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Comparing the impacts of drip irrigation by freshwater and reclaimed wastewater on the soil microbial community of two citrus species

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ABSTRACT

The search for new water resources for irrigation is a mandatory requirement in Mediterranean agroecosystems. The impacts of irrigation with water from different origins were evaluated in the soil microbial community and plant physiology of grapefruit and mandarin trees in the south-east of Spain. The following treatments were considered: i) freshwater with an electrical conductivity (EC) of 1.0 dS m^{-1} from the "Tagus-Segura" water-transfer canal and well (TW); ii) reclaimed water (EC = 3.21 dS m^{-1}) from a wastewater-treatment-plant (RW); iii) irrigation with TW, except in the second stage of fruit development, when RW was utilized (TW$_c$); and iv) irrigation with RW except in the second stage, when TW was used (RW$_c$). Phospholipid fatty acids (PLFAs) revealed that microbial biomass was higher in the grapefruit soil than in the mandarin soil. In grapefruit soil, TW treatment showed a lower bacterial PLFA content than RW, RW$_c$, and TW$_c$, while RW showed the lowest values in the mandarin soil. In grapefruit soil, β-glucosidase and cellobiohydrolase activities were greater in RW and TW$_c$ than in TW and RWc. Under mandarin, the greatest activity of these enzymes was recorded in the TW$_c$ treatment. The saline stress caused lower net photosynthesis (*A*) and stomatal conductance (g_s) in plants of RW, RW$_c$ and TW$_c$ than in plants of TW treatment. The annual use of reclaimed water or the combined irrigation with TW$_c$ benefited microbial biomass and enzyme activities of grapefruit soil. In contrast, the microbial community of mandarin soil seemed more affected by the annual irrigation with reclaimed water.

1. Introduction

In Mediterranean regions, water availability is predicted to decrease in the coming decades. The region of Murcia, located in the south-east of Spain, is characterized by a deficit of water resources that reaches 606 Mm3 (Ibor et al., 2011). In these conditions, farmers need to handle the deficit of water or consider non-conventional water resources for irrigation (Mounzer et al., 2013). The use of water reclaimed from wastewaters is a feasible option for agriculture. This water has the problem of containing an excess of salts that may increase the electrical conductivity and the risk of soil salinization (Becerra-Castro et al., 2015) and containing contaminants; either of these factors could affect the productivity of agroecosystems (Ibekwe et al., 2010). Conversely, reclaimed water has readily available sources of organic matter that could improve the productivity in agricultural areas (Chen et al., 2008). In this sense, several studies have revealed that the use of reclaimed water has benefits on the productivity and physiology of *Citrus* sp. crops (García-Orenes et al., 2015; Pedrero et al., 2015; Nicolás et al., 2016). Nevertheless, the salinity and the heavy metal content of wastewaters must be considered for the irrigation of *Citrus* sp. (Pereira et al., 2011; Grattan et al., 2015). Paudel et al. (2016) found that treated wastewater negatively affected *Citrus* sp. root system, while these effects were strongly dependent on soil texture, so meaning that soil properties must be carefully considered when wastewater irrigation is utilized. Alternatively, irrigation with combined wastewater and freshwater can be an adequate solution to the problems commonly related to wastewater use in *Citrus* sp. agroecosystems, such high salinity and boron concentration (Pedrero and Alarcón, 2009).

Soil is the fundamental substrate of agriculture. However, the profound evaluation of the impacts of wastewater irrigation in soil has been not frequently carried out together with plant responses. Within soil, microorganisms are greatly responsible for the dynamics of organic matter which remain fundamental to crop yield and soil sustainability (Acosta-Martínez et al., 2003; Zornoza et al., 2015). Moreover, soil microbial properties can act as early-warning indicators of changes in ecosystems (Nannipieri et al., 1990; Bastida et al., 2008a; Tejada and Benítez, 2014, Zornoza et al., 2015). In this sense, the potential negative effects of wastewaters in plants can be early detected by soil

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microbial parameters. Traditionally, the activity of microorganisms has been evaluated by microbial-ecosystemic indicators such as soil respiration and enzyme activities involved in the cycles of C, N, and P (Bastida et al., 2008a; Rodríguez-Morgado et al., 2015). Furthermore, phospholipid fatty acids (PLFAs) can be used for evaluating the impact of agricultural practices on the biomass and structure of the soil microbial community (Frostegard et al., 1993; Bastida et al., 2008b; Torres et al., 2015). Nevertheless, the impacts of irrigation with reclaimed water in soil microbial community are controversial and further studies are required. For instance, several studies found that long-term irrigation with reclaimed water in a semiarid soil fostered the activity of the soil microbial community without negative effects in microbial biomass (Meli et al., 2002; García-Orenes et al., 2015), while other studies reported increases of soil microbial biomass (Adrover et al., 2012) or an absence of variations in soil microbial activity (Elifantz et al., 2011). In some cases, changes in soil microbial activity after irrigation with reclaimed water have been related to variations in the composition of the soil microbial community (Wafula et al., 2015). However, Frenk et al. (2015) observed that reclaimed water did not impact the composition of the soil microbial community. In a previous study, the impact of regulated deficit irrigation and water quality – freshwater *vs* saline reclaimed water – in the soil microbial community of a grapefruit orchard was evaluated (Bastida et al., 2017). However, there are no studies that have evaluated the responses of the soil microbial community to the alternative irrigation with different water sources (i.e. freshwater or reclaimed water) through the growing season. This strategy could minimize the negative impacts of the continuous irrigation with reclaimed water and, the same time, avoids an excessive utilization of freshwater which is limiting in South-East Spain.

