



Microbial assessments of soil with a 40-year history of reclaimed wastewater irrigation

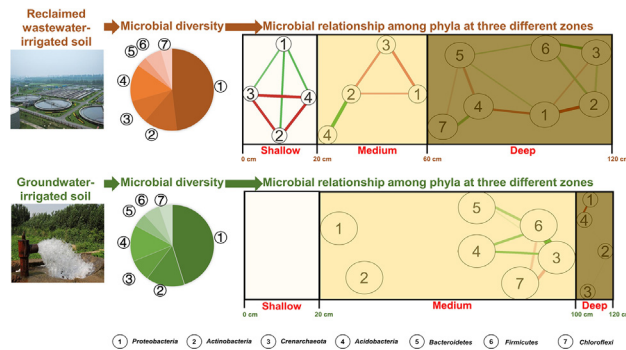
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HIGHLIGHTS

- Microbial variation in soil irrigated with reclaimed wastewater for 40 years were studied.
- Dominant phylum in reclaimed wastewater or groundwater irrigated soil is Proteobacteria.
- No significant influence of reclaimed wastewater on soil microbial quantity was observed.
- Positive influence of reclaimed wastewater on vegetable yield was identified.
- Microbial inter-species cluster profiles in two irrigated soils appeared to vary.

GRAPHICAL ABSTRACT



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ABSTRACT

The long-term effects on soil microorganisms from 40 years of irrigating soil with reclaimed wastewater was investigated by determining the quantity, composition, and inter-species connection of microorganisms. No significant difference in microbial quantity and composition were identified in the reclaimed wastewater- and groundwater-irrigated soils. The dominant bacterial phylum in both irrigation water sources and soils was Proteobacteria, which commonly exists in soil. From the analysis of four (4) alpha diversity metrics, including the observed number of operational taxonomic units (OTUs), Chao1, and the Shannon and Simpson diversity, there was no significant difference between the reclaimed wastewater- and groundwater-irrigated soils. Three zones (shallow, medium and deep) were identified in the reclaimed wastewater- and groundwater-irrigated soils based on the taxonomic networks and clusters generated by graphical lasso and random walk algorithm. The cluster profiles (shallow, medium and deep zones) appear to be different in the reclaimed wastewater- and groundwater-irrigated soils. Soil irrigated with reclaimed wastewater showed less depth of clustered profile in medium zone than that in soil irrigated with groundwater (20–60 cm of reclaimed wastewater-irrigated soil compared to 20–100 cm of groundwater-irrigated soil), although the significance of such a variance (the depth of medium zone of reclaimed wastewater-irrigated soil decreased 40 cm than that of groundwater-irrigated soil) is not clear at this time. Positive influence has been identified in the growth and yield of eggplant, tomato and cucumber between the reclaimed wastewater- and groundwater-irrigated soils, suggesting that reclaimed

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wastewater irrigation can potentially substitute groundwater irrigation, despite the variance in inter-species clustering profiles in soil microbes in certain soil zones. Nevertheless, the possible negative influence of pathogens, organic compounds and pharmaceuticals should be seriously considered during the reclaimed wastewater irrigation.

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1. Introduction

Reclaimed wastewater has become globally an important irrigation water source (Oki and Kanae, 2006; US-EPA, 2012; Tran et al., 2016), especially in Northern China (Wang et al., 2017a, 2017b). Reclaimed wastewater irrigation is one solution for the increasing demand of food production with increasingly scarce water resources. The reuse of reclaimed wastewater from wastewater treatment facilities (WWTFs) generates substantial economic and water conservation benefits; however, reclaimed wastewater irrigation may pose human and environmental health risks from toxic substances that may potentially remain in the treated wastewater. Considering these risks, recent research has focused on the influence of reclaimed wastewater irrigation on the growth and yield of crops and its impacts on the environment (e.g., soil, groundwater) and human exposure (Xu et al., 2010; Pereira et al., 2011).

Irrigation using reclaimed wastewater can change soil and groundwater properties, such as nutrient concentrations, salinity and sodicity, heavy metal concentrations, organic carbon content, and enzyme activities (Mathan, 1994; Gupta et al., 1998). Alkhamisi et al. (2011) reported that forage irrigated with reclaimed wastewater had a higher water usage efficiency (3.7 kg/m³ of dry matter) than forage irrigated with fresh water (2.9 kg/m³ of dry matter). Irrigating with reclaimed wastewater also resulted in the highest green forage yields of 72.1 and 59.4 t/ha with reference evapotranspirations of 1.4 and 1.0, respectively. Enhanced growth of treated wastewater-irrigated crops, in comparison with conventional water-irrigated crops, has been attributed primarily to higher nutrient contents (Chen et al., 2015); however, increasing public concerns have focused on the negative influence of reclaimed wastewater irrigation on the environmental and human health due to the higher concentrations of salts (Yadav et al., 2002; Chen et al., 2013), heavy metals (Singh et al., 2012; Chen et al., 2015; Zhang et al., 2018), organic compounds (Zhang et al., 2013; Qin et al., 2015), pharmaceuticals (Bhalsod et al., 2018), and pathogenic bacteria (Wang et al., 2014; King et al., 2017; Ibekwe et al., 2018).

