11–13]. The practice is also gaining popularity globally. The composition of reclaimed water is complex and rich in nutrients, which can increase soil organic matter, nitrogen, phosphorus, and potassium. To some extent, it can replace fertilizers and reduce land pollution caused by fertilization[14,15]. Reclaimed water contains many exogenous microorganisms, and irrigation into the soil will cause changes in the diversity of the soil microbial communities[16–18]. Numerous studies have shown that irrigation of soil with reclaimed water can increase the diversity of soil microorganisms[19–21].

Dwarf millennial red tomato (*Lycopersicon esculentum Mill.*) is a tomato variety that has become more popular in recent years for cultivation, with the advantages of low cost and considerable economic benefits[22]. In 2016, China already accounted for one-third of the world's total tomato production value, and the trend continues to grow[23]. Tomatoes are also one of the most common types of vegetable in China, they have a significant share in the country's vegetable industry, being one of the largest contributors to the growth of China's total agricultural production value. As a crop that teaches easy uptake and accumulates cadmium contamination, combined with the related studies of the PSC strategy[24] (pollution-safe cultivar), it was found that there is less literature studying the relationship between tomato and cadmium. Cadmium, a heavy non-ferrous metal naturally occurring in nature, is a common material in the manufacture of batteries. Because of its own toxicity and that of its compounds, it is potentially harmful to soil, crops, and humans. Cadmium is hard to degrade (a common characteristic of heavy metals), and when it enters the soil, it is difficult to degrade by soil microorganisms and other methods, resulting in its prolonged retention in the soil. When the content of cadmium exceeds the range that the soil can bear by itself, it will cause damage to its own fertility, and will also

damage the quality of the ecological environment and cause serious pollution consequences. Cd-contaminated soil microorganisms also change the relative abundance of different bacteria in the soil under long-term cadmium stress and change the diversity of their community structure[25–28]. The stability of the soil microbial community and its components has a crucial impact on the sustainable development, safety, and soil quality of soil [29–31]. Microbes not only participate in nutrient cycling, such as carbon and nitrogen cycling in soil, but also take part in detecting soil fertility[32,33].

In this study, we conducted pot experiments in slight to moderate Cd-contaminated soils under irrigation with reclaimed water coming from a municipal sewage treatment plant. The purpose is to explore the possibility of reclaimed water utilization in cadmium contaminated soils and reveal the mechanism of reclaimed water's safe reuse, so as to promote agricultural recycling use of reclaimed water.

2. Materials and Methods

2.1. Experimental Site

The experiment was carried out in a greenhouse (35.27′ N, 113.93′ E, 73.2 m above sea level) of the Agricultural Water and Soil Environment Field Scientific Observation and Experiment Station in Xinxiang, Henan, Chinese Academy of Agricultural Sciences. The average precipitation of Xinxiang city is 588.8 mm with large inter-annual variation, while the precipitation during the high flow years is 3 to 4 times greater than the low flow years. Moreover, the precipitation within the year is uneven, with maximum precipitation (70% of the annual precipitation) reported during the months of July to September. The annual average evaporation was recorded at 2000 mm in 2021 in Xinxiang.

2.2. Experimental Design

The soil used in this study was collected from a sewage irrigation area in Henan province. This area has been irrigated with sewage for historical reasons, resulting in cadmium contamination of the soil. The soil contained a certain heavy metal cadmium (Cd) content. The soil samples were naturally air-dried. The stones, plant fine roots, and biological residues visible to the naked eye were removed and then passed through a 5 mm sieve. According to the concentration of the first serious metal Cd within the soil, identical soil background is organized to confirm the consistency of soil physicochemical properties and soil microorganisms. We added Cd in the form of $CdCl_2 \cdot 2H_2O$ in the air-dried soil in the form of a water solution for a one-month passivation period to meet the configuration concentration. Soil cadmium content is configured according to soil environmental quality survey on soil pollution level classification (CHINA, 2014). The setting range of Cd concentration is Cd1-no pollution (less than 0.60 mg/kg), Cd2-slight pollution (0.60–1.20 mg/kg), Cd3-light pollution (1.20–1.80 mg/kg), Cd4-moderate pollution (1.80–3.00 mg/kg), and Cd5-moderate pollution (1.80–3.00 mg/kg). According to laboratory determination, the final Cd1-Cd5 contents were 0.30 mg/kg, 0.69 mg/kg, 1.20 mg/kg, 2.07 mg/kg, and 2.74 mg/kg, respectively.

The 10 treatments were marked with FCd1, FCd2, FCd3, FCd4, FCd5, DCd1, DCd2, DCd3, DCd4, and DCd5 (Table 1), with five replicates per treatment and 50 pots in total. Using a diameter of 38 cm, a bottom diameter of 30 cm, and a 40 cm high PVC material flower pot for potted plants. Each pot contained 36 kg of soil. We used dwarf millennial red tomato (*Lycopersicon esculentum Mill*.) plants as a model plant in our study. The tomato plants were one per pot. The planting time is from March 2021 to July 2021.

Table 1. Design explanation of different treatments.

Treatment	Irrigation Level	Irrigation level Symbol	Cadmium Pollution Concentration	
FCd1		F	Cd1	no pollution
FCd2		F	Cd2	slight pollution
FCd3	Full irrigation	F	Cd3	light pollution
FCd4		F	Cd4	moderate pollution
FCd5		F	Cd5	moderate pollution
DCd1		D	Cd1	no pollution
DCd2		D	Cd2	slight pollution
DCd3	Deficit irrigation	D	Cd3	light pollution
DCd4		D	Cd4	moderate pollution
DCd5		D	Cd5	moderate pollution

The present study was conducted by using two irrigation amounts: (1) full irrigation (F) means irrigation amount according to crop needs; and (2) the irrigation amount of deficit irrigation (D) is 70% of that of full irrigation. The reclaimed water used in this study was taken from the Luotuowan Urban Domestic Sewage Treatment Plant. The sewage source is mainly urban domestic wastewater. The treatment process was the A/O^2. The reuse of urban recycling water—Quality of farmland irrigation water (GB 20922-2007) and Standard for irrigation water quality (GB 5084-2021). After laboratory analysis of the physicochemical properties, it was determined that the reclaimed water had a pH value of 7.33, an EC value of 1298 $\mu s \cdot cm^{-1}$, a total nitrogen content of 8.9 $mg \cdot L^{-1}$, a total phosphorus content of 0.75 $mg \cdot L^{-1}$, a COD content of less than 15 $mg \cdot L^{-1}$, and a Cd content of less than 0.0001 $mg \cdot L^{-1}$.

The upper and lower limits of the irrigation water process were set at 90% and 60% of the soil moisture content, respectively. There was a HOBO measurement of water content. When the soil moisture content value from the HOBO measurement was equal to or less than the soil moisture lower limit value, the irrigation water volume per pot was 1 L in the full irrigation treatments and 0.7 L in the deficit irrigation treatments. The total amount of irrigation in the whole tomato growth period is 34 L for full irrigation and 24.1 L for deficit irrigation.

2.3. Measurement Items and Methods

2.3.1. Sample Collection

We performed rhizosphere soil collection in each replicate pot according to the rhizosphere soil collection method[34]. When collecting rhizosphere soils, it was done by replicate for each treatment, and then five replicate rhizosphere soil samples from each treatment were pooled into one sample, so a total of 10 samples were collected. Samples were placed in clean self-sealing bags and each composite sample was sieved using a 2 mm sieve, and then divided into two parts: one part was stored at room temperature and air-dried to determine basic physical and chemical properties of the soil, and the other one was for subsequent assessment of microbial community diversity in triplicate.

