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Impacts of disinfected wastewater irrigation on soil characteristics, microbial community composition, and crop yield

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ABSTRACT

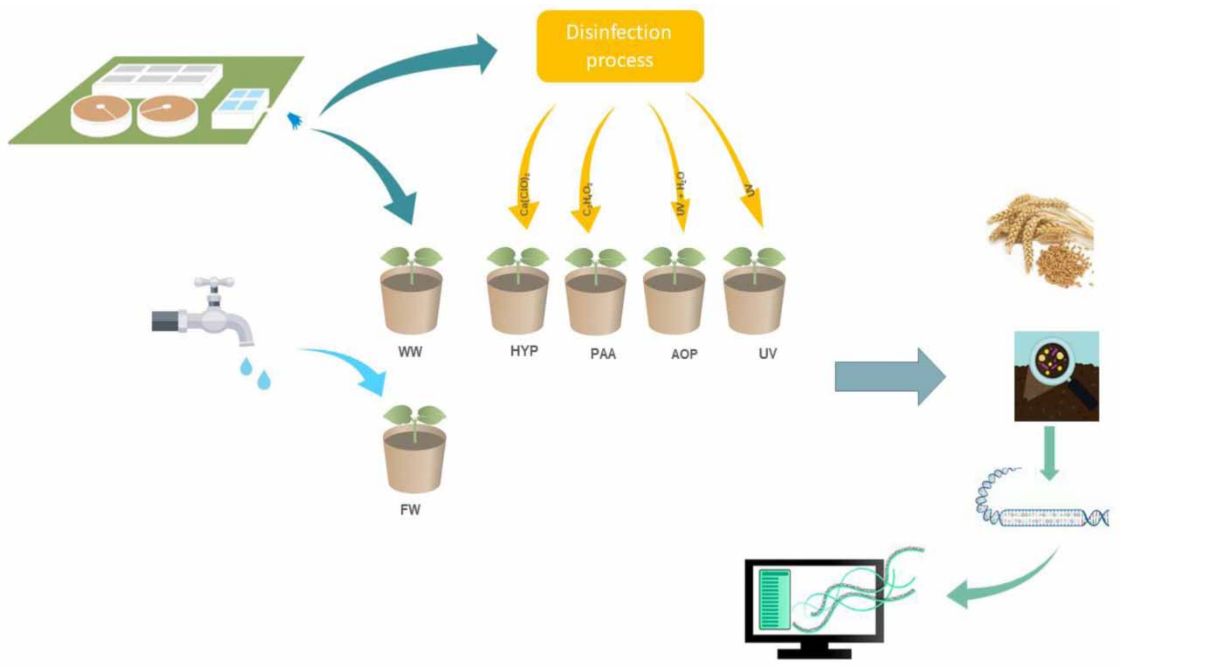
For agricultural reuse, the disinfection treatment must be efficient to inactivate the resistant pathogens and must not generate harmful byproducts for the soil and crop production. Thus, the aim of this work was to evaluate the possible impacts caused by the irrigation with wastewater disinfected with sodium hypochlorite, peracetic acid, ultraviolet radiation, or the oxidation process UV radiation combined with hydrogen peroxide over soil physicochemical properties and microbial community composition, as well as over the wheat crop yield in the short term. A pot essay was performed in a greenhouse, and at the end the main alterations observed in soil physicochemical properties were due to water type, not to the disinfection treatments. The crop yield was influenced by the water type, but not by the disinfectant treatments. Irrigation with wastewater improved almost 5 times the wheat grains yield, compared with freshwater. Wastewater irrigation increased the abundance of families involved in organic matter degradation and nitrogen cycle, and some pathogenic bacteria. Among the disinfectant treatments, the UV disinfection played an important role in shaping soil bacterial community structure.

Key words: agriculture reuse, crop yield, microbial diversity, soil properties, wastewater disinfection

HIGHLIGHTS

- The treatment influenced the prokaryotic community composition.
- Some wastewater typical bacteria persisted in the soil.
- The disinfectant treatment did not impact the crop yield.

GRAPHICAL ABSTRACT



INTRODUCTION

Since the beginning of the civilizations, wastewater (WW) has been used for crop irrigation as the solution to maintain agriculture in dry regions (Al-Hammad *et al.* 2014; Tal 2016; Jaramillo & Restrepo 2017; Angelakis *et al.* 2018; Dery *et al.* 2019). However, due to the rapid population growth, the consequent urbanization and the increasing demand for food, nowadays, irrigation projects with reclaimed water have been implemented all over the world as a strategy to save fresh water for more noble uses (Paranychianakis *et al.* 2015; Hara *et al.* 2016; Lyu *et al.* 2016; Perry & Praskievicz 2017; Agnelo *et al.* 2020). According to a United Nations report, at least 50 countries around the world use WW for irrigation, with 10% of the total irrigable areas on the planet being irrigated with sewage, treated or not (WWAP 2017).

Crop irrigation with WW, in addition to saving freshwater (FW), protects the aquatic ecosystems from sewage discharge and converts the WW into a valuable resource, since, in addition to water, it also supplies minerals, organic matter (OM), and nutrients for the plant, improving the crop yield (Bedbabis *et al.* 2010; Segal *et al.* 2011; Cirelli *et al.* 2012; Marinho *et al.* 2013, 2014; Mok *et al.* 2014; Becerra-Castro *et al.* 2015; Gatta *et al.* 2016; Pedrero *et al.* 2018, 2020; Erel *et al.* 2019). The sustainable adoption of agricultural reuse requires considerations regarding impacts on crop productivity, soil physicochemical and microbiological properties, as well as public health (Gabrielli *et al.* 2015; Ibekwe *et al.* 2018; Leonel & Tonetti 2021).

The choice of the method to produce reclaimed water suitable for agricultural irrigation is very important. The proper treatment must be a balance between effectiveness in removing or inactivating pathogens, cost-effectivity, and production of water with adequate quality for crop irrigation (Marinho *et al.* 2014; Becerra-Castro *et al.* 2015).

One of the major concerns about agricultural reuse is public health safety. Outbreaks of waterborne diseases have been associated with the consumption of vegetables and cereals irrigated with non-properly treated WW (Melloul *et al.* 2001; Gupta *et al.* 2009; Fallah *et al.* 2012), as well as the high prevalence of gastrointestinal diseases in farmers who use untreated or partially treated WW for irrigation (Blumenthal *et al.* 2001; Devaux *et al.* 2001; Ensink *et al.* 2005; Amoah *et al.* 2016; Fuhrimann *et al.* 2016). Therefore, the main guidelines for WW reuse in agriculture recommend a disinfection step to protect the consumers and the farmers (WHO 2006; USEPA 2012; EC 2018).

For agricultural reuse, the disinfection treatment must be efficient to inactivate the resistant pathogens and must not generate harmful byproducts for the soil and crop production. Due to its relatively low-cost and well-known bactericidal properties, chlorination is the most applied disinfection technology for WW treatment and reclaimed water production around the world (Marinho *et al.* 2013; Norton-Brandão *et al.* 2013; Lonigro *et al.* 2017).

However, there is an emerging concern about the use of chlorinated WW for agricultural irrigation, considering the harmful disinfection byproducts (DBP) formed when chlorine reacts with WW OM, and their potential accumulation in the edible parts of the plants (Lonigro *et al.* 2017; López-Gálvez *et al.* 2018; Garrido *et al.* 2020). In addition, there is an increased risk of soil salinization when chlorinated WW is used for irrigation, once the chlorine released by the oxidation reaction can be converted into chloride salts (Li *et al.* 2014). Furthermore, chloride salt deposition can impact nutrient accumulation, diminishing soil fertility (Li *et al.* 2014). Thus, some regulations for agricultural reuse have restricted the use of chlorine (Kistemann *et al.* 2008; Antonelli *et al.* 2013).

Peracetic acid, ultraviolet (UV) radiation, and, recently, the advanced oxidation processes have been studied as alternatives to chlorination for WW reclamation for different purposes, inclusive crop irrigation. These technologies showed very good disinfection capabilities and no phytotoxic DBP formation to date (Liberti *et al.* 2003, 1999; Kitis 2004; Antonelli *et al.* 2013; Chevremont *et al.* 2013, 2012; Rizzo *et al.* 2020; Sgroi *et al.* 2021). However, little is known about the impacts of reuse with disinfected effluent on the soil properties, on crop productivity, and over the soil–plant system. There is a preponderant concern with the destruction of pathogens and a lack of attention to the harm that disinfectant agents and their byproducts can cause to the soil and its ecosystem.

In this context, the present study aimed to evaluate the impact caused by agricultural reuse of treated and disinfected WW on the soil physicochemical properties, the composition of soil microorganism community, and crop yield, in the short term. For the disinfection treatment, we choose four disinfectant agents: two well-known ones (chlorine and UV radiation) and two emerging ones (peracetic acid and hydrogen peroxide associated with UV radiation). We also evaluated the impact of non-disinfected WW, compared to FW irrigation.

METHODS

The experiment consisted in a pot assay. It was conducted in a greenhouse located at the University of Campinas *campus*, in the city of Campinas, São Paulo state Brazil (22°49′09.5″S 47°03′40.3″W), between April and August 2019, corresponding to the dry winter months. The average temperature inside the greenhouse during the assay was 24.5 ± 14.0 °C.

The pots used were made of polyurethane, with holes in the base, size 15. Each pot received 1.6 kg of soil, from a non-agricultural region of the university *campus,* classified as Eutrophic Red Argisol, with clay texture according to the Brazilian Soil Classification System (SiBCS) (Santos *et al.* 2018). The initial soil physicochemical characterization is shown in Table 1.

Before planting, the wheat seeds (*Triticum aestivum* L.) cultivar IAC 389 ATAKAMA were placed to germinate in Petri dishes (Ø 15 cm), lined with filter paper, moistened with 5 mL of tap water, remaining in a BOD-type incubator, with the temperature controlled at 25 °C. After 5 days, six pre-germinated seeds were planted per pot.

The pots were placed randomly on a table to minimize interference from insects and other organisms from the external soil, and their position was changed, randomly every 15 days, to minimize any variations in light and temperature that can occur in the greenhouse (Figure 1).

