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Interactions between soil microbial communities and agronomic behaviour in a mandarin crop subjected to water deficit and irrigated with reclaimed water

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ABSTRACT

The structural deficit of water resources in Mediterranean areas forces us to search for new sources of water for irrigation as a mandatory requirement for a sustainable agriculture. However, given their critical role in soil fertility, the impacts of irrigation in soil microbial communities must be carefully considered alongside the crop responses. Here, we evaluate the impacts of irrigation with water from different origins in the soil microbial community and on the tree physiology and fruit yield in a Mediterranean mandarin agroecosystem. Two sources of water for irrigation were considered: i) fresh water, with an electrical conductivity (EC) of 1.2 dS m^{-1}, from the Tajo-Segura canal (transfer water, TW); and ii) reclaimed water (EC=3.4 dS m^{-1}) from a wastewater treatment plant (RW). Further, the two types of water were applied using two different regimes: control irrigation (C), to fully satisfy the crop water requirements (100% ET$_c$), and regulated deficit irrigation (RDI), in which the trees received half the amount of water applied to the C trees (50% ET$_c$) during the second stage of fruit development. In the case of TW, ~~the results show that~~ RDI increased~~mproved~~ bacterial biomass, and urease and β-glucosidase activities in soil. In contrast, in the case of RW, RDI did not increase bacterial biomass in comparison to control (RW-C). Irrigation with RW caused a reduction in yield in comparison to TW treatments. The combined evaluation of the plant and soil responses to different~~stinct~~ irrigation ~~approaches~~ strategies is essential in water-limited Mediterranean areas ~~which are~~ used to grow citrus crops that require~~are~~ less water and nutrients ~~demanding~~ than other crops. Further, this crop ~~and that~~ can be favoured by the use of low to moderate vigorous rootstocks~~ of low-moderate vigor~~. Our results demonstrate that RDI does not have a~~has not~~ drastic negative impacts o~~i~~n crop yield when RW is used, and that there~~it~~ may be~~have~~ some positive effects in soil microbial communities when TW ~~was~~is used for irrigation.

Keywords: soil quality; treated wastewater; microbial biomass; regulated deficit irrigation; *Citrus*

1.Introduction

The scarcity of rainfall and the most frequent occurrence of drought events as a consequence of climate change might compromise the availability of water in Mediterranean agroecosystems (IPCC, 2013). In southeastern (SE) Spain, agricultural production is the engine of the economy. However, the predicted reduction in rainfall for the whole Iberian Peninsula due to climate change, together with political concerns, endangers the supply of water for agriculture. Indeed, the availability of water for irrigation in SE Spain strongly relies on the transfer of water from the Tagus river, 500 km away. In these circumstances, there is an urgent need for alternative water sources. One option is the use of water from wastewater treatment plants, so called reclaimed water (RW) (Mounzer et al., 2013; Nicolás et al., 2016). However, RW is not exempt from risks. It usually has high salinity (Grattan et al., 2015; Bastida et al., 2017; Zolti et al., 2019) and salts may affect plant growth (Romero-Trigueros et al., 2014) and soil quality (García and Hernández, 1996). On the positive side, RW contains soluble organic matter that may benefit both soil fertility and the biomass and activity of the soil microbial community (Gelsomino et al., 2006; Adrover et al., 2012; Bastida et al., 2017). In addition, there is another way of saving water: regulated deficit irrigation (RDI), consisting of the reduction of the volume of irrigation water applied during the development stages when the plant is less sensitive to water stress (Chalmers et al., 1981). The reducing irrigation of *Citrus* species in summer does not cause significant negative impacts in terms of plant physiology and yield, and hence allows the saving of water when it is less available (Ruiz-Sánchez et al., 2010). However, while some studies have evaluated the influence of the combination of RDI and RW on plant physiology and yield (Mounzer et al., 2013; Pedrero et al., 2014; 2015; Romero-Trigueros et al. 2017a,b), the impacts ~~i~~on the soil microbial communities are less understood (Bastida et al., 2017, Starke et al., 2017). This limits our knowledge of the functioning of plant-soil interactions in Mediterranean agroecosystems subjected to different irrigation strategies~~approaches~~.