2.3.2. Soil Physicochemical Analysis

The soil physical and chemical properties were measured according to Bao Shidan's "Soil Agrochemical Analysis" [35]. Firstly, the pH of the soil with a 5:1 water–soil ratio was measured by using pH meter (Orion-star A211, Waltham, MA, USA). Secondly, the EC of soil was measured by using a DDB-303A portable conductivity meter (Shanghai INESA Scientific Instrument Co., Ltd.Shanghai, China). Later, the available potassium content of soil was measured by NaOH fusion-flame photometry, the available phosphorus content of soil was measured by $NaHCO_3$ extraction-molybdenum antimony anti-

colorimetric method, and total nitrogen and total phosphorus contents of soil were measured by the continuous flow analysis method (Auto Analyzer 3, BRAN LUEBBE, Hamburg, Germany, sensitivity 0.001 AUFS). Finally, the organic matter content of soil was measured by the low temperature external thermal potassium dichromate oxidation-colorimetric method (sensitivity 0.001 AUFS).

2.3.3. DNA Extraction

Soil DNA was extracted using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manual. The extracted DNA was assayed for DNA quality and concentration using a Nanodrop2000 (ThermoFisher Scientific, Inc., Waltham, MA, USA). The quality-checked samples were stored at −20 °C for subsequent experiments.

2.3.4. PCR Amplification

The primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGAC-TACHVGGGTWTCTAAT-3′) were used to amplify the bacterial 16S rRNA gene's V3-V4 region. An 8 bp barcode sequence was added to the 5′ end of each of the upstream and downstream primers to distinguish between different samples. PCR reaction system (total system of 25 μL): 12.5 μL 2xTaq Plus Master Mix II (Vazyme Biotech Co., Ltd., Nanjing, China), 3 μL BSA (2 ng/μ reaction parameters: 95 °C pre-denaturation for 5 min; denaturation at 95 °C for 45 s, annealing at 55 °C for 50 s, extension at 72 °C for 45 s, and extension at 72 °C for 28 cycles). Amplification was performed on an ABI 9700 PCR instrument (ThermoFisher Scientific, Inc., Waltham, MA, USA), and the PCR products were detected by 1% agarose gel electrophoresis to detect the size of the amplified target bands. The amplified bands were analyzed with an Agencourt AMPure XP (Beckman Coulter, Inc., Pasadena, CA, USA) nucleic acid purification kit.

2.3.5. Library Construction and MiSeq Sequencing

The PCR products were used to construct microbial diversity sequencing libraries using the NEB Next Ultra II DNA Library Prep Kit (New England Biolabs, Inc., Ipswich, MA, USA) library construction kit. Paired-end sequencing was performed using the Illumina Miseq PE300 (Illumina, Inc., Santiago, CA, USA) high-throughput sequencing platform at Beijing Allwegene Company Co. The sequenced raw sequences were uploaded to NCBI's SRA database. Different samples use the same specification sequencing information, and the library types are: library strategy is amplicon, library source is other, library selection is PCR, and library layout is paired. The platform sequencing instrument is Illumina, the instrument model of which is Illumina MiSeq. The design description sequencing region is 16S V3-V4, and the filetype original file format is fastq.

2.4. Data Analysis

The downstream data were filtered and spliced by QIIME (v1.8.0) software(Gregory Caporaso, Flagsta, AZ, USA) by splitting the samples according to barcode sequences. The raw data were filtered using Pear (v0.9.6) software [36]. The data were filtered and spliced using Pear (v0.9.6) software, and the scores below 20, containing ambiguous bases and primer mismatches, were removed. The minimum overlap was set to 10 bp and the mismatch rate was 0.1. After splicing, sequences less than 230 bp in length were removed using Vsearch (v2.7.1) software [37], and chimeric sequences were removed by comparing with the Gold Database using the UCHIME method [38]. OTU clustering (Operational Taxonomic Units) was performed on the quality sequences using the Vsearch (v2.7.1) software uparse algorithm with a sequence similarity threshold of 97%. Comparison with the Silva138 database was performed using the BLAST algorithm with an e-value threshold set to 1×10^{-3} to obtain the species classification information corresponding to each OTU. The α-diversity index analysis (including Shannon, Simpson, and Chao1 indices) was then

performed using QIIME (v1.8.0) software. Based on the species annotation and relative abundance results, histogram analysis of species composition was performed using R (v3.6.0) software(Robert Gentleman&Ross Ihaka, NJ, USA). The beta diversity distance matrix was calculated using QIIME (v1.8.0), and the cluster heat map and PCoA analyses were performed using R (v3.6.0) software based on Weighted Unifrace distances. The metastatic intergroup variance analysis was performed using Mothur (v.1.34.4) software(Ann Arbor, MI, USA), and LEfSe analysis was performed using Python (v2.7) software(Python Software Foundation, The Kingdom of the Netherlands).

3. Results

3.1. Changes in Physical and Chemical Properties of Reclaimed Water under Different Irrigation Levels

The soils were contaminated with different cadmium concentrations under the conditions of different irrigation levels of reclaimed water. The pH of deficit irrigation treatments showed a trend of first increasing, then decreasing with the concentration of Cd(Table 2). Compared with deficit irrigation, under full irrigation treatments, the pH value of the soil did not increase due to the large amount of irrigation. On the contrary, when the Cd concentration was 0.30 mg/kg, the pH values were lower than those of the full irrigation treatment, but the pH values of other cadmium concentrations of the deficit irrigation treatments were higher than those of the full irrigation treatments. Although the Cd concentrations were 1.20, 2.07, and 2.74 mg/kg, there were significant differences between the full irrigation and the deficit irrigation ($p < 0.05$). However, the overall pH ranged from 8.2 to 8.7. The EC values of full irrigation with reclaimed water were higher than those of deficit irrigation with reclaimed water in soils with different Cd concentrations. Compared with full irrigation, the deficit irrigation EC reduction ranged from 2.89% to 42.90%, and all reached a significant difference level ($p < 0.05$). With the increase of Cd concentration, the total N content of all treatments with reclaimed water showed a decreasing trend, but the difference in total N content between full irrigation and deficit irrigation was not significant, which could be due to the large temporal variability of the nitrogen content of soil. The organic matter content of the deficit irrigation treatment with reclaimed water was higher than that of the full irrigation treatment with reclaimed water, and the organic matter content showed a decreasing trend with increasing cadmium concentration. Compared with full irrigation, the organic matter content of deficit irrigation increased by 6.31–12.10%, but there was no significant difference between treatments ($p < 0.05$). The total phosphorus content of full irrigation reclaimed water was higher than that of deficit irrigation treatments. Although there were differences in the content of available phosphorus and available potassium among the treatments, the differences were not significant. The cadmium content of each treatment under the conditions of different irrigation levels of reclaimed water irrigation didn't exceed the range set in the original experiment. No significant difference was shown between treatments ($p < 0.05$). The irrigation of each different concentration of Cd-contaminated soil with different irrigation levels of reclaimed water did not aggravate soil Cd contamination.

Table 2. Physical and chemical properties of soil used in treatment.