To ensure that the plants' water demand would be completely met every day, an automated irrigation system was set up, providing crop irrigation twice a day, at 7:00 am and 7:00 pm, by drip irrigation systems. Each day, an amount of 100 mL was applied in each pot, keeping the soil moisture at the field capacity. The drippers were monitored periodically, and when clogging was detected, a new dripper was employed. The reservoirs filled with irrigation water were supplied weekly.

On day 130, at the end of the wheat crop cycle, the plants were harvested. The wheat grains produced in each pot were separated, counted, and weighted to determine the yield. In addition, soil in all treatments was destructively sampled to evaluate available N (ammonium, nitrate), available P, pH, electrical conductivity (EC), OM, and soil microbial composition.

The experimental design was completely randomized, with six irrigation treatments (four repetitions each):

- WW: secondary WW without disinfection step;
- AOP: secondary WW disinfected with hydrogen peroxide associated with UV irradiation;
- HYP: secondary WW disinfected with chlorine, as calcium hypochlorite;
- PAA: secondary WW disinfected with peracetic acid (PAA);
- UV: secondary WW disinfected with UV irradiation;
- FW: freshwater.

Table 1 | Soil physicochemical characteristics before the irrigation

Parameter	Value
pH	6.6
Phosphorus (P)	16.0 mg dm^{-3}
Potassium (K)	4.4 mmol dm^{-3}
Calcium (Ca)	105.0 mmol dm^{-3}
Magnesium (Mg)	11.0 mmol dm^{-3}
Copper (Cu)	3.8 mg dm^{-3}
Iron (Fe)	14.0 mg dm^{-3}
Manganese (Mn)	5.7 mg dm^{-3}
Zinc (Zn)	1.8 mg dm^{-3}
OM	82.0 g dm^{-3}
Hydrogen + Aluminum (H + Al)	24.0 mmol dm^{-3}
Sum of bases (SB)	120.4 mmol dm^{-3}
Cation exchange capacity (CTC)	144.4 mmol dm^{-3}
Base saturation (V%)	83.0% (V%)
Clay <0.002 mm	506.0 g kg^{-1}
Silt 0.002–0.053 mm	106.0 g kg^{-1}
Total sand 2.0–0.053 mm	388.0 g kg^{-1}

Irrigation water characteristics

The WW was from the Wastewater Treatment Plant (WWTP) 'Barão Geraldo' located in Campinas-SP – Brazil, which consists of an up-flow anaerobic sludge bed (UASB) followed by a trickling biofilters and decanters. The FW was from the *campus* drink water network, supplied by the Campinas city sanitation water supply company (SANASA). The physicochemical characteristics of the irrigation waters are shown in Table 2.

Figure 1 | Greenhouse experiment.

The treated WW was collected weekly and immediately taken to the laboratory of sanitation from the School of Civil Engineering, Architecture, and Urban Design of UNICAMP to undergo the disinfection processes. The disinfectants concentration, contact time, and UV radiation dose used were determined in a previous study developed by our research group (Leonel & Tonetti 2021) considering their efficacy to inactivate resistant pathogens (Table 3).

Microbial community characterization

To evaluate the soil microbial composition, total DNA was extracted from 500 mg of soil from each pot with the DNeasy® PowerSoil kit (QIAGEN, Hilden, Germany), according to the protocol recommended by the manufacturer.

The V4 region of the 16S rRNA structural gene was amplified using the 16S universal primers for bacteria and archaea 515F (Parada) (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (Apprill) (5′-GGACTACNVGGGTWTCTAAT-3′) (Caporaso *et al.* 2011; Apprill *et al.* 2015; Parada *et al.* 2016). The sequencing was performed using the Illumina MiSeq platform (2 × 150 bases for paired-end reads).

Raw sequences were analyzed using the bioinformatic pipeline FROGS (Find Rapidly OTU with Galaxy solution), a Galaxy workflow designed to produce an operational taxonomic unit (OTU) count matrix from high depth sequencing amplicon data (Escudié *et al.* 2018). Briefly, on the Galaxy platform, the software merged the paired-end reads using Flash (version 1.2.11) based on the overlapping regions and, filtered the sequences using Cutadapt (version 1.8) to remove those not between 250 and 340 bp length, those with ambiguous bases and those that did not contain the full primer sequences. Clustering was then performed using the algorithm Swarm (v2.1.2) with an aggregation maximal distance of three bases. In addition, chimeric sequences were removed using VSEARCH (v2.9.115) with cross-sample validation. The affiliation was performed using best blast hit against the database silva132 16S pintail100. And finally, a phylogenetic tree was constructed using FastTree (v2.1.1018) (Escudié *et al.* 2018).

Statistical analysis

All statistical analyses were performed using R (v4.0.2; R Core Team 2020). We used Student's *t*-test to evaluate the effects of water type (FW vs. WW) and analysis of variance (ANOVA) to test for effects of disinfectant treatments (WW, AOP, HYP, PAA, and UV) over soil physicochemical characteristics. Differences between

Table 2 | Physicochemical characteristics of the irrigation waters

Parameter	FW	WW	AOP	HYP	PAA	UV
Chemical oxygen demand (COD) (mg/L)	–	62.5	95.0	85.2	150.5	72.5
Total organic carbon (TOC) (mg/L)	–	14.8	15.7	18.1	45.7	19.2
Nitrogen (mg/L)	1.5	55.0	80.7	87.5	36.7	47.8
Phosphorus (mg/L)	–	2.9	4.4	4.1	5.3	3.9
Electrical conductivity (EC) (dS/m)	–	0.75	0.74	0.78	0.74	0.75
pH	7.9	8.0	8.1	7.8	7.1	8.0
Turbidity (NTU)	0.29	8.1	10.0	7.9	8.0	9.5

Performed in accordance with the Standard Methods for water and WW analysis (APHA 2012).

Table 3 | Disinfection conditions

Disinfectant	Concentration (mg L^{-1})	Radiation dose (mJ cm^{-2})	Wavelength (nm)	Contact time (min)
Calcium hypochlorite	40.0	NA	NA	30
Peracetic acid	40.0	NA	NA	30
	NA	100	255/280	45
UV radiation	NA	103	255/280/405	15
UV radiation + Hydrogen peroxide	1.5	103	255/280/405	15

NA, not applied.

disinfectant treatments were assessed with Tukey's HSD *post hoc* tests. All variables showed normal distribution (Shapiro–Wilk tests $p > 0.05$).

For crop yield, the variables presented a non-normal distribution, so we used Wilcoxon rank-sum exact test to evaluate the effects of water type and the Kruskal–Wallis rank-sum test for disinfectant treatment evaluation.

The PhyloSeq package (McMurdie & Holmes 2013) was used for phylogenetic and biostatistics analysis. Chao1 index, corresponding to the sum of the number of observed species and of the estimated number of unobserved species (Chao 1984), Shannon index, evenness of the species abundance distribution (Shannon 1948) and inverse Simpson (InvSimpson), a dominance index (Simpson 1949), were calculated to estimate richness and diversity within the samples. Two-way ANOVA was then performed on these three indexes to assess water type (FW or WW) and disinfection side effects. Beta diversity was evaluated by calculating Bray-Curtis distances between samples. Multivariate ANOVA (PERMANOVA) was then performed using the Adonis function with 9999 permutations.

To visualize the influence of the disinfection treatments on the beta diversity, a principal component analysis (PCA) was implemented with FactoMineR package (Lê *et al.* 2008). The OTU count data were first normalized with centered log-ratio (CLR) transformation. To evaluate the influence of the soil environmental conditions on microbial beta diversity, soil environmental variables were projected as vectors onto the PCA ordinations.

DESeq2 package (Love *et al.* 2014) was used to identify OTUs that were differentially abundant between WW compared to FW on the one hand, and between each disinfection treatment compared to WW on the other hand. The geometric means of the OTU count data were calculated prior to estimated size factors.

RESULTS AND DISCUSSION

Soil physicochemical characteristics

We evaluated the effects of the water type and of the disinfectant over soil physicochemical parameters, after one wheat crop cycle in pots. The main alterations observed were due to water type, not to the disinfection treatments (Table 4). Comparing FW-irrigated soil with WW-irrigated soil, pH, EC, and nitrate concentration (NO_3) were significantly different between the treatments. Comparing WW-irrigated soil with the soils that received disinfected WW (AOP, HYP, PAA, and UV), the physicochemical parameters were generally within the same ranges for all treatments (Table 4).

The salinity of the WW-irrigated soil (expressed as EC) increased in the end of the experiment, compared to the FW-irrigated soil (Table 4). The increase in soil salinity, is one of the main concerns related to irrigation with WW and have been reported by several countries (Farhadkhani *et al.* 2018; El Moussaoui *et al.* 2019; Erel *et al.* 2019; Pedrero *et al.*; Zolti *et al.* 2019; Chaganti *et al.* 2020; De las Heras & Mañas 2020). Continuous accumulation of salt can adversely affect soil permeability, soil structure, hydraulic conductivity, and the activity of the soil microorganisms, reflecting negatively on (Leonel & Tonetti 2021). Furthermore, some researchers have pointed out salt accumulation and, consequential leaching, leading to significant nitrogen losses from the root zone and contamination of deep soils and groundwater with nitrates (Segal *et al.* 2011; Erel *et al.* 2019; Pedrero *et al.* 2020). Indeed, several countries and the World Health Organization (WHO) have set limits for EC (>3.0 dS/m) and SAR (>10)

Table 4 | Soil properties after irrigation

	Soil properties						
Treatment	**EC (dS m^{-1})**	**pH**	**P (mg dm^{-3})**	**TN (g kg^{-1})**	**NH_3 (mg kg^{-1})**	**NO_3 (mg kg^{-1})**	**OM (g dm^{-3})**
FW[a]	0.425 ± 0.095*	6.2 ± 0.2*	26.0 ± 7.0	1.5 ± 0.1	8.5 ± 2.3	3.6 ± 2.0*	38.0 ± 2.6
WW[a,b]	1.150 ± 0.173	6.5 ± 0.1**	30.0 ± 9.3	1.5 ± 0.1	11.0 ± 2.3	21.0 ± 21.8	39.0 ± 1.6
AOP[b]	1.375 ± 0.221	6.7 ± 0.1	40.8 ± 3.5	1.6 ± 0.0	11.4 ± 1.7	31.8 ± 6.7	39.5 ± 1.3
HYP[b]	1.325 ± 0.613	6.6 ± 0.1	36.8 ± 4.6	1.6 ± 0.0	10.9 ± 2.7	16.0 ± 9.3	38.2 ± 1.5
PAA[b]	1.050 ± 0.238	6.7 ± 0.1	33.5 ± 3.0	1.5 ± 0.1	9.3 ± 1.3	19.4 ± 5.9	40.8 ± 1.7
UV[b]	0.875 ± 0.287	6.8 ± 0.1**	32.5 ± 3.3	1.6 ± 0.1	11.4 ± 1.5	18.5 ± 6.8	38.75 ± 1.5

[a]For water type evaluation, WW was compared to FW by Student's *t*-test.
[b]For disinfectant treatment evaluation, WW was compared with AOP, HYP, PAA and UV by one way ANOVA.
*p-value < 0.05.
**Tukey's HSD post hoc tests p-value <0.05.

in the case of WW reuse in agriculture (Common Ministerial Decision no 145116 (354B)/2011; Italian Ministry Decree 185/2003, WHO 2006; Paranychianakis *et al.* 2015).