The soil microbial community is critical for the maintenance of soil fertility because microbes are responsible for the cycles of soil nutrients, including C, N, and P, and organic matter decomposition (Burns et al., 2013). Given their high sensitivity, soil microbial parameters are commonly utilized as soil quality indicators (Bastida et al., 2008; Zornoza et al., 2015). For instance, several studies have evaluated the impacts of wastewater on soil microbial communities and observed positive effects on the activity (Elifantz et al., 2011; Adrover et al., 2012) and biomass of Gram-positive bacteria (García-Orenes et al., 2015), and changes in community composition (Bastida et al., 2017; Zolti et al., 2019). Many of these changes were associated with the soluble organic matter content and high salinity of such water (Elifantz et al., 2011; Adrover et al., 2012; Morugán-Coronado et al., 2013; Frenk et al., 2014; Bastida et al., 2017, 2018). However, little information is available about the interaction between the use of RW and RDI regarding the soil microbial community. Previous studies found that RDI had negative impacts on plant productivity, soil microbial biomass, and enzyme activities in summer, and that bacterial biomass, rather than fungal biomass, was more sensitive to RDI when RW was used (Bastida et al., 2017). However, these findings were for a grapefruit ecosystem, more water-demanding and productive than a mandarin crop (Bastida et al., 2017), the subject of this study. This is particularly important if we consider that there are specific relationships between plants and the belowground community ~~which can be shaped by plant species~~ (Haichar et al., 2008). Moreover, it is critical to highlight that each crop species and/or rootstock may have differential sensitivity to salinity and drought, and, consequently, the impacts of RDI and water quality on the plant-microbial community relationships can be different depending on the species and its rootstock. Many authors have demonstrated that the rootstock can increase the resistance of *Citrus* sp. crops to biotic and abiotic stressors (García-Sánchez et al., 2007; Rodríguez-Gamir et al., 2010; Machado et al., 2013). For instance, the Carrizo citrange rootstock is more drought-resistant than the rootstock citrus macrophylla used in other RDI studies for grapefruit (Pedrero et al., 2015), since the latter is more vigorous (Robles et al., 2017). Thus, both the plant species and the rootstock can determine the responses of the soil microbial community, which in turn is highly influenced by the soil water content and irrigation water quality (Hawkes et al., 2011; Bastida et al., 2017, 2018; Moreno et al., 2019). Here, we evaluate the effects of RW and RDI on the sustainability of a mandarin agroecosystem by utilizing sensitive microbial community indicators, as well as their relationship with crop productivity and plant physiology. Given that the rootstock utilized in this study is drought tolerant (Pedrero et al., 2015), we hypothesize that, while the mandarin yield will not be affected significantly by RDI, the use of RW will be reflected in both the plant productivity and the belowground microbial community in terms of biomass and activity, in comparison to TW.

2. Materials and Methods

2.1. Experimental area and design

The experiment was performed during three years (2016-2018) in a commercial 0.5-ha orchard cultivated with 17-year-old mandarin trees (*Citrus clementina* cv. ‘Orogrande’) grafted on Carrizo citrange (*Citrus sinensis* [L.] Osb. × *Poncirus trifoliata* [L.]) rootstock, with a tree spacing of 5 m × 3.5 m. This site is located in Campotéjar-Murcia, Spain (38º07’18”N; 1º13’15”W) and is characterized by a Mediterranean semi-arid climate with warm, dry summers and mild winter conditions. The average annual reference evapotranspiration (ET$_0$) and rainfall in the last decade are 1337 and 296 mm, respectively. However, the year 2017 was very dry, the rainfall (204 mm) being below the historical average and around half that of 2016 (449 mm) and 2018 (402 mm). The ET$_0$ hardly changed between 2016 and 2018, with an annual average value of 1398 mm in this period (Fig. S1). We have provided all climate data for 2018, in consistency with climate data provided for 2016 and 2017. However, only climate data for January-2018 will be used to discuss ~~soil~~the results in agreement with ~~the~~ ~~same~~ soil sampling time~~ for soil~~. The 2016-climate data offer a perspective on the climate situation before starting the soil sampling and plant measurements in 2017 and 2018. Climate in 2016 is fundamental for explaining plant production responses in 2017.