Treatment	pH	EC Value/μs·cm⁻¹	Organic Matter Content/g·kg⁻¹	Total N Content/mg·g⁻¹	Total P Content/mg·g⁻¹	Available P Content/mg·kg⁻¹	Available K Content/mg·kg⁻¹	Total Cd Content/mg·kg⁻¹
FCd1	8.53 ± 0.03 bc	515.67 ± 7.77 d	29.34 ± 2.76 abc	0.73 ± 0.04 a	0.97 ± 0.03 a	50.72 ± 1.12 a	254.73 ± 1.85 b	0.27 ± 0.02 f
FCd2	8.22 ± 0.02 f	758.33 ± 8.33 a	26.53 ± 0.57 abcd	0.66 ± 0.02 b	0.71 ± 0.06 b	40.76 ± 3.03 c	220.34 ± 2.22 c	0.61 ± 0.16 e
FCd3	8.35 ± 0.02 e	627.00 ± 9.17 b	22.7 ± 1.64 cd	0.52 ± 0.02 c	0.55 ± 0.01 c	35.86 ± 0.26 de	201.4 ± 1.57 de	1.10 ± 0.09 d
FCd4	8.34 ± 0.01 e	598.67 ± 9.50 c	22.07 ± 2.9 e	0.51 ± 0.02 c	0.54 ± 0.02 c	32.89 ± 1.43 f	185.74 ± 0.63 g	1.89 ± 0.15 c
FCd5	8.47 ± 0.02 d	472.67 ± 6.51 e	21.93 ± 0.15 e	0.48 ± 0.01 c	0.57 ± 0.03 c	32.89 ± 1.57 f	197.31 ± 1.27 ef	2.51 ± 0.06 b
DCd1	8.52 ± 0.01 c	438.33 ± 2.08 g	29.48 ± 0.64 a	0.75 ± 0.02 a	0.96 ± 0.01 a	46.15 ± 1.26 b	260.88 ± 7.37 a	0.27 ± 0.02 f
DCd2	8.52 ± 0.02 c	451.67 ± 4.51 f	29.44 ± 0.28 ab	0.63 ± 0.02 b	0.67 ± 0.01 b	42.58 ± 1.26 c	218.19 ± 0.46 c	0.55 ± 0.05 e
DCd3	8.69 ± 0.01 a	358.00 ± 6.56 h	25.45 ± 2.31 abcd	0.51 ± 0.02 c	0.50 ± 0.01 c	34.32 ± 1.67 def	216.24 ± 1.4 c	1.09 ± 0.11 d
DCd4	8.55 ± 0.01 b	471.67 ± 6.03 e	24.38 ± 3 abcd	0.49 ± 0.01 c	0.53 ± 0.04 c	36.66 ± 0.48 d	202.37 ± 2.96 d	1.96 ± 0.08 c

DCd5	8.47 ± 0.02 d	459.00 ± 9.00 f	23.31 ± 7.72 bcd	0.52 ± 0.03 c	0.55 ± 0.08 c	33.64 ± 0.41 ef	196.27 ± 1.15 f	2.68 ± 0.15 a

Note: The lowercase letters in the same row indicate significant differences among different treatments.

3.2. Analysis of Bacterial Community Structure and Diversity

All the sequences were clustered and formed into 8287 OUTs based on a 97% similarity level, and the final number of OTUs was 8061 after the leveling process. These included: 42 phylums, 128 classes, 295 orders, 411 families, and 724 genera. It was observed that the richness and diversity of rhizosphere soil microbial communities were increased by full irrigation with reclaimed water. The abundance and diversity of microbial communities in the rhizosphere soil of full irrigation treatments with reclaimed water were increased. The Chao1 index, PD whole tree, and Shannon index were higher in the full irrigation treatments than in the deficit irrigation treatments at each Cd concentration, but didn't have significant difference levels were presented ($p < 0.05$) (Table 3). The Simpson index of 1 for each treatment indicated that the microbial community diversity was high. The observed species index, which was used to calculate the number of OTUs actually observed in the community with increasing sequencing depth, and the goods coverage index, which represented the depth of observation, were significantly not different, reflecting that the sequencing results can represent the true situation and consistency of microorganisms in all samples, then can describe the microbial community of the samples more accurately.

Table 3. Bacterial community α diversity indices for different samples.

Treatment	Chao1	Goods Coverage	Observed Species	PD Whole Tree	Shannon	Simpson
FCd1	4632.02 ab	0.96 ab	3230.37 ab	258.02 abcd	9.78 ab	1 a
FCd2	4978.66 a	0.96 b	3406.77 a	267.94 ab	10.02 a	1 a
FCd3	4798.4 ab	0.96 ab	3304.3 ab	273.03 a	9.95 ab	1 a
FCd4	4424.68 b	0.96 a	3073.63 b	248.33 cd	9.65 b	1 a
FCd5	4757.18 ab	0.96 ab	3299.2 ab	264.26 abc	9.98 ab	1 a
DCd1	4587.39 ab	0.96 ab	3174.1 ab	244.97 d	9.78 ab	1 a
DCd2	4759.15 ab	0.96 ab	3297.1 ab	258.81 abcd	9.87 ab	1 a
DCd3	4567.93 ab	0.96 ab	3117.7 ab	250.78 bcd	9.8 ab	1 a
DCd4	4644.97 ab	0.96 ab	3147.27 ab	252.89 bcd	9.84 ab	1 a
DCd5	4389.39 b	0.96 ab	3080.23 b	248 cd	9.78 ab	1 a

Note: The lowercase letters in the same row indicate significant differences among different treatments.

Information on the community composition of rhizosphere soil microorganisms at the phylum taxonomic level and genus taxonomic level for each treatment is shown in Figure 1. At the phylum classification level, the bacterial community structure composition similarity among treatments was relatively high. The main dominant microflora include *Proteobacteria* (23–27.7%), *Acidobacteria* (13.6–30.5%), *Actinobacteria* (12.8–20.1%), *Chloroflexi* (8.3–11.2%), *Chloroflexi* (8.3–11.2%), *Gemmatimonadetes* (5.56–10%), and *Bacteroidetes* (4.72–7.35%). The relative abundance of these six bacterial groups accounted for over 80% of the microorganism community in the rhizosphere soil. Among them, *Proteobacteria* accounted for the highest proportion. There were differences in bacterial community composition changes in tomato rhizosphere soil irrigated with reclaimed water, but the overall trend was consistent. The relative abundances of *Proteobacteri* and *Actinobacteria* in different treatments were little different. The relative abundance of *Acidobacteria* decreased with increasing Cd concentration, at a Cd concentration of 0.30 mg/kg, the relative abundance of *Acidobacteria* in the full irrigation treatment with reclaimed water was 1.96% higher than that in the deficit irrigation treatment, while the relative abundance of *Acidobacteria* in the deficit irrigation treatment with other Cd concentrations was higher than

that in the full irrigation treatment, with the difference ranging from 0.02–5.99%. The relative abundance of *Chloroflexi* was higher in all of the full irrigation treatments with reclaimed water than in the deficit irrigation treatments, with the difference ranging from 0.11–2.88%, and the relative abundance in the full irrigation treatment (8.69%) was slightly lower than in the deficit irrigation treatment (9.70%) when the Cd concentration was 2.74 mg/kg. The relative abundance of *Gemmatimonadetes* was both higher in the treatment with full irrigation of reclaimed water than in the treatment with deficit irrigation of reclaimed water, with the difference ranging from 0.66–2.15%. The relative abundance of *Bacteroidetes* in the full irrigation treatment with reclaimed water was lower than that of the deficit irrigation treatment, but the difference in relative abundance was not significant (0.01–0.06%), but at a Cd concentration of 2.74 mg/kg, the relative abundance of *Bacteroidetes* in the full irrigation treatment (6.18%) was slightly higher than that of the deficit irrigation treatment (6.15%).
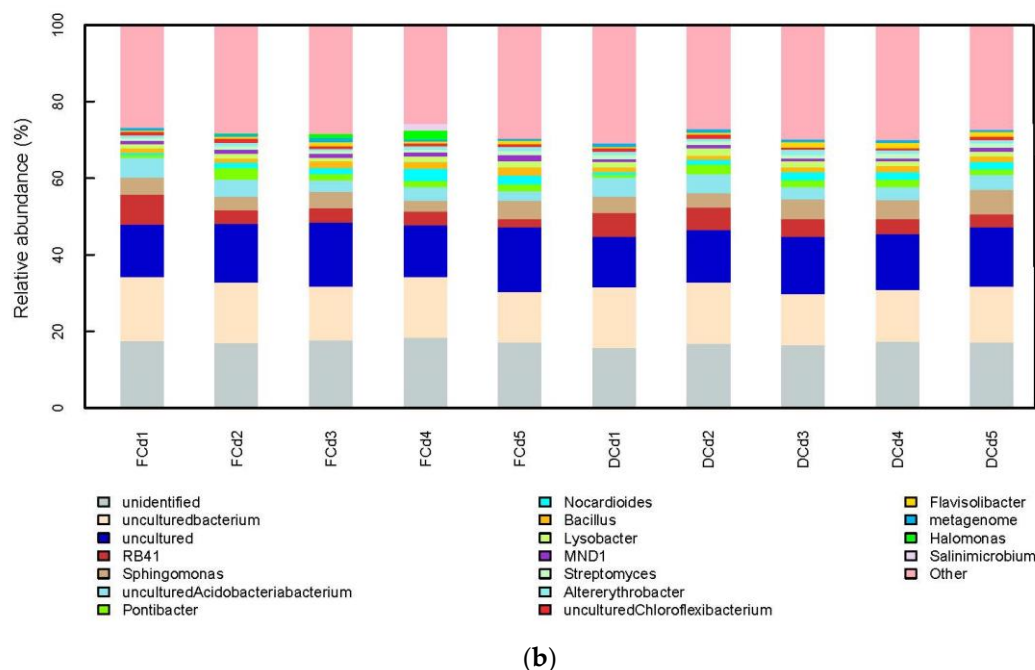