In the present study, we used WW within the limits established by agriculture reuse guidelines (Table 2), and at the end of the experiment, the WW-irrigated soil could be still considered as a non-saline soil (EC < 2 dS/m) (Abrol *et al.* 1988). The salinity increase caused by the WW irrigation (expressed as EC), compared to FW (Table 4), was probably due to the pot assay, performed in a greenhouse. In an open field condition, the leaching by the rainfall would avoid salt accumulation. However, the 2.7 times higher soil salinity in WW-irrigated soils could be a problem in arid and semi-arid regions where the evaporative demand is high and the natural precipitation is low (Muyen *et al.* 2011). The soil salinization rate does not depend only on water quality, but also on some soil characteristics such as transmissivity, OM content, and land drainage; and external factors such as irrigation, precipitation, and evaporation rates (WHO 2006). Therefore, monitoring soil salinity periodically at the site is important to guarantee a sustainable reuse practice in agriculture irrigation.

The disinfection treatments did not change the EC of the WW (Table 2), and consequently, the EC values of the soils irrigated with WW were within the same ranges, no matter the disinfectant treatment applied (Table 4). There is some concern about an increased risk of soil salinization when chlorinated WW is used for irrigation, once the chlorine released by the oxidation reaction can be converted into chloride salts (Li *et al.* 2014). The high chlorine demand of the WW used in this research (Silva *et al.* 2020), due to the high ammonia content, avoided the accumulation of free chlorine in the WW, and consequently the accumulation of chloride salts. The reaction between chlorine and ammonia is almost instantaneous in domestic effluent, where the pH is close to neutrality, forming chloramines and reducing the free chlorine available. The same was observed by Li *et al.* (2014), using chlorine concentration below 50 mg L^{-1}.

Regardless of the statistical significance ($p = 0.036$), for all the six treatments evaluated, the soil pH (Table 4) was within the optimum range for plant development (6.2–6.8) (Rechcigl 2017), and did not change from the soil initial pH (Table 1), perhaps due to high buffering capacity of the soil. The same was observed by Farhadkhani *et al.* (2018) and Guo *et al.* (2017).

In this study, we did not observe a significant increase ($p = 0.084$) in the available phosphorus concentration, in the soil irrigated with WW, compared with that irrigated with FW. The low P concentration (2.9 ± 2.3 mg L^{-1}) of the secondary WW used in this research, combined with a low hydraulic load rate per pot (100 mL day^{-1}) resulted in an applied P rate of approximately 1 kg P ha^{-1} $year^{-1}$. As we did not add any other source of P, the plant probably used a considerable part of the P supplied by the WW for its development, avoiding its accumulation in the soil. An increase in available soil phosphorus was reported by Adrover *et al.* (2012), Bedbabis *et al.* (2010), Mohammad & Mazahreh (2003), in treated WW-irrigated soils, reflecting the high phosphorus content in the WW used. According to Elliott & Jaiswal (2012), effluents containing elevated total P levels (6–15 mg P L^{-1}) will almost assuredly result in excess P for all common cropping scenarios, even at low hydraulic loading rates, especially in the long term.

In the same way, the total nitrogen (TN) followed a similar pattern for both water type treatments (FW and WW) ($p = 0.050$) (Table 4), even if the TN concentration was much higher in WW (55 ± 12.2 mg L^{-1}) than FW (around 1 mg L^{-1}). The same was observed by Guo *et al.* (2017), suggesting that most of the N in the WW was in forms that could be easily uptaken by the plant: NO_3^- and NH_4^+ (Glass *et al.* 2002; Guo *et al.* 2019). The high concentration of NH_4^+ in the WW used in this study was previously reported by Silva *et al.* (2020).

Although we have not observed an increase in NH_4^+ concentration in WW-irrigated soil, compared with FW ($p = 0.132$), the NH_4^+ content in the WW seems to have stimulated the soil nitrification rate, resulting in NO_3^- accumulation in WW-irrigated soil, compared with FW. This is in accordance with Crecchio *et al.* (2004) and Elifantz *et al.* (2011), who observed a higher nitrification rate in soils irrigated with treated wastewater. Rates of nitrification generally respond quickly to ammonium additions (Norton & Stark 2011). In WW-irrigated soil, the nitrification rate was probably higher than the nitrate uptake rate by the wheat, resulting in nitrate accumulation. The high nitrate (NO_3^-) concentration in soils can be beneficial for crops, but in excess, it could be leaching, thereby contaminating deeper soil and groundwater (Leonel & Tonetti 2021).

The disinfectant treatment did not provide a significant difference ($p < 0.05$) in the nutrient content of the soil. However, over time, the chlorine deposition by irrigated water can impact nutrient accumulation, diminishing soil fertility (Li *et al.* 2014).

We did not observe an increase of the OM content in WW-irrigated soils, compared to FW-irrigated ones, probably due to the limited duration of the experiment or to the fast decomposition of the effluent OM added to the pots, as similarly reported by Kayikcioglu (2012). The soil OM improvement is usually observed when low-quality WW is used for irrigation (Adrover *et al.* 2012; Orlofsky *et al.* 2016; Bastida *et al.* 2017; Dang *et al.* 2019; El Moussaoui *et al.* 2019; Zolti *et al.* 2019), which is not the case in this study.

Importantly, this was an initial short-term study, with little alteration in soil physicochemical properties, but previous research works have shown that WW irrigation effects become stronger with time (Pedrero *et al.* 2010; Adrover *et al.* 2012; Morugán-Coronado *et al.* 2013; Wafula *et al.* 2015; Bastida *et al.* 2017; Jaramillo & Restrepo 2017; Erel *et al.* 2019). It calls for attention to the need of periodic monitoring of the soil that receives reclaimed water, to ensure a sustainable reuse practice in agriculture.

Impact on wheat crop yield

Like the soil properties, the crop yield was influenced by the water type ($p = 0.028$), but not by the disinfectant treatments ($p = 0.642$) (Table 5). Irrigation with WW improved almost 5 times the wheat grains yield, compared with FW ($p < 0.05$). The wheat grains production was, on average, of 8.64 g pot^{-1} in WW treatment and 1.78 g pot^{-1} in FW-irrigated plants. The higher yield obtained in WW-irrigated pots was probably a consequence of the nutrient supply, such as N and P, key elements required for plant growth (Zohar *et al.* 2010; Guo *et al.* 2019; Wierzbowska *et al.* 2020). While the FW-irrigated plants did not receive any fertilizer, the total N and P applied in each WW-irrigated pot was around 742.0 and 43.0 g, respectively, throughout the experiment. Crop yield increases have been frequently associated with nutrient content in the WW used for irrigation (Bedbabis *et al.* 2010; Cirelli *et al.* 2012; Martínez *et al.* 2013; Marinho *et al.* 2014, 2013; Gatta *et al.* 2016).

Nitrogen and phosphorus are the main wheat yield-limiting elements. Insufficient N and P supply reduces tillering, and consequently the number of heads per square meter; it causes flower abortion and reduction in floret primordium initiation, decreasing the number of grains per head; it results in the disturbance of normal cell growth division, and a decrease in the rate and extent of protein synthesis (Römer & Schilling 1986; Jeuffroy & Bouchard 1999). Supplementary Material, Figure S1 shows the phenotypic differences between the plants irrigated with WW and FW, and the lack of nutrients is clear.

The irrigation with chlorinated WW (HYP) did not prejudice the wheat development or yield (Table 5), probably due to the low residual chlorine concentration in the HYP reservoir (<1.0 mg L^{-1}). A previous study suggested that the greater the residual chlorine, the worst is the plant damage (Lonigro *et al.* 2017). Lonigro *et al.* (2017) reported that lettuces irrigated with chlorinated water showed typical symptoms of stress, such as chlorosis, leaf necrosis, and reduced crop yield, mainly when the free chlorine concentration was higher than 10.0 mg L^{-1}. In addition, chlorination also reduced the yields and quality of chlorine-sensitive tobacco and potato (Song *et al.* 2019).

The absence of a negative effect on wheat yield does not mean that HYP irrigation is safe. The accumulation of DBP such as chlorate and organohalogenated compounds was reported in the edible part of fresh lettuce and baby spinach irrigated with chlorinated WW (Lonigro *et al.* 2017; López-Gálvez *et al.* 2018; Garrido *et al.* 2020). The DBP may have genotoxic effects and adverse impacts on human health (Gil *et al.* 2016; Lonigro

Table 5 | Crop yield average per treatment

	Crop yield	
Treatment	Grain weight (g pot^{-1})	Number of grains per pot
FW[a]	1.9 ± 0.5*	44 ± 12
WW[a,b]	8.6 ± 4.1	195 ± 93
AOP[b]	6.4 ± 1.5	157 ± 38
HYP[b]	6.3 ± 2.0	215 ± 67
PAA[b]	7.9 ± 6.2	188 ± 147
UV[b]	5.4 ± 1.6	127 ± 38

[a]For water type evaluation, WW was compared to FW by the Wilcoxon rank-sum exact test.
[b]For disinfectant treatment evaluation, WW was compared with AOP, HYP, PAA and UV by the Kruskal–Wallis rank-sum test.
*p-value = 0.028.

et al. 2017). In the current work, we did not evaluate the DBP formation, however, it is important to draw attention to the risk associated with these compounds when chlorinated WW is used for crop irrigation. in the world (USEPA 2012), thus chlorine doses must be managed to maximize pathogen inactivation and minimize the formation of harmful DBP.