Irrigation was scheduled on the basis of crop evapotranspiration (ET$_c$), as described by Nicolás et al. (2016) and Romero-Trigueros et al. (2017a). Two types of water were used. The first one was pumped from the Tajo-Segura canal (transfer water, TW) and the second one was obtained from the Molina de Segura tertiary wastewater treatment plant (reclaimed water, RW). Water samples were analyzed as described by Bastida et al. (2017). Also, we established two irrigation treatments for each water source: control (C), with irrigation to fully satisfy the crop water requirements (100% ET$_c$), and regulated deficit irrigation (RDI), in which the trees received half the amount of water applied to the C trees (50% ET$_c$) during the second stage of fruit development (Romero-Trigueros et al., 2017a). The total amount of water applied was quantified with inline water flow meters and in the C treatments were 7.463 and 7.653 m^3 ha^{-1}, whilst in the RDI treatments were 6.210 and 6.322 m^3 ha^{-1} for 2016 and 2017, respectively. Hence, RDI treatments meant reductions of about 17% of water per year. The irrigation was controlled automatically by a head-unit programmer and electro-hydraulic valves. All treatments included application of the same amounts of fertilizers ($N–P_2O_5–K_2O$), applied through the drip irrigation system: 215–100–90 kg ha^{-1} year^{-1} for mandarin trees. A layout depicting the drip lines is available in Mounzer et al. (2013).

The experimental was laid out in a ~~design consisted of four~~ completely randomized block~~s,~~ with four replications. Each experimental unit consisted of~~plots per block and~~ 12 trees, from which~~ per treatment within each plot. In each plot,~~ the two central trees of the middle row (named “inner trees”) were used for measurements and the other trees were the border (guard trees).

2.~~1~~2. Water analyses.

Four water samples from each irrigation source were collected monthly from 2016 to 2018 in glass bottles, transported in an ice chest to the laboratory and stored at 5 ºC before being processed for physical and chemical analyses. An inductively coupled plasma mass spectrometer (ICP-ICAP 6500 DUO Thermo, England) was used to determine the concentration of Ca, Mg, K, Na and B. Anions (Cl^-, NO_3^-, PO_4^{3-} and SO_4^{2-}) were analyzed by ion chromatography with a liquid chromatograph (Metrohm, Switzerland). Electrical conductivity of water (EC_w)~~-~~ was determined using a PC-2700 meter (Eutech Instruments, Singapore) and pH was measured with a Crison 507 pH-meter (Crisom Instruments S.A., Barcelona, Spain).

2.~~2~~3. Soil characterization and sampling. Chemical, biochemical and microbial soil analyses.

Soil was collected in the four replicate plots for each treatment~~ (n=4)~~. A composite soil sample from under the canopy of two trees per treatment and plot was taken in January 2017, September 2017, and January 2018, to a depth of 20 cm. Soil sampling in January and September (when RDI is being applied) allowed us to compare the effects of this water management in soil. The selection of two January sampling times was due to the important variation in rainfall conditions although ET_0 was very similar in the different years (2016, 2017 and 2018). Thus, 2017 was a very dry year respect to 2016. Therefore, we measured soil parameters in both January 2017 and 2018 in order to consider the possible effect of rainfall variation for both

Con formato: Sin Superíndice / Subíndice years. Thus, sampling in January 2017 and 2018 offered us the possibility to compare two very different years in terms of rainfall that could affect to soil chemistry and soil microbial communities.