(**a**)

(**b**)
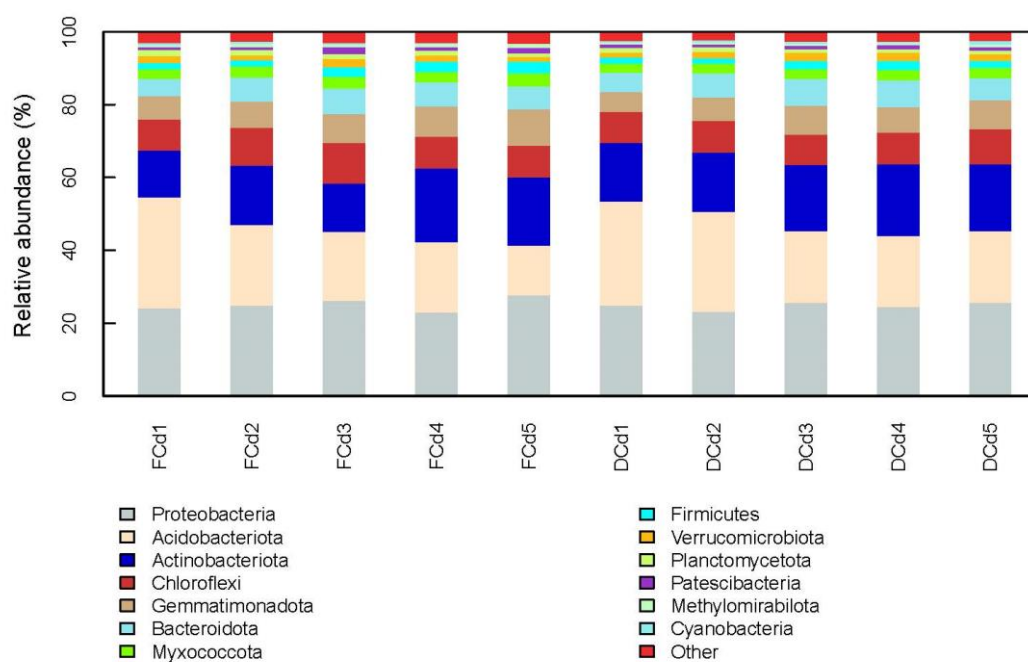
Figure 1. Histograms of the relative abundances of bacterial communities in the rhizosphere soil at the phylum and genus levels ((**a**) = phylum level, (**b**) = genus level).

At the genus classification level, the dominant genera with top 20 abundance accounted for about 70% of the relative proportion of genera, and the top three advantage bacterium groups among treatments were all unidentified genera and uncultured genera (44.7–48.5%), with the remaining genera *RB41* (2.24–7.87%), *Sphingomonas* (2.84–6.51%), *Bacillus* (1.01–2.06%), *Pontibacter*, (0.76–2.87%), and *Nocardioides* (0.58–2.03%), and the relative abundance of these dominant genera varied considerably among treatments.

3.3. Correlation Analysis of Bacterial Community Clustering Characteristics and Environmental Factors

PCoA analysis based on Bray–Curtis distance was used to investigate the similarities and differences in bacterial community composition of rhizosphere soils from different treatments. The different shape legends in Figure 2 represent tomato rhizosphere soil samples from different treatments, and the PC1 and PC2 axes explained 28.88% and 11.56% of the results, respectively. The results showed that the bacterial community in the rhizosphere soil was significantly different with different Cd concentrations. With the increase of cadmium concentration, the dispersion distance of samples with adequate irrigation of reclaimed water showed an increase and then shortened. The same phenomenon was also shown for deficit irrigation. When the Cd concentration was 0.30 and 0.69 mg/kg, the dispersion distance between the full irrigated and deficit irrigated samples intergroup of reclaimed water was farther, and when the Cd concentration was 1.20, 2.07.2.74 mg/kg, the dispersion distance between the full irrigated and deficit irrigated samples intergroup of reclaimed water was closer. It shows that the microbial community composition between all treatment intergroups of reclaimed water when the cadmium concentration is low (unpolluted and slight polluted) is significantly different from that when the cadmium concentration is high (light polluted and moderate polluted). This indicates that the magnitude of soil Cd concentration had a greater effect on the composition of the rhizosphere soil bacterial communities compared to the irrigation water source.
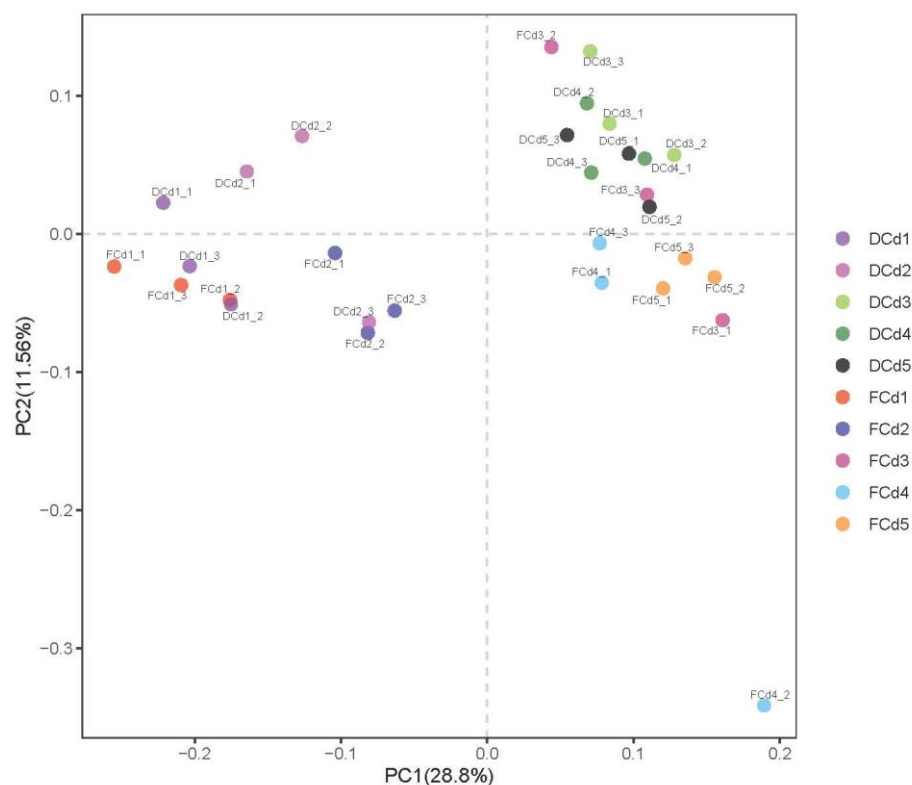
Figure 2. Principal coordinate analysis (PCoA) of bacterial communities in the rhizosphere of different samples.