PAA, UV, and POA also did not prejudice the wheat crop yield, corroborating with previous studies that pointed out these treatments as good alternatives to chlorination, since they showed very good disinfection capabilities and no phytotoxic DBP formation (Liberti *et al.* 2003, 1999; Kitis 2004; Antonelli *et al.* 2013; Chevremont *et al.* 2013, 2012; Rizzo *et al.* 2020; Sgroi *et al.* 2021).

Microbial community characterization

A total of 1,759,806 sequences were generated through Illumina Miseq platform sequencing from the 24 analyzed soil samples. After quality filtering and rare OTU removal, a total of 1,255,661 sequences, with an average number of sequences per sample of 52,319 (Supplementary Material, Figure S2), remained for the microbial diversity analyses. The sequences were clustered into 1,857 OTUs. The rarefaction curve of OTUs reached the plateau, showing that the sequencing depth was sufficient (Supplementary Material, Figure S3).

The richness (Chao 1 index) and the alpha diversity (Shannon and InvSimpson indexes) of the microbial community were not significantly associated with the water type, neither with the disinfection process side effects ($p > 0.05$) (Figure 2). This agrees with other studies, in which the water irrigation quality had an insignificant impact on the overall alpha diversity and richness of the soil microbiome (Elifantz *et al.* 2011; Broszat *et al.* 2014; Frenk *et al.* 2014; Ibekwe *et al.* 2018; Krause *et al.* 2020).

The OTUs were affiliated to 25 phyla: 23 from Bacteria and 2 from Archaea. Acidobacteria (23%), Proteobacteria (19%), Actinobacteria (15%), and Chloroflexi (15%) were the most abundant phyla across all the 24 soil

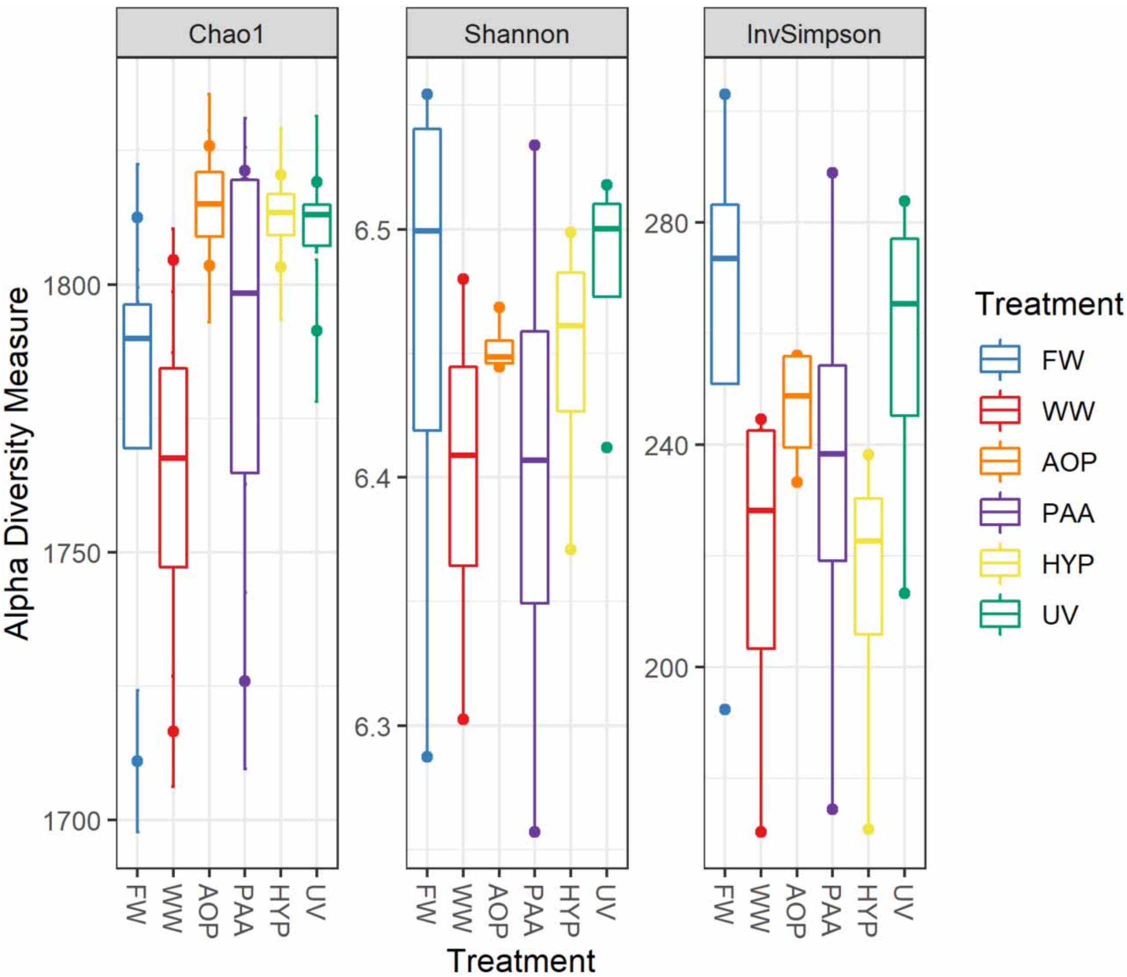


Figure 2 | Alpha diversity indexes per treatment.

samples, following the common soil microbial distribution (Kim *et al.* 2016; Reese *et al.* 2017; Ibekwe *et al.* 2018; Kulkarni *et al.* 2018; Miranda *et al.* 2018; Wang *et al.* 2018; Dang *et al.* 2019; Song *et al.* 2019; Uddin *et al.* 2019; Zolti *et al.* 2019; Krause *et al.* 2020); moreover, the irrigation treatments did not shift the relative abundance of the 10 dominant phyla (Figure 3).

However, the Bray-Curtis dissimilarity index (beta diversity) demonstrated that the water type (PERMANOVA, $R^2 = 0.094$; $p = 0.0058$) and the disinfectant treatments (PERMANOVA, $R^2 = 0.345$; $p = 0.0003$) influenced the soil community composition. Previous studies also observed community structure differences between soils irrigated with different water sources (Frenk *et al.* 2014; Wafula *et al.* 2015; Bastida *et al.* 2017; Dang *et al.* 2019; Zolti *et al.* 2019).

We removed the samples from the FW treatment and performed a PCA to visualize the effect of the disinfectant treatments on the beta diversity and to identify the main soil variables that explained the variance in microbial community structure. The PCA biplot (Figure 4) revealed some relationship between the microbial community and the treatments.

The PCA's first two axes explained 36.7% of the variance. Along PC1, the samples were separated according to the treatments, with WW and PAA samples on the positive side and AOP, HYP, and UV samples on the negative side.

Despite the little effect caused by the disinfectants on the soil physicochemical properties, the biplot (Figure 4) clearly shows that several physicochemical parameters of the soil were linked with the soil microbial community composition, according to in each type of disinfectant treatment. The physicochemical parameters were represented as supplementary variables, so that the vectors are the gradients of the soil variables in the 2D plane defined by PC1 and PC2.

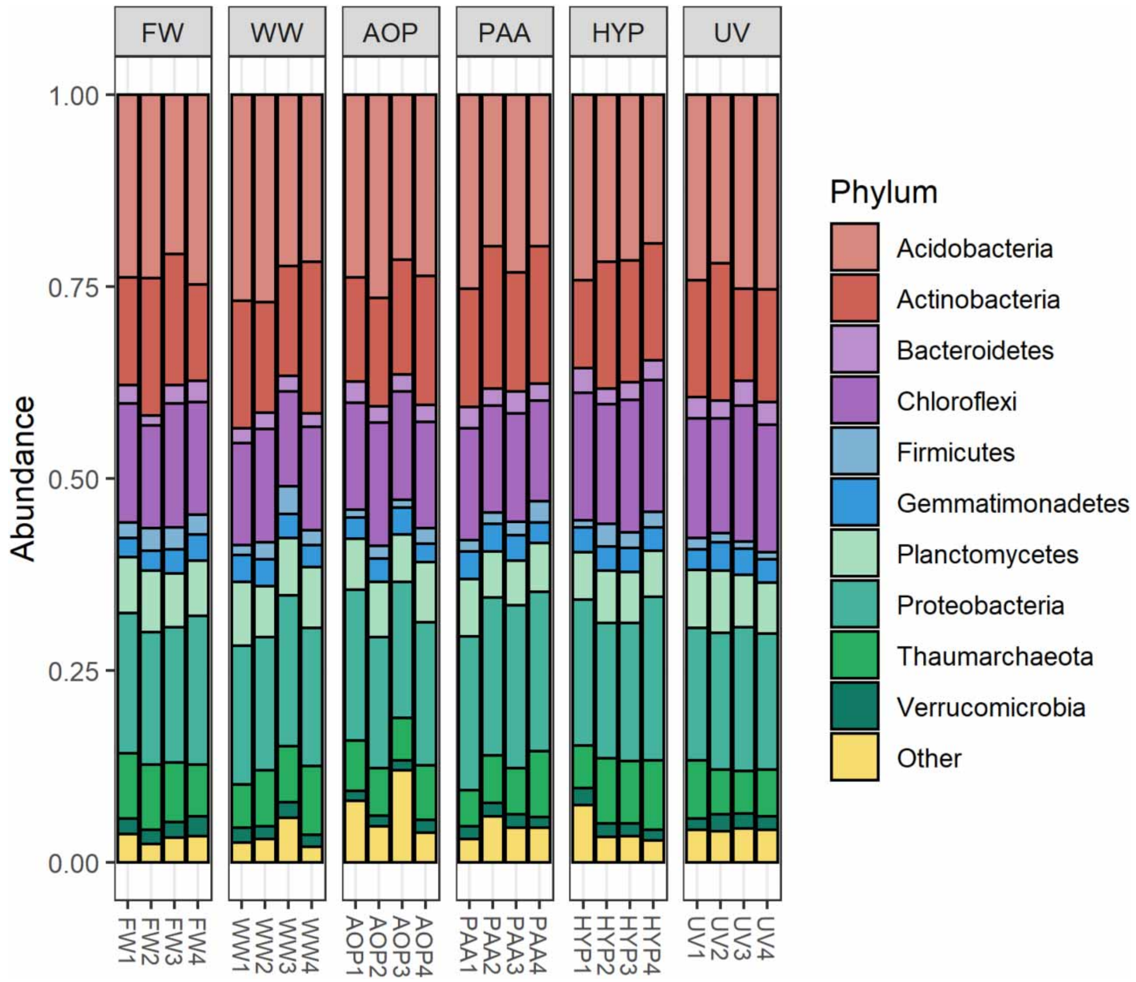


Figure 3 | Relative abundance of the top 10 phyla, in each sample.