Reclaimed wastewater irrigation is known to have an impact on indigenous soil microorganisms (Hamilton et al., 2006; Cui et al., 2017; Guo et al., 2018; Piña et al., 2018). By comparing the impacts of drip irrigating with freshwater and reclaimed wastewater on the soil microbial communities, Bastida et al. (2018) determined that irrigating with reclaimed wastewater did not negatively impact the soil microbial community of semi-arid soils growing grapefruit and mandarin orange crops. The annual use of reclaimed wastewater or the combined irrigation with fresh water and reclaimed wastewater positively influenced the microbial biomass and biogeochemical activities of microbial communities in soil growing grapefruit. From microbial biomass carbon analysis, Chen et al. (2015) determined that reclaimed wastewater irrigation could significantly improve soil microbial activities. In the Xiaoqing River Valley (Shandong, China), which has a 30-year agricultural history of irrigating with reclaimed wastewater, Zhang et al. (2008) investigated the microbial community structure of agricultural soil using Biolog and fatty acid methyl esters (FAME). The Biolog results indicated that with increasing amount of reclaimed wastewater irrigation, microbial functional diversity also slightly increased; however, the FAME results indicated that the amount of epiphyte decreased. Xu et al. (2012) investigated the influence of 20 years of reclaimed wastewater irrigation on the quantity and distribution of soil microorganisms in a soil collected from an area in Xu Zhou City (Jiangsu, China). Using

most probable number (MPN) methods to quantify different microorganisms, they determined that the quantities of the soil actinomycetes, fungi, nitrite oxidizing bacteria (NOB), nitrate bacteria (NB), and denitrifying bacteria (DB) decreased after reclaimed wastewater irrigation, while ammonia oxidizing bacteria (AOB) and aerobic cellulose decomposing bacteria (CDB) increased after reclaimed wastewater irrigation.

However, Yin et al. (2018) reported that reclaimed wastewater should not be recommended as irrigation water for fresh produce due to the high populations of the *E. coli* that exceeded the limit of Food Safety Modernization Act regulations. In addition, Gong et al. (2014) reported that reclaimed wastewater irrigation had little effect on the microbial biomass and Shannon diversity in soil collected at 0–20 cm while simultaneously decreasing the Pielou evenness index and increasing the Margalef richness index. Six endemic genera were observed in batch-type columns irrigated with reclaimed wastewater and four were observed in groundwater-irrigated columns. In a green land with a reclaimed wastewater irrigation history of 2 years, Chen et al. (2014) observed that *Bacillus* was the dominant bacteria genus in the soil, while *Pseudomonas* was the dominant genus in the groundwater-irrigated area. The soil microbial evenness index and the probability of interspecific encounter in the reclaimed wastewater-irrigated soil were high while the Shannon index, Pielou evenness index, Margalef richness index, and Patrick richness index were low. Based on the 16S ribosomal RNA (16S rRNA) sequence analysis of the reclaimed wastewater-irrigated soil from a greenhouse, Guo et al. (2017) observed that Proteobacteria, Gemmatimonadetes, and Bacteroidetes were more abundant in soil irrigated with reclaimed wastewater than in soil irrigated with groundwater. The type of irrigation water also had a greater influence on the structure of the soil microbial community than a nitrogen fertilizer treatment. By investigated the impact of reclaimed wastewater for irrigation on soil microbial communities, Ibekwe et al. (2018) found that no significant differences in microbial diversity between soils irrigated with reclaimed wastewater and fresh water, and most of the sequences from pyrosequencing including nitrifying bacteria, nitrogen-fixing bacteria, carbon degraders, denitrifying bacteria, potential pathogens, and fecal indicator bacteria were more abundant in reclaimed wastewater-irrigated soil than in fresh water-irrigated soil. Reclaimed wastewater may contain bacteria that are very active in many soil functions, as well as some potential pathogens.

In order to evaluate the influence of reclaimed wastewater irrigation on the quantity, composition and inter-species connection of microorganisms in soil after 40 years of reclaimed wastewater irrigation, soil samples were collected from an area near Beijing, China, and 16S rRNA sequence analysis was used to investigate the microbial variation in soil samples.

2. Materials and methods

2.1. Study area and soil sampling

The study area was located southeast of Beijing, China, where reclaimed wastewater has been used for irrigation since 1975, and the typical crops grown in the area included wheat and corn. Vegetable crops that included eggplant, tomato, cucumber, carrot, potato, celery, cabbage, and chives have also been grown in the same soil. Fig. 1 shows the location and layout of the study area as well as the sampling

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