Correlation between community composition of bacterial genus levels and different environmental factors in rhizosphere soil samples of different treatments revealed by RDA (Figure 3). RDA plots explained 26.28% (PC1) and 9.98% (PC2) of the variation in sample intergroup, respectively, and the total interpretation rate exceeded 30%. The results showed that organic matter and heavy metal cadmium content had significant correlations ($p < 0.05$) with changes in bacterial communities in rhizosphere soil samples. Soil pH and total phosphorus content had highly significant correlations with changes in bacterial communities in rhizosphere soil samples ($p < 0.01$), indicating that environmental factors have a great influence on the diversity of rhizosphere soil bacterial communities and are also important components that participate in altering microbial community outcomes. The pH and OM, OM and AK, AP, TN, and TP showed a sharp angle shape, indicating a positive correlation between environmental factors, Cd content showed a blunt angle shape with other environmental factors, indicating that Cd content was negatively correlated with environmental factors. EC showed a blunt angle shape with pH and OM, and a sharp angle shape with AP, TP, and TN, indicating that EC had a positive correlation with pH and OM environmental factors, and a negative correlation with AP, TP, and TN environmental factors.
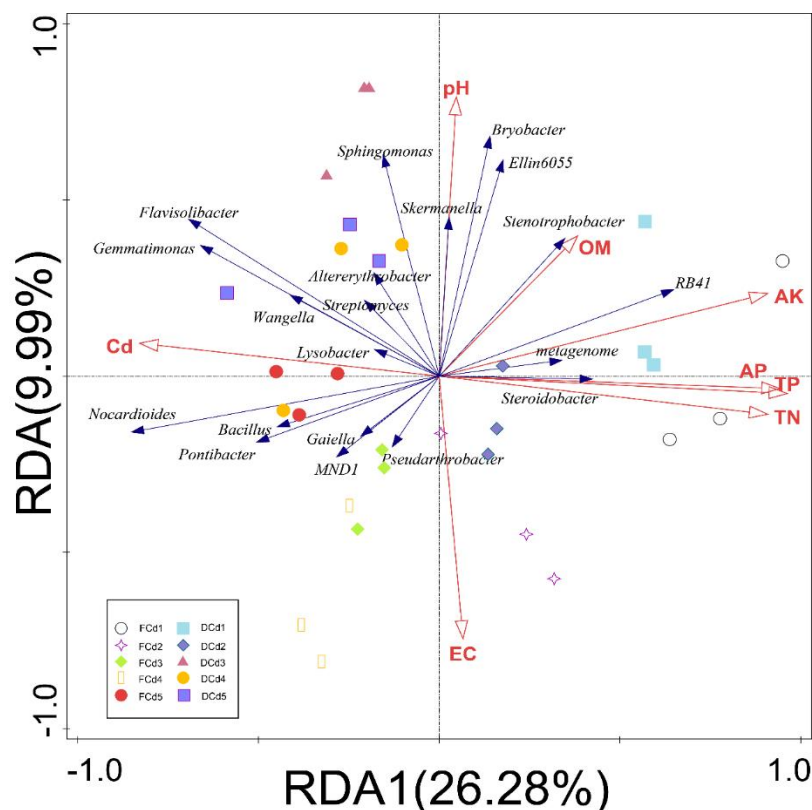
Figure 3. Redundancy analysis of bacterial communities and environmental factors in different samples.

The effects of different environmental factors on the genus-level community composition of bacteria in the rhizosphere soil were analyzed by the correlation heatmap (Figure 4). *RB41* was significantly positively correlated with AK (r = 0.534, *p* = 0.002) and AP (r = 0.512, *p* = 0.003), TN (r = 0.521, *p* = 0.003), and Cd (r = -0.538, *p* = 0.003) 0.002) was significantly negatively correlated. It was positively correlated with pH (r = 0.387, *p* = 0.034) and TP (r = 0.430, *p* = 0.018). *Sphingomonas* was significantly negatively correlated with EC (r =-0.468, *p* = 0.009), and positively correlated with pH (r = 0.441, *p* = 0.015) and Cd (r = 0.393, *p* = 0.032). *Nocardioides* was significantly positively correlated with Cd (r = 0.759, *p* = 1.17 × 10^{-6}), TP (r = −0.767, *p* = 7.54 × 10^{-7}), AK (r = −0.778, *p* = 4.18 × 10^{-7}), AP (r = −0.781, *p* = 3.48× 10^{-7}), and TN (r = −0.741, *p* = 2.82 × 10^{-6}) was significantly negatively correlated. OM (r = −0.455, *p* = 0.012) was negatively correlated. *Bacillus* was significantly negatively correlated with TP (r = -0.476, *p* = 0.008), AK (r = -0.530, *p* = 0.003), TN (r = -0.636, *p* = 0.0001), and OM (r = −0.601, *p* = 0.0004), and had a negative correlation with AP (r =-0.420, *p* = 0.02), but a positive correlation with Cd (r = 0.439, *p* = 0.015). *Flavisolibacter* was significantly negatively correlated with TP (r = −0.688, *p* = 2.64× 10^{-5}), AP (r = -0.587, *p* = 0.000658), and TN (r = −0.680, *p* = 3.62× 10^{-5}). There was a negative correlation with AK (r = −0.462, *p* = 0.010), and a positive correlation with Cd (r = 0.633, *p* = 0.000172). *Metagenome* was significantly positively correlated with AK (r = 0.547, *p* = 0.002) and AP (r = 0.545, *p* = 0.002). There was a positive correlation with TN (r = 0.440, *p* = 0.145) and TP (r = 0.363, *p* = 0.049), and a negative correlation with Cd (r = −0.451, *p* = 0.012). *Gemmatimonas* was significantly negatively correlated with TP (r = −0.555, *p* = 0.001), AP (r = −0.575, *p* = 0.001), and TN (r = −0.597, *p* = 0.001). With Cd (r = 0.616, *p* = 0.0002), it showed a significant positive correlation. *Wangella* was significantly negatively correlated with TN (r = −0.465, *p* = 0.009), negatively correlated with TP (r =−0.407, *p* = 0.026) and AP (r =−0.391, *p* = 0.033). Then, with Cd (r = 0.447, *p* = 0.013), showed a positive correlation.

According to Spearman's correlation coefficient and *p*-value, the correlation between bacterial community and environmental factors was divided into different groups

(Group1–Group3). Among them, Group1 is a bacterial genus that has a significant positive/negative correlation with environmental factors at the same time. They are *RB41, Nocardioides, Gemmatimonas*, and *Ellin6055*. Group2 is a bacterial genus that exhibits a\positive/negative correlation with environmental factors at the same time. They are *Bacillus, MND1, Flavisolibacter, Metagenome, Bryobacter,* and *Wangella*. Group3 is a bacterial genus that exhibits significant positive/negative correlation or significant positive/negative correlation with environmental factors. They are *Sphingomonas, Lysobacter, Altererythrobacter, Stenotrophobacter*, and *Steroidobacter*. The above results (Figure 3 and 4) indicated that the bacterial community in the rhizosphere soil was influenced by the soil environment, and this difference was related to the irrigation water source being reclaimed water and the quality of soil contaminated with different concentrations of cadmium.
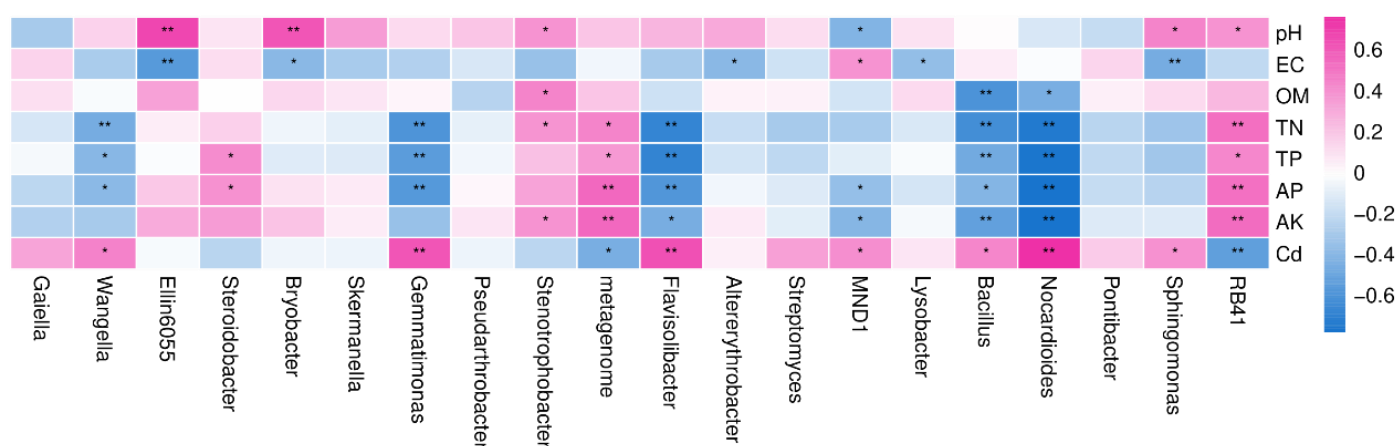