Each composite soil sample was composed of 10 subsamples (5 subsamples per tree). The samples were sieved at < 2 mm. A fraction of each sample was stored at room temperature for chemical analysis and the rest was stored at 4ºC until the biochemical and microbial analyses were performed. Before the assay, the soil was classified as a Typic Haplocalcid, according to Soil Survey Staff (2014). The soil within the first 90 cm depth had a loamy texture (24% clay, 33% silt, and 43% sand), with an average bulk density of 1.37 g cm^{-3}. Before the experiment, the soil electrical conductivity (EC) was 2.1 dS m^{-1}. The pH and EC were measured in a 1/5 (w/v) aqueous soil extract, pH using a pH meter (Crison mod. 2001, Barcelona, Spain) and EC with an electrical conductivimeter (Crison micro CM2200). The total nitrogen (N) and total organic C (TOC) contents were determined using a C/N Flash EA 1112 Series elemental analyzer (Thermo Finnigan EA-1112, Thermo Fisher Scientific Inc., MA, USA). Water-soluble elements from the soil were quantified in the aqueous extracts mentioned above using an inductively coupled plasma mass spectrometer (ICP-ICAP 6500 DUO Thermo, UK). Anions (NO_3^- and SO_4^{2-}) were analyzed by ion chromatography with a liquid chromatograph (Metrohm, Switzerland).

The urease activity in the soil was determined by the method of Kandeler & Gerber (1988). The phosphomonoesterase and β-glucosidase activities were analyzed by following the methods described by Tabatabai & Bremmer (1969) and Eivazi & Tabatabai (1988), respectively. Phospholipids were extracted from 6 g of soil with a chloroform:methanol:citrate buffer (1:2:0.8 v/v/v) (Bligh and Dyer, 1959), and afterwards were fractionated and quantified as described by Frostegard et al. (1993). Phospholipids were transformed into fatty acid methyl esters (FAMES) by alkaline methanolysis and designated as described by Frostegard et al. (1993). Further details for the analysis of fatty acids and their assignment to microbial groups (bacteria, fungi, Gram-positive bacteria, and Gram-negative bacteria) can be found in Bastida et al. (2017).

Código de campo cambiado

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2.~~3~~4. Plant physiology. Potentially phytotoxic elements and yield.

Twenty leaves per tree were sampled from non-fruiting twigs in the two central trees of each replicate for each treatment, twice in each year (in January and at the end of the RDI period). Potentially phytotoxic elements (Na and B) were determined by inductively coupled plasma mass spectrometry (ICP-ICAP 6500 DUO Thermo, UK). To prepare the leaves for ICP analysis, they were washed with a detergent (Alconox, 0.1 %), rinsed with tap water, cleaned with 0.005% HCl, rinsed with distilled water, and left to drain on filter paper before being oven-dried for at least 2 days at 65 ºC. The dried leaves were ground and then digested with a mixture of nitric acid (4 ml) and hydrogen peroxide (1 ml).

The stem water potential (Ψ_s) was measured at midday using a pressure chamber (model 3000; Soil Moisture Equipment Corp., Santa Barbara, USA) and following the recommendations of Turner (1988). Monthly, two mature leaves per tree in the eight inner trees of each treatment were selected. At least 2 h before the measurement, the leaves were covered with aluminum foil and enclosed within polyethylene bags (McCutchan & Shackel, 1992). Also, measurements of net photosynthesis (A) and stomatal conductance (g_s) were made with a portable photosynthesis system (LI-6400 Li-Cor, Lincoln, NE, USA) on the same dates as those of midday stem water potential. Leaf gas exchange was measured on eight young, fully expanded leaves per treatment, placed in a 2-cm^2 leaf cuvette at mid-morning (9:00 GMT). The CO_2 concentration in the cuvette was maintained at 400 µmol mol^{-1} (≈ ambient CO_2 concentration). Measurements were performed at a saturating light intensity of 1200 µmol m^{-2} s^{-1} and at ambient temperature and relative humidity. In each harvest season (in November), the eight inner trees per treatment were evaluated to determine the total yield, in kilograms per tree. The irrigation water productivity (WP_i) was calculated for each treatment as the ratio between the annual yield (kg ha^{-1}) and the applied water (m^3 ha^{-1}) during the same period (Nicolás et al., 2016).

2.45. Statistical analyses

Normality and the homocedasticity of the variables were checked by the Kolmogorov-Smirnov and Levene tests, respectively. The variables were log-transformed when necessary. The data were analyzed using three-way ANOVA with water source (RW or TW), water quantity (C vs RDI), and sampling time as the main factors. With the purpose of assessing pair-wise differences between the treatments, for both soil and plant parameters, the data were analyzed using one-way ANOVA followed by the Tukey *post-hoc* test (HSD, $P<0.05$).