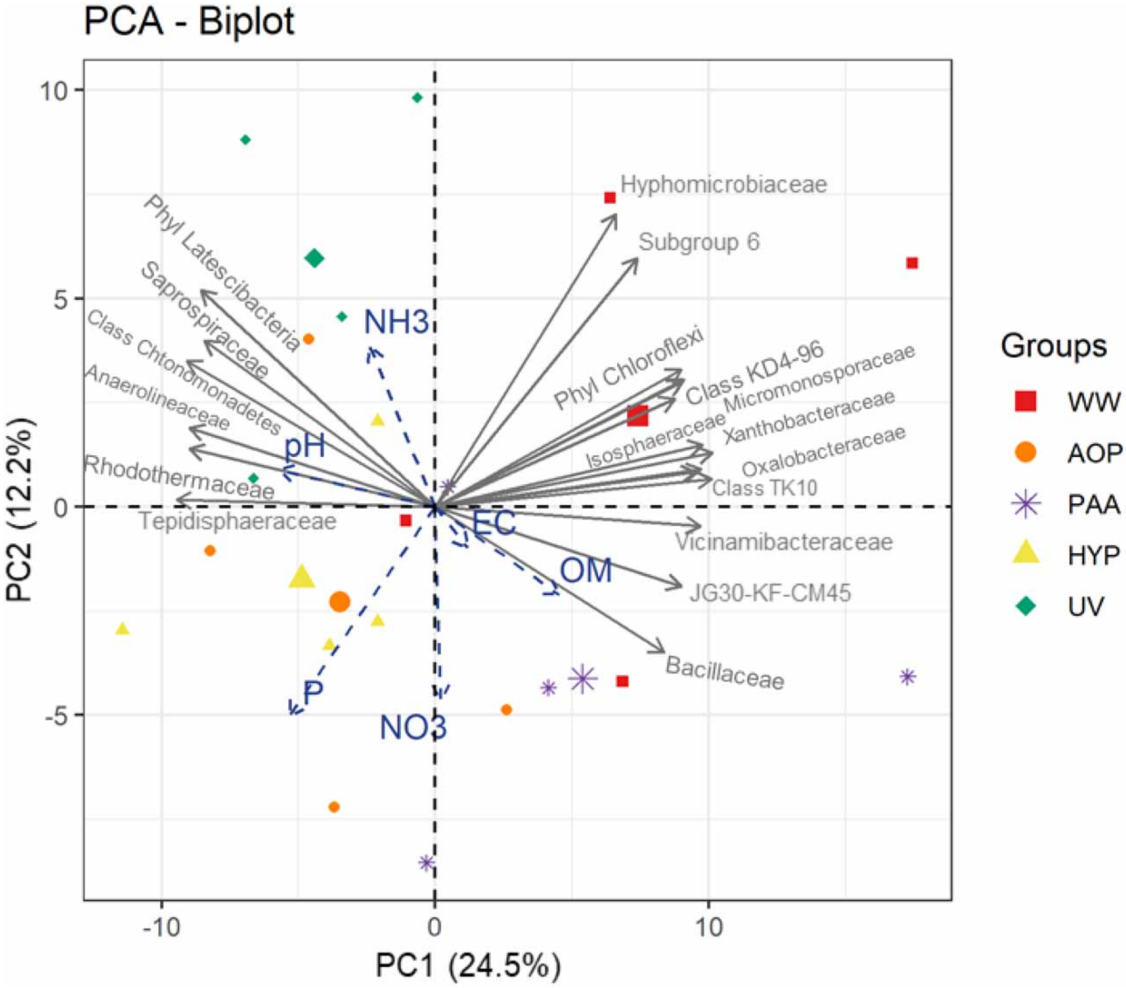


Figure 4 | PCA biplot illustrating the soil microbial composition based on disinfectant treatment. Physicochemical soil parameters (supplementary variables) such as OM (organic matter); EC (electrical conductivity); P (phosphorus); pH (hydrogen potential); NH_3 (ammonia); NO_3 (nitrate) are shown in blue. For this analysis, the samples from FW treatment were removed. Only the 20 most abundant OTU are plotted and their taxonomic affiliation at the family level is shown. The bigger shapes indicate the barycenter of each corresponding group.

OTUs from the bacterial families Saprospiraceae (Bacteroidetes), Anaerolineaceae (Chloroflexi), Rhodothermaceae (Bacteroidetes), Tepidisphaeraraceae (Planctomycetes), and from one unidentified family of each phylum Armatimonadetes (Class Chthonomonadetes) and Latescibacteria (unidentified class) were negatively related to EC and OM and positively related to NH_3 and pH. OTUs belonging to Bacillaceae (Firmicutes), JG30-KF-CM45 (Cloroflexi), and Vicinamibacterceae (Acidobacteria) showed opposite trends. OTUs from Hyphomicrobiaceae (Proteobacteria), Thermomicrobiales (Chloroflexi), Gemmataceae (Planctomycetes), Isospharaceae (Planctomycetes), Xanthobacteraceae (Proteobacteria), Oxalobacteraceae (Proteobacteria), from two unidentified Chloroflexi families (Classes TK10 and KD4-96), and from one unidentified Acidobacteria family (Class SubGroup 6) were negatively correlated to P.

Various correlations between the disinfectant treatments and the 20 most abundant OTUs were also visible (Figure 4). To further understand the effect of water type and disinfectant treatments over the prokaryote taxa selection, we performed a differential analysis of OTU count data based on negative binomial, comparing FW with WW and WW with AOP, HYP, PAA, and UV individually. Figure 5 shows the relative abundance of the differentially abundant OTUs ($p < 0.05$) in each comparison. The impact of the water type and disinfection treatment was more significant in sub-dominant OTUs, corresponding to less than 15% of the community relative abundance.

To visualize the taxa selected by water type and disinfectant treatments, we built scatter plots based on differential abundance analysis. Figure 6 shows the differentially abundant OTUs in the soil irrigated with both water