Figure 4. Spearman rank correlation heatmap used to study the environmental factors and bacterial community compositions. *: 0.01 < *p* ≤ 0. 05, positively/negative **:*p* ≤ 0.01 significant positive/negative.

We selected the top 20 genus-level species in absolute abundance for species correlation analysis. Then, we drew a network diagram according to the correlation between species (Figure 5) that was used to reflect the interaction between species in the sample. The genus-level correlation network map reveals that there are significant interactions between different genera, with the size of the dots representing the magnitude of abundance and the thickness of the line representing the magnitude of correlation. The color of the dots represents the phylum to which they belong. A pink line indicates a positive correlation, and a green line represents a negative correlation. *Proteobacteria* and *Acidobacteriota* are closely related to other species. While *Myxococcota* and *Actinobacteriota* were less related to other species. The degree of correlation among the 20 genera was different, of which six were positively correlated and 10 were negatively correlated. *Gemmatimonas* and *Flavisolibacter* and *Flavisolibacter* and *Sphingomonas* showed the greatest positive correlation (r^2 = 0. 719, r^2 = 0.683).
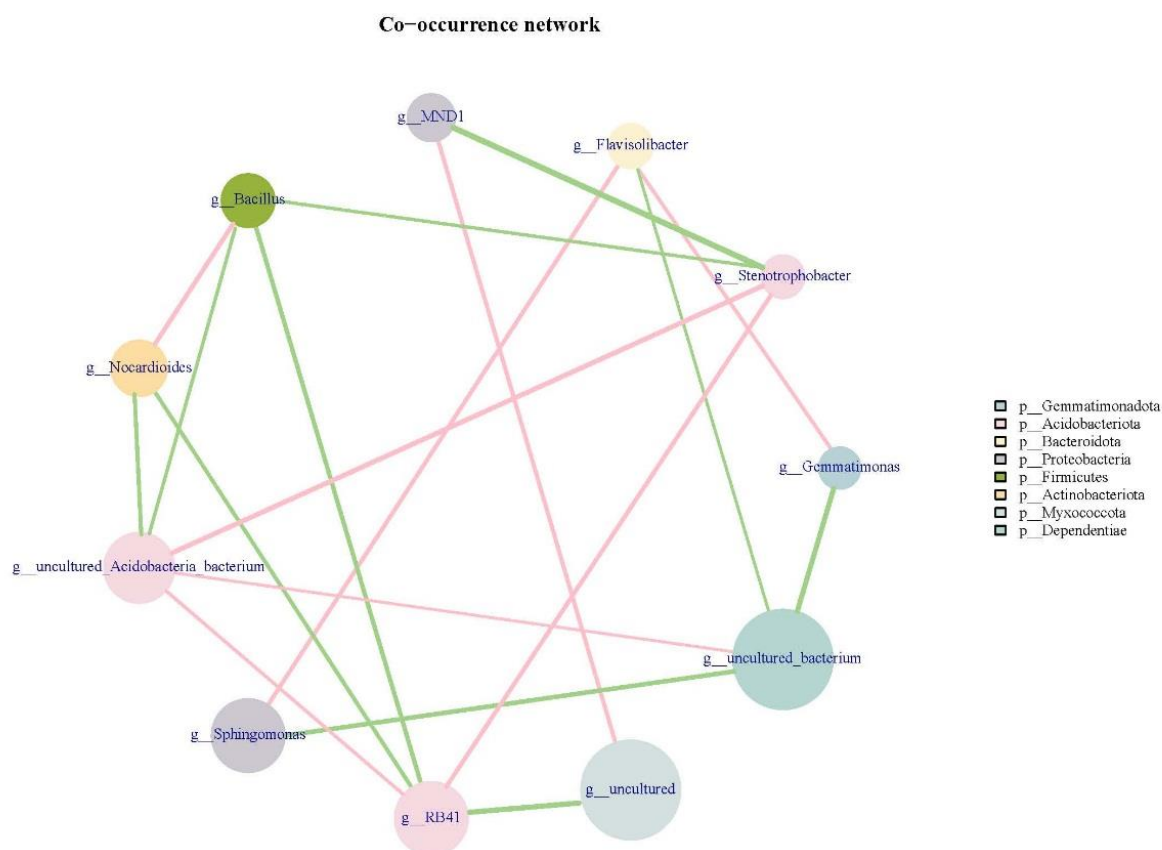
Co−occurrence network

Figure 5. Species-level network analysis.