3. Results

3.1. Water analyses

Reclaimed water (RW) showed significant differences from transfer water (TW) regarding their chemical parameters in the three years studied years (2016, 2017, and 2018). Thus, averaging the values of the three years, RW had higher salinity and sodicity, with an average electrical conductivity (EC_w) of 3.4 dS m^{-1} and an average sodium absorption ratio (SAR) around 6.4 [meq $L^{-1}]^{0.5}$; TW had an average EC_w of 1.2 dS m^{-1} and an average SAR of 2.2 [meq $L^{-1}]^{0.5}$. Moreover, RW had higher concentrations of nutrients (NO_3^-, K, Ca, Mg) and of elements potentially phytotoxic for citrus (Cl^-, Na, B) than TW (Table 1).

3.2. Plant physiology and yield, and the content of toxic elements (Na and B)

The Ψ_s levels were similar for the two types of water at the end of the summer period, in both the C (-1.0 MPa) and RDI (around -1.33 MPa) treatments. In contrast, leaf gas exchange showed significant differences between the treatments. Thus, for RW, the values of A and g_s were lower at the end of the summer period (September 2017) - by around 17% for both parameters - with respect to TW. However, in winter only A was lower with respect to TW (by 42%) and the values of g_s were similar for both types of water. In the RDI treatments, the leaf gas exchange rates were more reduced at the end of the summer (September 2017) with TW than with RW. Thus, A decreased by around 55% in TW-RDI compared to 29% in RW-RDI, and g_s by 76% in TW-RDI compared to 53% in RW-RDI (Table S1).

The leaf concentrations of potentially phytotoxic elements (Na and B) in the RW treatment were significantly higher than those in the TW treatment (Table S2). The yield was significantly affected by the water quality. Thus, for both 2016 and the average values of 2016-2017, significant reductions in yield only occurred with the treatments involving irrigation with RW (RW-C and RW-RDI) with respect to TW-C (Table 2). However, 2017 showed a different pattern. Thus, TW-RDI gave a significant reduction with respect to TW-C and a yield similar to that of RW-C. Furthermore, the significantly lowest ~~among the irrigation treatments, the~~ yield was observed under~~lowest, significantly so, for~~ RW-RDI (Table 2). The yield reductions observed in RW treatments as averaged for 2016-2017 period did~~were~~ not correspond to the reductions in the WP$_i$. RW-RDI did not show significant differences in WP$_i$ values when compared~~respect~~ to the TW-C treatment, being TW-RDI treatment the one with~~which had~~ the highest WP$_i$ values (Table 2).

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3.3. Chemical soil properties and the content of water-soluble elements

The source of water (TW *vs* RW), but not the quantity (C *vs* RDI), influenced the electrical conductivity (EC$_s$) (Table S3), which tended to increase with time in soils treated with RW (Fig. S1). The sampling time influenced significantly the EC$_s$, as well as the TOC and total N contents (Table S2). Overall, the interaction between the water source and quantity did not influence the pH, EC$_s$, or element contents. The water source (TW *vs* RW) also influenced significantly (P<0.05) the water-soluble contents of B, Na, P, and NO_3^- (Table S3).

Unlike the water source, the quantity of water (C *vs* RDI) influenced significantly (P<0.05) the contents of TOC and total N in the soil. The TOC and total N contents were significantly greater in TW-RDI than in TW-C in January 2018, but not in January-2017. Further, no significant differences were observed between RW-RDI and RW-C at any time for TOC and total N contents (Fig. 2). Furthermore, the quantity of water applied influenced significantly the contents of water-soluble B and P in the soil: water-soluble B was higher in the RDI treatments in comparison to the controls. The contents of water-soluble B and Na were higher in the soils irrigated with RW (except in the case of soluble-Na in January-2018), while water-soluble P and NO_3^-, tended to be higher in the soils irrigated with TW (Fig. S2).