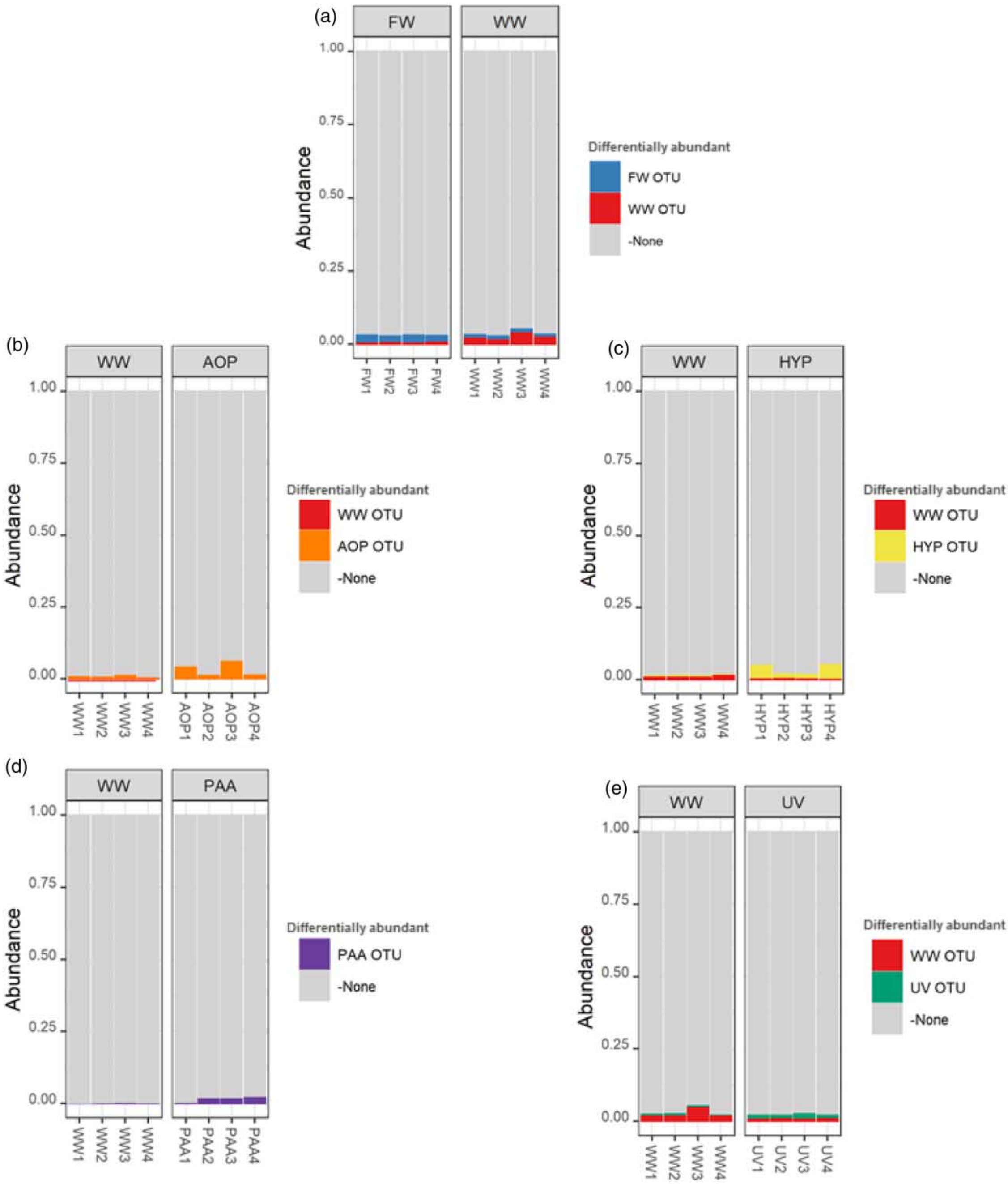


Figure 5 | Relative abundance of differentially abundant prokaryotic OTUs ($p < 0.05$), based on negative binomial. (a) Comparison between FW-irrigated soil and WW-irrigated soil; (b) comparison between WW-irrigated soil and AOP-irrigated soil; (c) comparison between WW-irrigated soil and HYP-irrigated soil; (d) comparison between WW-irrigated soil and PAA-irrigated soil; (e) comparison between WW-irrigated soil and AOP-irrigated soil. FW OTU: OTUs significantly more abundant in FW-irrigated soil; WW OTU: OTUs significantly more abundant in WW-irrigated soil; HYP OTU: OTUs significantly more abundant in WW disinfected with HYP-irrigated soil; PAA OTU: OTUs significantly more abundant in WW disinfected with PAA-irrigated soil; AOP OTU: OTUs significantly more abundant in WW disinfected with AOP-irrigated soil; UV OTU: OTUs significantly more abundant in WW disinfected with UV-irrigated soil.

types (WW vs. FW). Positive log2 Fold change values correspond to OTUs more abundant in WW-irrigated soil, and the negative values in the FW-irrigated one.

Comparing WW with FW, most of the differentially abundant OTU in WW-irrigated soil were affiliated to Proteobacteria families (Figure 6), such as Cellvibrionaceae, a cellulolytic family, usually found in soils (Ranalli *et al.* 2019); Micropepsaceae, a family involved in carbon cycling (Behnke *et al.* 2021); Burkholderiaceae, an OM degrading family, ubiquitous in diverse environments polluted by sewage, that has also been linked to

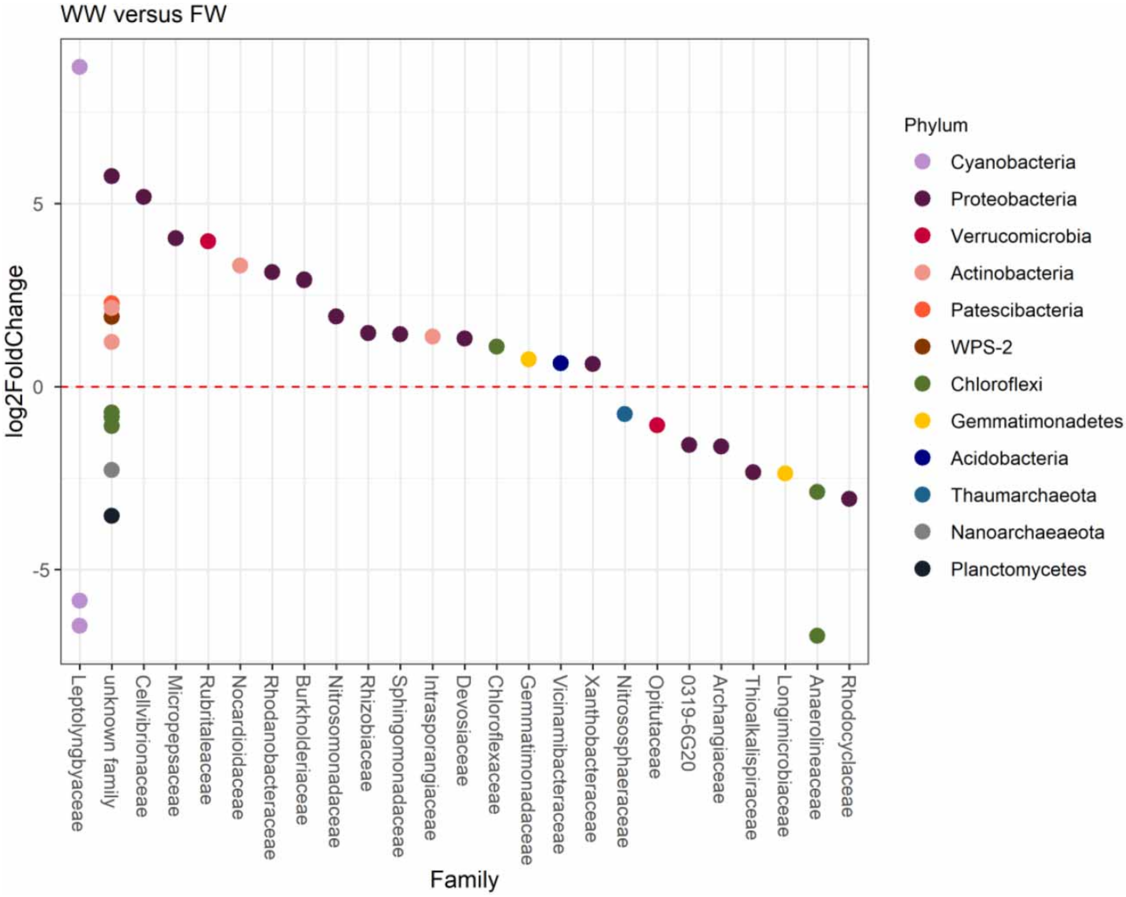


Figure 6 | Differentially abundant prokaryotic OTUs ($p < 0.05$) in WW-irrigated soil compared to FW-irrigated soil, based on negative binomial, shown according to their taxonomic affiliation. The positive log2 Fold change values correspond to the OTUs which are significantly more abundant in WW-irrigated soil. The negative log2 Fold change values correspond to the OTUs which are significantly more abundant in FW-irrigated soil.

contaminant degradation in the rhizosphere (Kator & Rhodes 2003; Carrión *et al.* 2018; Worrich *et al.* 2018); Sphingomonadaceae, associated to nutrient-enriched soil (Yang *et al.* 2019), Nitrosomonadaceae, a nitrifying bacteria family (Prosser *et al.* 2014); Rhodanobacteraceae, comprising bacteria capable of performing heterotrophic denitrification (de Almeida Fernandes *et al.* 2018); and three nitrogen-fixing families from the orders Rhizobiales: Rhizobiaceae, Devosiaceae, and Xanthobacteraceae (Franche *et al.* 2009; Oren 2014a).

Wu *et al.* (2021) observed a positive correlation between the increase in the relative abundance of families Sphingomonadaceae, Xanthobacteraceae, Rhizobiaceae in the wheat rhizosphere, and N fertilization levels. The authors also correlated Nitrosomonadaceae abundance with the concentration of NO_3 in the soil (Wu *et al.* 2021). In the current study, the FW-irrigated soil did not receive any nitrogen supply, while the WW ones received on average 742.0 g of nitrogen per pot, throughout the experiment. In addition, the NO_3 accumulated in the FW-irrigated soil was 5 times lower, which can explain the highest abundance of the above-mentioned families in WW-irrigated soil.

Proteobacteria are typically copiotrophic and abundant in soils rich in easily available OM (Nemergut *et al.* 2010; Dang *et al.* 2019). Its abundance increase is usually reported in soils irrigated with WW (Broszat *et al.* 2014; Frenk *et al.* 2014; Bastida *et al.* 2017; Guo *et al.* 2017; Ibekwe *et al.* 2018; Dang *et al.* 2019). However, Proteobacteria being a diverse metabolic group, we also observed some OTUs affiliated to Proteobacteria families that were significantly more abundant in FW-irrigated soil (Figure 6), such as Thioalkalispiraceae, a sulfur oxidizer family, Rhodocyclaceae that oxidize a range of simple sugars and organic acids (Oren 2014b), Archangiaceae, a nitrogen-fixing bacteria commonly distributed in soil environments (Masuda *et al.* 2020); and two uncultivated families from the classes Gamma and Deltaproteobacteria.

Chloroflexi is another metabolic and environment-diverse phylum, identified as differentially abundant in both water type treatment (Figure 6). Members of the autotrophic photosynthetic family Chloroflexaceae were more abundant in WW-irrigated soil (Gupta 2013), and members of the poorly characterized orders Ardenticatenales, SBR1031 and RBG-13-54-9 and family Anaerolineacea in FW-irrigated soil (Figure 6). Members of these last groups have been previously related to low-nutrient soils (Behnke *et al.* 2021; Frey *et al.* 2021).

We identified members of the Cyanobacteria family Leptolyngbyaceae in both water type irrigated soil (Figure 6). The genus *Leptolyngbya* was more abundant in WW-irrigated soil, the species of this genus have been reported in WW treatment plants and in dripper biofilms, when WW was used for irrigation; furthermore, it is associated with salinity tolerance (Furtado *et al.* 2009; Maity *et al.* 2014; Lequette *et al.* 2019). The genus *Oscillatoria*, more abundant in FW-irrigated soil, can promote carbon fixation and nutrient accumulation (Zhang *et al.* 2018). The presence of *Oscillatoria* in FW-irrigated soil can be an adaptation to N limitations, as previously observed in rice roots in paddy soil (Hoque *et al.* 2001).

The family Rubritaleaceae, from the phylum Verrucomicrobia, is more abundant in WW-irrigated soil while the family Opitutaceae is in higher proportion in FW-irrigated ones (Figure 6). Verrucomicrobia is found in many diverse biomes, including soil, nevertheless, the adaptations and functions of their members are enigmatic since the proportion of cultured members is low (Bünger *et al.* 2020).