3.4. Functional Prediction of Microbial Communities between Different Treatments

For the pathway, the PICRUSt software was used to obtain three levels of information on the metabolic pathways and their abundances in the tomato rhizosphere soil irrigated by reclaimed water with different Cd concentrations (Figure 6). Pathway level 1 has six metabolic pathways. Pathway level 2 has 37 metabolic pathways. Pathway level 3 has 185 metabolic pathways. Under similar environmental conditions of Cd stress, the metabolic pathways of microbial communities in the rhizosphere soil irrigated by reclaimed water were similar. However, the abundance values of metabolic pathways are different. On the pathway of metabolism, the abundance values of microbial metabolic pathways in the rhizosphere soil irrigated with deficit reclaimed water were higher than those in the soil irrigated with full reclaimed water (Figure 6). This indicated that the metabolic function potential of the bacterial community in the rhizosphere soil irrigated with deficit reclaimed water was higher than that in the rhizosphere soil irrigated with full reclaimed water.
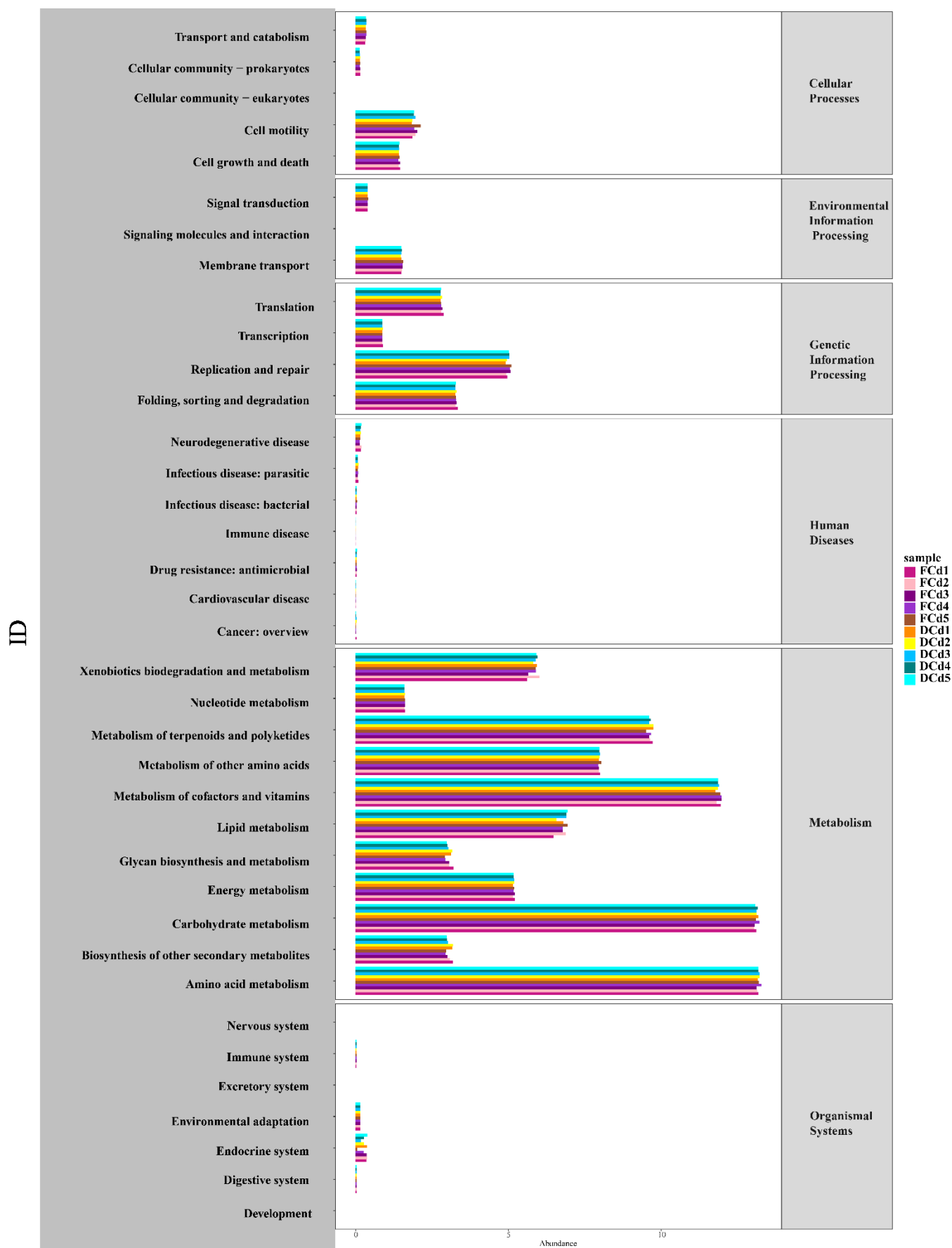
Figure 6. Prediction of PICRUst function in rhizosphere soil of full irrigated with recycled water and deficit irrigation of reclaimed water in the pollution with different cadmium concentrations.

4. Discussion

4.1. Effect of Reclaimed Water Irrigation Amount on the Physicochemical Properties of Rhizosphere Soil with Different Cadmium Concentrations

The trends of soil physicochemical properties under full irrigation treatment and deficit irrigation treatment with different Cd concentrations of reclaimed water were different. The pH values in the deficit irrigation treatment were higher than those in the full irrigation treatment compared to the deficit irrigation treatment. The higher pH values in the soil may be due to the neutralizing effect of the water quality components of the irrigation water source and the alkaline soil, highlighting the acid-alkaline buffering characteristics of the soil[39]. However, some scholars have found a uniform increase or decrease in soil pH values in soils irrigated with reclaimed water[40,41]. This phenomenon of pH differences in experimental results is strongly related to the duration of irrigation with reclaimed water, soil type, and other important environmental conditions. Other important environmental conditions are strongly related. Meanwhile, the effectiveness of Cd in soil is largely regulated by soil pH [42,43],the effectiveness of Cd decreases significantly with increasing soil pH[44,45]. The soil pH of the deficit irrigation treatment with reclaimed water is higher than that of the full irrigation treatment.

The EC values of full irrigation soil with reclaimed water were significantly higher than those of deficit irrigation with reclaimed water ($p < 0.05$) (Table 2). The amount of irrigation water for deficit irrigation set in the experiment was 70% of the irrigation water for full irrigation, so the higher irrigation water for full irrigation led to a significant increase in soil soluble salts[46,47]. The total nitrogen and phosphorus content of the soil should increase after irrigating the tomato, especially because the irrigation volume of full irrigation is greater than that of deficit irrigation. However, the total nitrogen content of full irrigation and deficit irrigation did not show a significant difference ($p < 0.05$) (Table 2), probably because of the large spatial and temporal variability of nitrogen content in the soil, resulting in a non-significant difference in the total soil nitrogen after treatment. Mohamad et al. [48] irrigated peas and maize in farms with treated reclaimed water, focusing on changes in soil fertility, the results showed that the treated reclaimed water irrigated soil added a large amount of nitrogen, phosphorus, and potassium, which to some extent can replace artificial application of nitrogen, phosphorus, and potassium fertilizers and mitigate soil fertilizer pollution to the maximum extent.

Chen et al. [49] irrigated several green areas in Beijing with reclaimed water to study the changes in soil quality. The organic matter content showed an increasing trend. However, the experimental results showed that the organic matter content of the deficit irrigation treatments of reclaimed water with small irrigation amounts were all higher than that of the full irrigation treatments (Table 2), accompanied by a decrease in organic matter content with increasing cadmium concentration. Probably because the organic matter content of the soil can significantly influence the presence of heavy metals in the soil form, the increase in organic matter content increases the content of the organic matter-bound state of heavy metals[50,51]. The organic matter-bound state is a heavy metal that is chelated with various organic matter in the soil, such as plant and animal residues, humus. and inclusions of mineral particles, etc. It is not very translocational and relatively stable. The organic matter content of the deficit irrigation treatment was higher than that of the full irrigation treatment (Table 2), indicating that the organic matter-bound state Cd content in soils with different Cd concentrations under the deficit irrigation treatment of reclaimed water was higher than that of the full irrigation treatment, and the soil safety of the deficit irrigation treatment was higher than that of the full irrigation treatment.

The reclaimed water was secondary treated effluent, which contains heavy metals but meets the irrigation standard. Table 2 shows that the Cd content of the full irrigation treatment with reclaimed water and the deficit irrigation treatment did not show significant differences ($p < 0.05$) and did not exceed the Cd content range set in the original experiment, which proves that the soil irrigated with reclaimed water for a short period of

time did not cause the accumulation of soil Cd content. However, there is a deficiency of long-term monitoring studies on heavy metals in reclaimed water[52–54].

4.2. Effect of Different Irrigation Levels of Reclaimed Water on the Structural Diversity of Rhizosphere Soil Microbial Communities Contaminated with Different Cadmium Concentrations

The rhizosphere is that part of the microdomain environment that is affected by plant root activity and is different from the soil in physical, chemical, and biological properties. The rhizosphere zone is generally defined as the area within a few millimeters from the surface of the root axis[55,56]. The diversity of its microbial community structure is affected by the irrigation water source[57] and also by soil contamination [58], which can lead to instability of its microbial community structure. The composition of the soil bacterial community in the rhizosphere of each treatment was the highest with *Proteobacteria* as a result of experimental sequencing, followed by *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes,* and *Bacteroidetes*, which is also consistent with the results of other scholars studying the diversity of species of soil microorganisms under irrigation with reclaimed water[59]. but there were differences in the relative abundance percentages. *Acidobacteria, Gemmatimonadetes,* and *Firmicutes* [60]. This indicates that the highest enrichment of the *Ascomycetes* phylum was observed under different regenerated water treatments.