3.4. Phospholipid fatty acids (PLFAs) analysis

The sampling time influenced significantly the bacterial and fungal PLFA contents (Table S3), the highest values being observed in January 2018 (Fig. 3). The water source (TW *vs* RW) did not influence the contents of representative PLFAs of bacteria and fungi, while the quantity (RDI *vs* C) influenced the content of bacterial PLFAs, including the Gram+ and Gram- biomarkers. The PLFA content was subjected to a temporal dynamic, being highest in January 2018 (Fig. 3). In September 2017 and January 2018, the bacterial PLFA content was higher for TW-RDI than for the rest of the treatments (P<0.05). However, in January 2017 it was highest for RW-C (Fig. 3). A similar pattern was observed for the contents of Gram+ and Gram- fatty acids. The Gram+-to-Gram- PLFAs ratio was higher in RW-C and RW-RDI soils than in soils irrigated with TW in September 2017 and January 2018 (Fig. 4). The fungal-to-bacterial PLFA ratio was highest for RW-C in January 2017 and for TW-C in January 2018.

3.5. Soil enzyme activities

The sampling time influenced significantly the measured activity of soil enzymes ($P<0.05$) (Table S3, Supporting Information), with the phosphatase and urease activities being greatest in January 2017 (Fig. 5). The water source (TW *vs* RW) influenced the activity of β-glucosidase and urease (Table S3): β-glucosidase activity was higher in TW-RDI soil than in the rest of the samples across all sampling times, while urease activity was higher in RW than in TW soil in January 2017. The quantity of water applied influenced the activity of β-glucosidase, phosphatase, and urease. In the case of urease, the RDI treatments resulted in greater activity than their corresponding controls ($P<0.05$) (Fig. 5), while TW-RDI soils had higher values of β-glucosidase activity than TW-C soils.

4. Discussion

High concentrations of B and Na in the soil solution are known to cause reductions in the CO_2 available for photosynthesis (García-Sánchez and Syvertsen 2006) and increase leaf ion accumulation (Mouhaya et al. 2010). However, despite the saline stress induced to plants irrigated by RW, no significant differences in g_s were observed (Nicolás et al., 2016) since Ψ~~□~~$_s$ regulated the g_s values. This result, agrees with other studies with Carrizo citrange seedlings irrigated with saline water (Pérez-Pérez et al. 2007) and reclaimed water (Nicolás et al. 2016), which indicated good water status for trees irrigated with TW or with RW. In the RDI treatments, whilst the Ψ_s levels were similar for the two types of water, the leaf gas exchange rates (A and g_s) were more reduced at the end of the summer (September 2017) with TW than with RW. Previous studies demonstrated that the salinity load of RW produced a considerable reduction in the available soil water~~available fraction of soil water~~ and subjected the root system to additional undesired osmotic stress and potential ion toxicity risks (Mounzer et al 2013), whilst ~~in~~ TW plants could be~~are~~ able to uptake a larger quantity of water from soil. ~~readily more available soil~~ water ~~water fraction.~~ Further, during the experimental period, the impact of the adverse salinity conditions observed in the soil was not proportionally reflected on the mandarin production capacity. The trees subjected to RW-RDI did not suffer significant yield reduction relative to the control treatment except during years of high fruit loads (Mounzer et al. 2013) and neither for 2016 and 2017, since even improved the WP$_i$. This is due ~~to the~~ to the dynamic activity of the plant root system which is able to compensate any partial reduction in water uptake either by increasing roots activity or by absorbing water from less saline soil layers (Homaee and Schmidhalter, 2008). Overall, these plant responses to the different type of water, as well as the yield production through the demands of water and nutrients in soil, can influence the belowground microbial community, as developed below.