OTUs affiliated to members of Actinobacteria were more abundant in WW-irrigated soil (Figure 6), unlike previous observations by other authors, who reported a decrease of Actinobacteria in soils irrigated with WW (Elifantz *et al.* 2011; Broszat *et al.* 2014; Frenk *et al.* 2014; Wafula *et al.* 2015; Bastida *et al.* 2017). Phylum level distribution does not indicate specific metabolic processes or a specific response to spatial patterns, thus, especially for a highly diverse group as Actinobacteria, most explanatory taxonomic rank must be used to further understand the microbial composition shifts (Frenk *et al.* 2014; van Bergeijk *et al.* 2020). Analyzing taxa at a finer level, our result is completely understandable: the differentially abundant OTUs in WW-irrigated soil are affiliated to Nocardioidaceae, Intrasporangiaceae, and an unidentified family from the class Acidimicrobiia, all of them already isolated from WWTP (Hu *et al.* 2018; Peng *et al.* 2018; Świątczak *et al.* 2019). This is in agreement with Bastida *et al.* (2017), who reported that some microbes inhabiting WW can persist in soil. OTUs affiliated to an unidentified family from the order Frankiales were also more abundant in WW-irrigated soil. Some members of this order are symbiotically fixing nitrogen within nodules of vascular plants, but to date, no study reported their association with wheat root (Franche *et al.* 2009; Normand & Fernandez 2020).

From the phylum Gemmatimonadetes, the family Gemmatimonadaceae was in higher proportion in WW-irrigated soil and the oligotrophic family Longimicrobiaceae (Pascual *et al.* 2016) in FW-irrigated soils (Figure 6). The Gemmatimonadaceae genus *Gemmatimonas* comprises exclusively N_2O-reducing organisms. N_2O is a potent greenhouse gas and ozone depletion agent, mainly produced by the biological transformation of N fertilizer applied to agricultural soils, even when fertigation with WW is used (Zhou *et al.* 2011; Wafula *et al.* 2015). Nitrification produces N_2O as a byproduct of ammonia oxidation, and denitrification emits N_2O as a stable intermediate or a final product (Park *et al.* 2017). The N_2O-reducing organisms are the only biological sink of N_2O in the environment, playing an important role in global warm control. The higher abundance of *Gemmatimonas* in WW-irrigated soil can indicate a balance in the soil that received nitrogen from wastewater, since, as reported before, we identified in the same soil bacterial families involved in the previous phases of the nitrogen cycle.

The order Saccharimonadales, from the Phylum Patescibacteria, usually correlates to nutrient-rich sites (Mason *et al.* 2021; Shi *et al.* 2021); it was more abundant in WW-irrigated soil. The same was observed for the family Vicinamibacteraceae, from the phylum Acidobacteria, a common soil clade (Huber *et al.* 2016). In WW-irrigated soil, we also identified a higher number of OTUs affiliated to the uncultured Bacteria phylum WPS-2 (recently proposed as Candidatus Eremiobacterota) (Figure 6).

An OTU affiliated to a poorly known family of the phylum Planctomycetes was more abundant in soils irrigated with FW (Figure 6), possibly corresponding to the one observed by Bastida *et al.* (2017).

OTUs affiliated to two Archaea phyla were more abundant in FW-irrigated soil (Figure 6). Regarding the ammonia-oxidizing family Nitrososphaeraceae, from the phylum Thaumarchaeota, ecophysiological studies of this taxon suggest adaptation to low ammonia concentrations and demonstrated a negative correlation with organic carbon (Pester *et al.* 2011; Kerou & Schleper 2016; Frey *et al.* 2021), which explains their higher abundance in FW-irrigated soils. Regarding the family Woesearchaeia, from the phylum Nanoarchaeaeota, their members are usually negatively associated with salinity, (Wang *et al.* 2019) consistent with its distribution in the present study.

Comparing HYP with WW, the irrigation with chlorine-disinfected WW (HYP) selected mainly OTUs from Cyanobacteria and Chloroflexi phyla (Figure 7). Some members of these phyla can degrade organohalogenated compounds, such as trihalomethane, a chlorine disinfection byproduct (Kuritz 1998; Robert *et al.* 2001; Kassouf *et al.* 2018; Maillard & Willemin 2019). For instance, in high nitrate environments the family Nostocaceae, from Cyanobacteria, use their nitrate-reduction system to metabolize chlorinated organic compounds (Kuritz 1998).

In WW-irrigated soil, we identified the highest abundance of two Proteobacteria families related to the nitrogen cycle: Rhodomicrobiaceae, nitrogen-fixing and Steroidobacteraceae, denitrifying; and also three Actinobacteria families, usually related to inorganic nitrogen input: Micromonosporaceae, Streptosporangiaceae, and

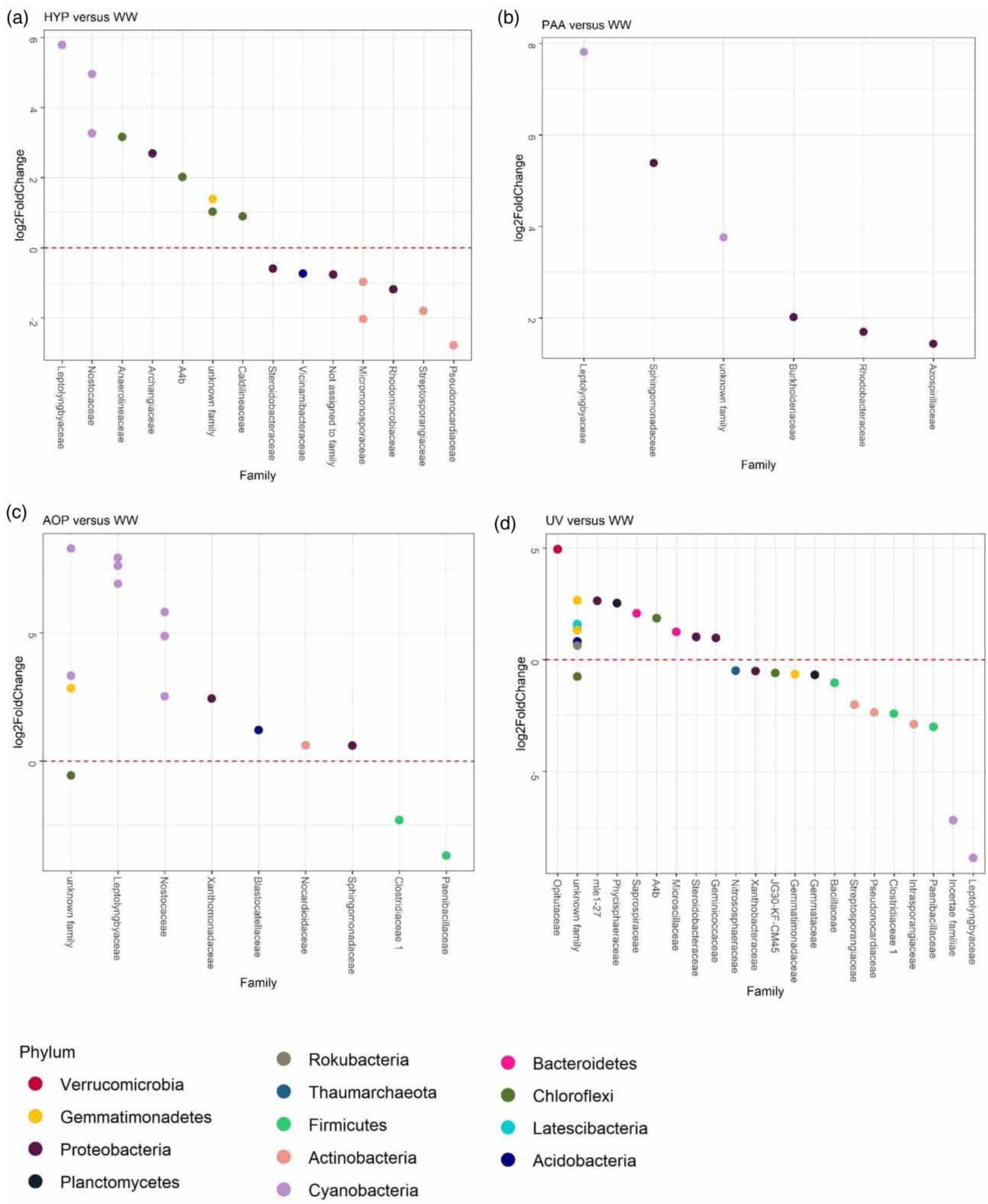


Figure 7 | Differentially abundant prokaryotic OTUs ($p < 0.05$) in soil irrigated with HYP (a), PAA (b), AOP (c), UV (d) compared to WW-irrigated soil based on negative binomial, shown according to their taxonomic affiliation. The positive log2 Fold change values correspond to the OTUs which are significantly more abundant in soil irrigated with HYP (a), PAA (b), AOP (c), UV (d); the negative log2 Fold change values correspond to the OTUs which are significantly more abundant in WW-irrigated soil.

Pseudonocardiaceae (Trujillo *et al.* 2014; Fernández-González *et al.* 2019) (Figure 7). This can suggest a reduced soil nutrient cycling and potential soil biological health problems in HYP-irrigated soil.

In addition, OTUs affiliated to the uncultured S0134 terrestrial group from Gemmatimonadetes and to the Archangiaceae family from Proteobacteria were more abundant in HYP-irrigated soil, and OTUs affiliated to the barely known Vicinamibacteraceae family from Acidobacteria was differentially abundant in WW-irrigated soil (Figure 7).

Surprisingly, the irrigation with HYP did not select chlorine-resistant bacteria, such as the Firmicutes genus *Clostridium* (Cui *et al.* 2020).

The community of the soil irrigated with peracetic acid-disinfected WW (PAA) presented only six differentially abundant OTUs, affiliated to the phyla Proteobacteria and Cyanobacteria, more abundant than in WW-irrigated soil (Figure 7). The Proteobacteria families Sphingomonadaceae, Burkholderiaceae, Rhodobacteraceae, and Azospirillaceae, are catalase-positive (Pujalte *et al.* 2014; Carrión *et al.* 2018; Onori *et al.* 2018; Sharma *et al.* 2021). Catalase is responsible for the protection, interception, and repair of microorganisms against hydrogen peroxide (H_2O_2), one of the decomposition products of peracetic acid. The catalase enzyme could contribute to these organisms' survival in an environment submitted to peracetic acid disinfection (Pardieck *et al.* 1992).