The advantage bacterium groups have a very powerful role in the soil, such as *Proteobacteria*, which participate in the nitrogen cycle in the soil and have nitrogen fixation; *Acidobacteria*, which belong to heterotrophic populations and are also closely related to heavy metal stress and will have a good potential to change the soil structure; *Actinobacteria*, which can promote the decay process of plant and animal remains in the soil; *Chloroflexi*, which participate in the process of carbon cycle in the soil and are also involved in soil respiration; *Gemmatimonadetes*, which can adapt to the low humidity environment is also an important part of the diversity of microbial communities in the soil; *Bacteroidetes,* which can secrete decomposing soil; and *Bacteroidetes*, wich are able to secrete active enzymes and participate in the process of soil nutrient cycling[61,62].

Acidobacteria are microorganisms that have received much attention in recent years and are characterized by their preference for acidic environments, low abundance, and it is difficult to separate single cultures [63]. Previous studies have found that *Acidobacteria* are the dominant group in acidic soils [64]. However, in the results of this experiment, the soil was alkaline and the relative abundance of *Acidobacteria* was dominant in each treatment. It is possible that *Acidobacteria* are specific to alkaline soils, or that the enrichment of *Acidobacteria* is also regulated by other soil properties. It has been suggested that the *Acidobacteria* phylum can be detected in alkaline soils [65]. So, this may be the reason why the *Acidobacteria* phylum was the dominant group in alkaline soil in the present study. Because of the acidophilic nature of *Acidobacteria*, they are more enriched in heavy metal content loci, resulting in stronger resistance to heavy metals, and it has been pointed out that the relative abundance of *Acidobacteria* and Cd content showed a positive correlation[66], contrary to the results of this study.

The relative abundance of *Acidobacteria* under the same treatment in this experiment decreases with increasing concentration of cadmium (Cd), while it has been pointed out that soil organic matter content and the number of *Acidobacteria* show a significant positive correlation[67], while the experimental results of organic matter content of each treatment of reclaimed water decreases with increasing concentration of heavy metals. In the experimental results, the organic matter content of each treatment of reclaimed water decreased with the increase of Cd concentration (Table 2). The organic matter content of the deficit irrigated treatment was higher than the full irrigated treatment (Table 2). The reasons for such experimental results may be the following: Firstly, the diversity of soil microbial metabolic functions decreases under long-term exposure to Cd stress[68]. Secondly, it has been demonstrated that irrigation with reclaimed water significantly increases the diversity of microbial communities [69] and also significantly increases the relative abundance

of *Acidobacteria* in the soil. The relative abundance of *Acidobacteria* [70], changes in soil physicochemical properties due to reclaimed water irrigation are caused by changes in microbial structural diversity. Cd-stressed environments produce some synergistic effects. Thirdly, reclaimed water is enriched with nutrients such as nitrogen and phosphorus. Crop root secretions after irrigating the crop affect changes in the dominant rhizosphere soil microflora.

The dynamics of the relative abundance of *Bacteroidetes*, *Chloroflexi*, and *Gemmatimonadetes* under each treatment after irrigation with reclaimed water could be the result of exogenous microbial inputs contained in the irrigation water source, nutrients required by the soil, and non-essential elements of the microecosystem. Depending on the different types of exogenous inputs, they can promote or inhibit certain characteristic microorganisms or biomarkers of their metabolic processes [71,72].

4.3. Correlation Analysis of Bacterial Community Clustering Characteristics of Rhizosphere Soil and Environmental Factors with Reclaimed Water Irrigation

Soil organic matter, total phosphorus, Cd content, and pH were the key environmental factors that influenced the structural diversity of microbial communities when irrigating soils with different Cd concentrations with reclaimed water in this experiment. The dominant group of *Sphingomonas* at the genus level showed a significant positive/negative correlation with the key environmental factors at the same time, and this genus is a group of bacteria with the ability to biodegrade and synthesize substances [73], which can degrade environmental pollutants in soil and can be widely used in environmental pollution management and is closely associated with environmental safety [74]. The *RB41* bacterial genus showed a highly significant positive/negative correlation with key environmental factors at the same time,it is one of the most abundant bacterial genus under the *Acidobacteria* phylum. It is a key factor in maintaining metabolic capacity if it has been present in contaminated soils for a long time [75]. The genus *Pseudomonas* includes many common pathogenic bacteria, which contain a variety of pathogenic bacteria that are pathogenic to human conditions and, to a lesser extent, common groups of plant pathogenic bacteria. CUI et al. [76] showed that irrigation with reclaimed water significantly increased the abundance of *Pseudomonas* in rhizosphere soils. The relative abundance of *Pseudomonas* in this study was higher in the full irrigation treatment with reclaimed water than in the deficit irrigation treatment, but was not among the dominant groups in the species annotation at the genus level, which is not consistent with the results of existing studies [59,76], probably because irrigation with reclaimed water has a Cd-contaminated soil some degree of regulation and buffering capacity, resulting in not increasing the invasion of soil pathogens.

The species correlation network diagram at the genus level reflects the interrelationship between different species in the dominant microflora and also indirectly reflects the role of key microflora in the rhizosphere soil [77] and how they respond to environmental changes. Different nodes (genera) play different roles in the network [78]. From the results, it can be concluded that in addition to the dominant genera of *RB41* and *Sphingomonas*, key genera such as *Flavisolibacter*, *Nocardioides*, and *Gemmatimonas* are also factors driving the structure of the rhizosphere soil bacterial community. The rhizosphere soil microbial community structure is affected by the soil microenvironment, and the magnitude of the effect of different genera varies slightly depending on the degree of soil Cd contamination and irrigation water source.

4.4. Prediction of Bacterial Community Function in Rhizosphere Soil under Cadmium Concentrations with Reclaimed Water Irrigation

According to the PICRUSt method analysis, there were differences in the gene functions of rhizosphere bacteria in different treatments. The abundance values of the metabolic pathways of microorganisms in the deficit irrigation rhizosphere soil with reclaimed water were all higher than those of microorganisms in the full irrigation soil with

reclaimed water, indicating that the potential of metabolic functions of bacterial communities in the deficit irrigation rhizosphere soil with reclaimed water is higher than that in the full irrigation rhizosphere soil with reclaimed water. We can reasonably speculate that the change in abundance of this metabolic pathway may lead to an increase in the abundance of either harmful or beneficial bacteria. This study may provide new research directions and perspectives for further studies on which pathways in rhizosphere soils differ significantly in reclaimed water irrigated soils with different Cd concentrations or the strength of the metabolic capacity of bacterial genera on the pathway.

5. Conclusions

When reducing irrigation water, the soil physicochemical properties of the reclaimed water deficit irrigation treatment did not change significantly when compared to the reclaimed water full irrigation treatment, but changed the trend of decreasing microbial diversity in rhizosphere soil under long-term heavy metal stress. The distribution characteristics of *Acidobacteria* in alkaline soil were different from those in acidic soil. That is to say, the factors affecting microbial diversity are not only soil pH, but also other physical and chemical properties such as soil organic matter.

Soil organic matter, total phosphorus, Cd content, and pH value were the key environmental factors affecting bacterial community structure in this study. It was also revealed by the presence of key genera in reclaimed water irrigated Cd contaminated soil, such as *RB41*, *Sphingomonas*, *Flavisolibacter*, *Nocardioides*, *Bacillus*, etc. These key genera were closely related to environmental factors. The relative abundance of *Pseudomonas* pathogenic genera did not increase. Based on these results, we can hypothesize that under deficit irrigation, the use of reclaimed water did not increase the abundance of pathogenic bacteria in soil.

In the metabolic pathway, the relative abundance of bacteria was significantly increased in each treatment, with a low relative abundance of potential human pathogenic bacteria, indicating that reclaimed water irrigation could be used to improve the productivity of cadmium contaminated soil.

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Data Availability Statement: The bacterial raw sequences of this study have been submitted to SRA of NCBI database, with the accession number PRJNA847168.

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