The TW-RDI soil showed higher values of bacterial biomass than TW-C. However, this pattern was not observed in the case of RW. In principle, it could seem illogical that a soil which received less water (TW-RDI) showed higher values of soil microbial biomass and some enzyme activities (i.e. β-glucosidase and urease) than TW-C. A possible explanation for the increase of bacterial biomass and some enzyme activities in TW-RDI compared to TW-C might be related to the dynamics of water-soluble C and N which were not measured in this study. These fractions represent labile and dynamic fractions of organic matter that are rapidly used by microbes. It is known that moisture and drought fluctuations critically influence these fractions. For instance, drought conditions have been shown to increase the content of water-soluble C content in semiarid conditions (Bastida et al., 2019) and an increased water-soluble C is generally associated to a greater microbial biomass (Bastida et al., 2006). However, the plant responses should also be carefully considered when studying the responses of the belowground microbial communities to soil and water management (Haichar et al., 2008; Bastida et al., 2017). The rootstock used here was Carrizo citrange, characterized by its high resistance to drought and oxidative stress (Yi-ling et al., 2015; Hussain et al., 2018). Indeed, the fruit yield of the RDI treatments was similar to that of their respective well-irrigated treatments in 2016 and for the average of the two years (2016 and 2017); this was also the case for the average of the years 2008-2010 (Pedrero et al., 2014). It should be borne in mind that mandarin is less nutrient-demanding than other *Citrus* spp. such as grapefruit (Romero-Trigueros et al., 2017a) and its combination with a less vigorous rootstock makes it less water-demanding compared to grapefruit grafted on citrus macrophylla (Robles et al., 2017).

In comparison to TW-RDI, the absence of an increase in the microbial biomass in RW-RDI compared to RW-C might be related to an accumulation of some toxic elements which are more abundant in reclaimed water (Becerra-Castro et al., 2015) and concentrated in soil. Thus, the soil content of water-soluble B was higher in RW-RDI than in RW-C, particularly in September when the RDI is applied. Indeed, it has been recently demonstrated that boron might have negative impacts in soil microbial communities (Vera et al., 2019). Interestingly, B content in plant leaves followed an opposite trend to that in soil, with RW-C having greater B-leaf content than RW-RDI in September 2017. These findings may indicate that B is partially entrapped in soil and is not totally adsorbed by plants. In fact, it is known that calcareous soils, such those in south-east Spain, have high affinity with boron (Majidi et al., 2010). A similar behaviour occurred for Na in plants in September 2017. Such interactions between boron and soil, reducing the bioavailability for plants, can partially explain the absence of negative responses in terms of crop yield (RW-C vs RW-RDI) in spite that net photosynthesis (A) was lower in RW-RDI and in RW-C in September 2017. Regardless of the differences between RW-C and RW-RDI for the content of toxic elements in plant leaves, RW treatments accumulated more Na and B than TW treated plants. In this sense, different authors have indicated the phytotoxic thresholds which provoked reductions in citrus yield; in the case of B, this was 100 µg g^{-1} in leaf dry matter (Romero-Trigueros et al., 2014). In the latter study, these phytotoxic levels affected only gas exchange, as in the current study.

Three-way ANOVA indicated that water type has a significant influence in soil electrical conductivity, as well as in the content of soluble Na and B in soil. Soils irrigated with RW had higher content of soluble Na and B in comparison to soils irrigated with TW, with potential consequences for the soil microbial community. Moreover, a high content of salt, and particularly Na in the soil may impact the soil microbial communities (García and Hernández, 1996; Wichern et al., 2006; Rath and Rousk, 2015). Further, recent evidences suggest that boron can alter the soil microbial community, with decreases or increases of soil microbial biomass depending on the boron chemical form (Vera et al., 2019). Indeed, when considering full irrigation, the biomass of bacteria was greater in RW-C than in TW-C in January. In addition, these changes in microbial biomass with the type of water occurred with an impact in the structure of the soil microbial community. Thus, the soils irrigated with RW had the highest

Gram+-to-Gram- biomass ratio in September 2017 and January 2018. This ratio is indicative of harsh conditions in soils because Gram+ bacteria are often more resistant to stressors (i.e. salts, boron, etc.) given the capacity of some of them for sporulation (de Vries et al. 2012). In addition, particularly in the case of Gram+ bacteria, the microbial biomass was greater for RW-C than for TW-C in spite of the fact that no significant differences appeared between these treatments for the soil organic C and N contents. Although these results varied across the sampling times, they could indicate that Gram+ bacteria are able to outcompete Gram- bacteria when RW is used for irrigation. This capacity of Gram-positive bacteria for triggering in RW occurs in despite Gram-negative bacteria are usually known ~~to be~~as fast grow microbes with the capacity to quickly use available nutrients (Fierer et al., 2007; de Vries et al., 2012), ~~which are~~as those preferentially contained in RW. ~~This is to say that t~~The resistance of bacteria, particularly Gram+ bacteria, against potentially harmful contaminants (i.e. Na, B) could be of paramount importance for the persistence of soil microbial biomass ~~they survival and growth~~ when RW is used for irrigation.