Cyanobacteria also have effective protection mechanisms, such as catalases, peroxidases, and superoxide dismutase, to avoid oxidative damage from potentially harmful reactive oxygen species (ROS) which include the hydroxyl radical, a strong oxidant generated by the H_2O_2 photocatalytic dissociation (Rastogi *et al.* 2014). This explains the differential abundance of this group of bacteria in PAA-irrigated soil, compared to WW-irrigated ones (Figure 8).

Similarly, we see eight OTUs affiliated to Cyanobacteria and more abundant in the soil irrigated with the WW disinfected by the advanced oxidative process that combines hydrogen peroxide (H_2O_2) with UV radiation (AOP) (Figure 7). Indeed, all the OTUs differentially abundant in AOP-irrigated soil were affiliated to bacterial families that present ROS-scavenging enzymes: Nocardioidaceae (Actinobacteria), Sphingomonadaceae (Proteobacteria) Xanthomonadaceae (Proteobacteria), Blastocatellaceae (Acidobacteria) (Denner *et al.* 2015; Yu *et al.* 2015; Wirgot *et al.* 2017; Du *et al.* 2020).

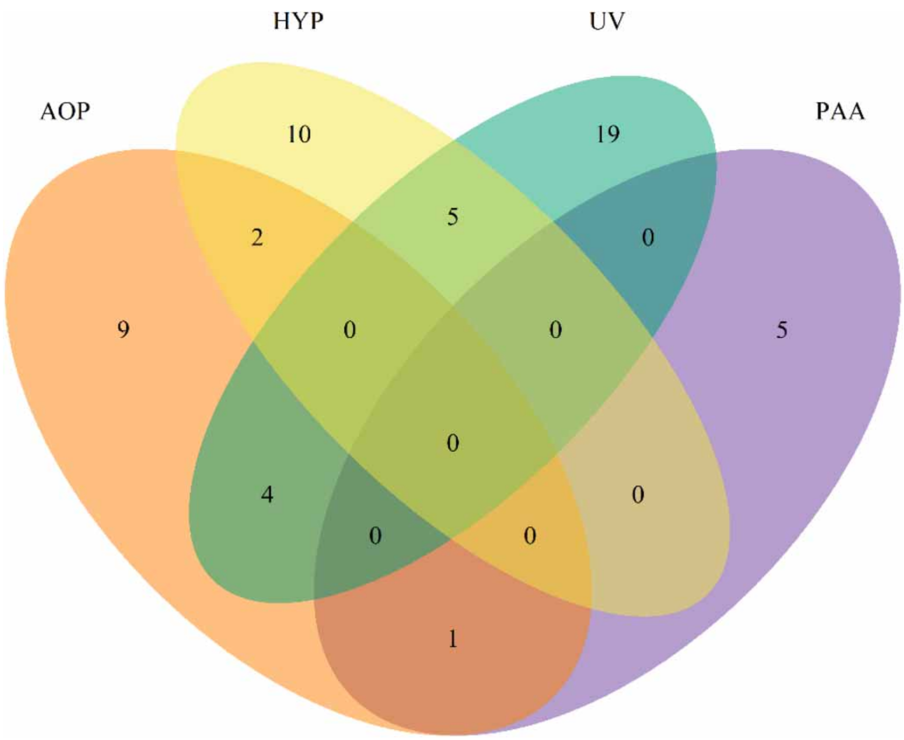


Figure 8 | Venn diagram to summarize the unique and shared differentially abundant OTUs among the soils irrigated with WW subjected to the different disinfectant treatments.

Only three OTUs were more abundant in WW-irrigated soil, compared to AOP ones. One unknown family from Chloroflexi phylum, and two affiliated to the phylum Firmicutes, genera *Clostridium* (Clostridiaceae) and *Aneurinibacillus* (Paenibacillaceae) (Figure 7). Both genera are spore-forming bacteria, usually found in WWTP (Nikaeen *et al.* 2015; Skariyachan *et al.* 2018). Their higher abundance in WW-irrigated soil can indicate that AOP treatment was effective against resistant microorganisms indigenous to wastewater. Previous studies also showed the low resistance of Firmicutes to advanced oxidative processes (Wang *et al.* 2017; Du *et al.* 2020).

The UV radiation seems to have similar effectiveness against Firmicutes since OTUs affiliated to this phylum were significantly more abundant in soils irrigated with WW, compared to that irrigated with UV (Figure 7). This observation is in agreement with Cui *et al.* (2020), who reported a significant decrease of Firmicutes members in WW disinfected with UV radiation. On the other hand, in UV-irrigated soil, we identified OTUs affiliated to bacteria resistant to UV radiation, such as members of the families Microscillaceae and Saprospiraceae, from phylum Bacteroidetes, and Phycisphaeraceae, from phylum Planctomycetes (Ordoñez *et al.* 2009; Viana *et al.* 2013). The last one is usually found in treated WW but seldom detected in agricultural soil (Becerra-Castro *et al.* 2015).

Besides Firmicutes, the OTUs identified as most abundant in WW-irrigated soil were: from the order Oxyphotobacteria (family Leptolyngbyaceae and one unidentified family) from Cyanobacteria; from the order TK10 (unidentified family) and from the uncultured family JG30-KF-CM45 from Chloroflexi; from the soil family Gemmataceae from Planctomycetes; and from some taxa related to nitrogen cycle: families Intrasporangiaceae, Streptosporangiaceae and Pseudonocardiaceae from Actinobacteria; family Xanthobacteraceae from Proteobacteria, family Gemmatimonadaceae from Gemmatimonadetes; and the archaeal phylum Thaumarchaeota, family Nitrososphaeraceae.

Interestingly, the UV-irrigated soil presented more OTUs affiliated to poorly described taxa such as Rokubacteria, order Rokubacteriales (unknown family); Acidobacteria, Subgroup 17 (unknown family), Proteobacteria, Geminicoccaceae, Chloroflexi, order Ardenticatenales (unknown family), Gemmatimonadetes, uncultured S0134 terrestrial group, an unidentified member of phylum Latescibacteria, Proteobacteria, uncultured mle1-27, and Gemmatimonadetes, uncultured AKAU4049, Verrucomicrobia, Opitutaceae (Figure 7).

To achieve a synthetic overview, a Venn diagram was constructed to help to understand the unique and shared differentially abundant taxa among the soils irrigated with the different disinfectant treatments (Figure 8). The treatment with UV selected the highest taxa number; 19 taxa were differentially abundant exclusively in the UV-irrigated soil. The possible reason might be that bacterial community shift during UV disinfection played an important role in shaping soil bacteria community structure through eliminating UV-sensitive and enriching UV-resistant bacteria, as observed by other authors (Hu *et al.* 2016; Huang *et al.* 2018; Pullerits *et al.* 2020).

Despite the little effects caused by the disinfectant treatment over soil physicochemical properties and crop yield, the treatments played an important role in shaping the microbial structure, mainly in the less abundant taxa.

As reported by Bastida *et al.* (2017), Broszat *et al.* (2014), and Ibekwe *et al.* (2018), we observed the persistence of some bacteria from WW in soil. However, the powerful disinfection treatment AOP and UV had an important role, eliminating some potential pathogenic high resistant spore-forming bacteria, from the phylum Firmicutes, usually found in WW samples. Ibekwe *et al.* (2018) also reported the presence of potential pathogenic members of the phylum Firmicutes, and other bacteria in soils irrigated with wastewater, calling attention to the hazard of the WW irrigation becoming a pathogen transmission source, without a proper disinfection treatment.

Overall, the WW irrigation increased the abundance of OM degrader organisms and mainly, families involved in the nitrogen cycle, in agreement with previous studies, due to the input of nitrogen compounds, compared to FW (Oved *et al.* 2001; Zhou *et al.* 2011; Tsiknia *et al.* 2013; Frenk *et al.* 2014; Becerra-Castro *et al.* 2015; Wafula *et al.* 2015; Ibekwe *et al.* 2018). However, the UV, as well as the HYP treatments, selected some bacteria related to the nitrogen cycle, which can affect soil fertility in the long term.

CONCLUSIONS

The changes in soil physicochemical properties were the more apparent when comparing irrigation with WW and with FW. The addition of a disinfection step with chlorine, peracetic acid, UV radiation, or UV radiation combined with hydrogen peroxide did not interfere directly in the soil evaluated characteristics. Despite the increase of the soil pH and EC caused by WW irrigation, compared to FW, the soil buffering capacity maintained the soil pH within the optimum range for plant development in all six treatments and, at the end of the experiment, the soil was classified as non-saline. However, as the salt accumulation can raise with a continuous WW irrigation, good management practices, considering precipitation rate, soil drainage and soil–plant system are essential to avoid soil salinization. The nitrate accumulation was due to the high ammonia concentration in the WW used for irrigation. The soil nitrification rate was higher than the nitrate uptake rate by the wheat crop, demonstrating the importance to consider not only the plant water demand, but also the nutrient demand, when WW is used for irrigation.

WW irrigation had a positive effect on wheat development and productivity, and the disinfectant treatment did not impact the crop yield. The non-accumulation of phosphorus and total nitrogen in the soil indicates that the plant used all the nutrient supplied by the WW for its development.

The water type and the disinfectant treatment influenced the prokaryotic community composition. WW irrigation increased the abundance of OM degrader organisms and, mainly, families involved in the nitrogen cycle, compared to FW. In addition, we observed the persistence of some WW typical bacteria in the soil, including some potential pathogenic groups. Among the disinfectant treatments, the UV disinfection played an important role in shaping soil bacterial community structure, 19 taxa were differentially abundant in the UV-irrigated soil. UV and AOP eliminated resistant pathogenic spore-forming bacteria, found in WW-irrigated soil, calling attention to the hazard of the WW irrigation becoming a pathogen transmission source, without a proper disinfection treatment. Bacterial groups with ROS-scavenging mechanism were more abundant in POA and PAA-irrigated soil and groups capable to degrade organohalogenated compounds in HYP-irrigated soil. One concern related to the disinfection step is that the UV and HYP treatments selected some bacteria related to the nitrogen cycle, which in the long term can affect soil fertility.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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