Here, we extent the knowledge on the adaptations to water scarcity in Mediterranean agroecosystems. For this purpose, we evaluate the impacts of combinations of water from different sources in the soil microbial community of two crops with distinct water demands: mandarin and grapefruit (Pedrero et al., 2015; Nicolás et al., 2016). The reason behind this objective is that *Citrus* spp. are less susceptibile to reclaimed water in summer (Nicolás et al., 2016); hence, the use of reclaimed water exclusively in summer, with freshwater being used the rest of the year, can be an adequate approach to save water in Mediterranean areas. However, the impacts of combined treatments in comparison to single water source irrigation on soil sustainability, including chemical and microbiological indicators, are not fully known. From a water management perspective, this study aims to answer to the following question: is it better the irrigation with wastewater alone or in combination with freshwater? We hypothesized that reclaimed water would increase the soil salinity and that the responses of the soil microbial community would be mediated by the sensitivity of each rootstock and crop to salinity. In this respect, combinations of water from different sources might represent a proper strategy for combating water limitations in Mediterranean agroecosystems while maintaining soil sustainability. Furthermore, given the different plant-water relationships of the two *Citrus* spp. studied here, soil microbial biomass and community structure were expected to differ between crops and between irrigation treatments.

2. Material and methods

2.1. Experimental area, irrigation treatments and soil sampling

The experiment was carried out in Campotéjar-Murcia, Spain (38°07′18″N; 1°13′15″W) – with a Mediterranean semiarid climate. The annual reference evapotranspiration (ET_0) and rainfall are, on average, 1326 and 300 mm, respectively. Within this area, an orchard of 1 ha was cultivated with 2 crops. One crop consisted of 16-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. The other crop consisted of 11-year-old 'Star Ruby' grapefruit trees (*Citrus paradisi* Macf) grafted on Macrophylla rootstock [*Citrus macrophylla*], with a tree spacing of 6 m × 4 m.

From 2005–2007 – three growing seasons – the field area was fully irrigated with freshwater transferred from the "Tagus-Segura" river channel (90%) and well (10%) (TW). After this, four irrigation treatments based on the source of irrigation water were established. The first treatment was based on irrigation with freshwater from the "Tagus-Segura" water-transfer canal (90%) and well (10%) (TW). The TW water had an average electrical conductivity (EC_w) of 1.0 dS m^{-1}. The second treatment consisted on irrigation with tertiary reclaimed water pumped from a nearby wastewater treatment plant (EC = 3.21 dS m^{-1}) (reclaimed water, RW). Treatments TW and RW were applied along the growing season for both citrus species from 2008 onwards. In the third and fourth treatments, the trees were irrigated by combining the water sources in different ways: either the trees were irrigated with TW, except in the second stage of fruit development (55–65 days between late-June and mid-September) when RW was applied (TW_c), or, conversely, the trees were irrigated with RW except in this second stage when TW was used (RW_c). From 2013, treatments TW_c and RW_c were applied to soils irrigated previously with TW. The experiment was carried out throughout the year 2015. All plant physiological measurements and yield were performed during 2015, and the soil sampling was done in October 2015 which corresponds to the harvest period for both species. The provided water analyses correspond to annual average values for 2014 and 2015 (n = 12).

The irrigation system consisted of a single drip line laid on the soil surface next to each tree row. This system provided three pressure compensating, in-line emitters per tree, each discharging 4 l h^{-1}, which were placed 0.85 m from the trunk and spaced 0.9 m apart in the mandarin trees and were placed 1 m from the trunk and spaced 1 m apart in the grapefruit trees. The irrigation doses were scheduled on the basis of the daily crop evapotranspiration (ET_c) accumulated during the previous week (Pedrero et al., 2015: Nicolás et al., 2016). ETc values were estimated as reference evapotranspiration (ET_0), calculated with the Penman–Monteith methodology (Allen et al., 1998) and a month-specific crop factor. This water quantity was arrived from an irrigation control head of the entire experimental area that was equipped with pumps, filters, fertigation system, electrovalves and an automatic irrigation programmer (NTC-Mithra Nutricontrol, Murcia, Spain). The frequency of irrigation depended on the climatic demand (ET_0) and varied from daily (since mid-May until early October) to 3 days per week in winter.

The trees were irrigated at 100% ET_c from January to December. The total amount of water applied was quantified with inline water flow meters. The amount of water applied was 5945 and 7531 m^3 ha^{-1} for grapefruit and mandarin, respectively. The irrigation was controlled automatically by a head-unit programmer and electro-hydraulic valves. All treatments included application of the same amounts of fertilizer ($N–P_2O_5–K_2O$), applied through the drip irrigation system: 215–100–90 kg ha^{-1} $year^{-1}$ for mandarin trees and 215–110–150 kg ha^{-1} $year^{-1}$ for grapefruit trees. Weeds were eradicated in the orchard by applying the farmers' commonly used pest control methods (Romero-Trigueros et al., 2017). A layout depicting the drip lines for mandarin and grapefruit is available in Mounzer et al. (2013) and Pedrero et al. (2015), respectively.

Three replicate plots (n = 3) for each treatment and crop were established. A composite soil sample under the canopy of one tree for each of the three plots was sampled in October 2015. Each composite soil sample was composed of six subsamples. 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 4 °C until the biochemical and microbial analyses were done.