Soil microbes secrete enzymes for the catabolism of complex compounds and these enzymes can be stabilized extracellularly in humic substances and mineral particles (Bastida et al., 2008; Burns et al., 2013). Soil enzymes represent the biogeochemical potential of soils and, in semi-arid environments, are strongly related with the content of organic matter and soil fertility (García et al., 1994; Bastida et al., 2008). The activities of β-glucosidase (related to the cycle of C) and urease (responsible for the hydrolysis of urea) were greater in TW-RDI than in TW-C. However, in particular for β-glucosidase activity, these differences were not observed when RW was applied, pointing to certain simil~~i~~arities~~tudes~~ with the behaviour of bacterial biomass. As discussed above, RDI may have reduced the water and nutrient demands of the trees, leading to a potential accumulation of water-soluble organic matter that contains substrates for the activity of these enzymes.

5.Conclusions

Our study demonstrates how the joint investigation of plant and soil microbial responses is central to evaluate the sustainability of irrigation management in Mediterranean agroecosystems. Overall, this study highlights a pattern of increases in bacterial biomass and some enzyme activities for the RDI treatments in comparison to the controls when transfer water (TW) is utilized for irrigation. In contrast, these positive results of RDI on certain microbial parameters were not clearly observed when reclaimed water is utilized. In the case of RW, the potential positive effects of their nutritional content can be counterbalanced by the content of toxic elements such Na and B in some microbial parameters, causing alterations of microbial community structure. In terms of plant responses, irrigation with RW reduced crop yield. However, when compared to full irrigation (RW-C), regulated deficient irrigation did not decrease crop yield when reclaimed water was used (RW-RDI) and even improved the WPi. Further, TW-RDI reduced significantly the crop yield only in one year (2017) in comparison to TW-C, but did not when the production was computed both in 2016 and 2017. Our investigation implies that, in the case of water shortages or the advent of extreme climatic events, RDI strategies, especially with non-saline water (TW), would maintain both mandarin yields and the soil biological quality in terms of its microbial biomass and activity.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Figure captions

Figure 1. Monthly values (mm) of reference evapotranspitarion (ET_0) (lines) and rainfall (bars) for the years 2016 (black), 2017 (red) and 2018 (blue).

Figure 2. Total organic carbon (A) and nitrogen (B) contents in the studied soil treatments and sampling times. For the all the irrigation treatments and years studied (TW = Transfer water; RW = Reclaimed Water; C = Control; RDI = Regulated Deficit Irrigation). Error bars represent standard deviation. For each time, different letters denote significant differences ($P < 0.05$).

Figure 3. The content of phospholipid fatty acids (PLFAs) representative of bacteria (A), Gram+ (B), Gram- (C) and fungi (D) in the studied soil treatments and sampling times. For the all the irrigation treatments and years studied (TW = Transfer water; RW = Reclaimed Water; C = Control; RDI = Regulated Deficit Irrigation). Error bars represent standard deviation. For each time, different letters denote significant differences ($P < 0.05$).

Figure 4. The ratio between fungal and bacterial (A), and Gram+ and Gram- (B) phospholipid fatty acids in the studied soil treatments and sampling times. For the all the irrigation treatments and years studied (TW = Transfer water; RW = Reclaimed Water; C = Control; RDI = Regulated Deficit Irrigation). Error bars represent standard deviation. For each time, different letters denote significant differences ($P < 0.05$).

Figure 5. β-glucosidase (A), phosphatase (B) and urease (C) activities in the studied soil treatments and sampling times. For the all the irrigation treatments and years studied (TW = Transfer water; RW = Reclaimed Water; C = Control; RDI = Regulated Deficit Irrigation). Error

bars represent standard deviation. For each time, different letters denote significant differences ($P < 0.05$).