THE IMPACTS OF IRRIGATION WITH TRANSFERRED AND SALINE RECLAIMED WATER IN THE SOIL MICROBIAL COMMUNITY OF TWO CITRUS SPECIES

F. Bastida, I.F. Torres*, J. Abadía, C. Romero-Trigueros, A. Ruiz-Navarro, J.J. Alarcón, C. García, E. Nicolás

ABSTRACT

The search for new water resources for the irrigation is a mandatory requirement in Mediterranean agroecosystems. The impacts of irrigation with water from different sources were evaluated in the soil microbial community and plant physiology of grapefruit and mandarin trees in the south-east of Spain. Four irrigation treatments were evaluated: i) water with an average electrical conductivity (EC) of 1.1 dS m^{-1} from the "Tagus-Segura" water-transfer canal (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 applied (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 under grapefruit than under mandarin trees. In the case of grapefruit, 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. In mandarin soil, the greatest activity of these enzymes was recorded for TW_c. The saline stress induced lower net photosynthesis (*A*) and stomatal conductance (g_s) in plants of RW, RW_c and TW_c in comparison with TW. The annual use of reclaimed water or the combined irrigation with TW_c positively influenced the soil microbial biomass and biogeochemical activities under grapefruit. In contrast, the mandarin soil community seemed more sensitive to the annual irrigation with RW.

KEYWORDS: irrigation; soil microbial community; enzyme activities; semiarid; plant physiology

1. INTRODUCTION

In Mediterranean regions, water availability is predicted to decrease in the coming decades (IPCC, 2013). 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 cheap and continuously available option for agriculture. This water has the drawback 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 impair 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 demonstrated that the use of reclaimed water has positive effects on the productivity and physiology of *Citrus* sp. crops (García-Orenes *et al.,* 2015; Pedrero *et al.,* 2015; Nicolás *et al.,* 2016), as well as in the soil microbial community (Adrover *et al.,* 2012; García-Orenes *et al.,* 2015).

Soil microorganisms are greatly responsible for the dynamics of organic matter which remain fundamental to crop productivity and soil sustainability (Acosta-Martínez *et al.,* 2003; Zornoza *et al*., 2015). Moreover, microbial properties are sensitive to impacts on ecosystems (Bastida et al., 2008a; Tejada & Benítez, 2014, Zornoza et al., 2015). Traditionally, the activity of microorganisms has been evaluated by microbial-ecosystemic indicators such as soil respiration and the activities of enzymes involved in the cycles of C, N, and P (Bastida *et al.,* 2008a; Rodríguez-Morgado *et al.,* 2015). Furthermore, phospholipid fatty acids (PLFAs) can constitute a suitable approach 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).

In a previous study, the impact of regulated deficit irrigation and water quality –transferred water *vs* reclaimed saline water - in the soil microbial community of a grapefruit orchard were evaluated (Bastida *et al.,* 2017). Here, we extent the knowledge on the adaptations to water scarcity by evaluating the impacts of combinations of water from differing sources in the soil microbial community of two crops with different water demands (Pedrero et al., 2015; Nicolás *et*

al., 2016) – mandarin and grapefruit – in Mediterranean countries. 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 transfer water 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 chemical and microbial properties are not fully known. We hypothesized that reclaimed water would increase the soil salinity but at the same time benefit microbially mediated processes related to the cycling of organic matter. In this respect, combinations of water of differing sources might represent a proper strategy for combating water limitations in Mediterranean agroecosystems. 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 (38º07'18"N; 1º13'15"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 to 2007 the field area was fully irrigated with water transferred from the river channel (TW). After this, four irrigation treatments based on the source of irrigation water were established. The first treatment was based on irrigation with water pumped from the "Tagus-Segura" water transfer canal, which supplies a large part of the water used in the Region of Murcia for both human consumption and irrigation (TW). The TW water had an average electrical conductivity (EC_w) of 1.1 dS m^{-1}. The second treatment was consisted on irrigation with tertiary reclaimed water pumped from a nearby wastewater treatment plant (reclaimed

91 water, RW). Treatments TW and RW were applied along the growing season for both citrus
92 species from 2008 onwards. In the third and fourth treatments, the trees were irrigated by
93 combining the water sources in different ways: either the trees were irrigated with TW, except in
94 the second stage of fruit development when RW was applied (TW_c), or, conversely, the trees
95 were irrigated with RW except in this second stage when TW was used (RW_c). Treatments TW_c
96 and RW_c were applied from 2013 to soils irrigated previously with TW. The irrigation system
97 consisted of a single drip line laid on the soil surface next to each tree row. It provided three
98 pressure compensating, in-line emitters per tree, each discharging 4 l h^{-1}, which were placed
99 0.85 m from the trunk and spaced 0.9 m apart in the mandarin trees and were placed 1 m from
100 the trunk and spaced 1 m apart in the grapefruit trees. The irrigation doses were scheduled on
101 the basis of the daily crop evapotranspiration (ET_c) for the previous week (Pedrero et al., 2015:
102 Nicolás et al., 2016).
103 The trees were irrigated at 100% ET_c from January to December. The total amount of water
104 applied was measured with inline water flow meters. The irrigation was controlled automatically
105 by a head-unit programmer and electro-hydraulic valves. All treatments included application of
106 the same amounts of fertilizer ($N–P_2O_5–K_2O$), applied through the drip irrigation system: 215–
107 100–90 kg ha^{-1} $year^{-1}$ for mandarin trees and 215–110–150 kg ha^{-1} $year^{-1}$ for grapefruit trees.

108 Three plots (n=3) for each treatment and crop were established. A composite soil sample under
109 the canopy of one tree for each of the three plots was sampled in October 2015. Each
110 composite soil sample was composed of six subsamples. The samples were sieved at < 2 mm.
111 A fraction of each sample was kept at room temperature for chemical analysis and the rest was
112 stored at 4ºC until the biochemical and microbial analyses were performed.

113 ***2.2. Water characterization, soil characteristics and sampling***

114 Water samples were analyzed as described by Bastida *et al*. (2017). The sand, silt, and clay
115 contents of the soil were 50, 26, and 24%, respectively, with an average bulk density of 1.37 g
116 cm^{-3}. It was classified as a Typic Haplocalcid, according to Soil Survey Staff (2014). Before the
117 experiment, the soil electrical conductivity (EC) was 2.1 dS m^{-1}. Samples were taken to a depth
118 of 20 cm in October 2015, before harvesting the fruit.

119

2.3. Chemical analyses, soil respiration, soil enzyme activities and phospholipid fatty acid (PLFA) analysis

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 content (N) and total organic C (TOC) were determined using a C/N Flash EA 1112 Series elemental analyzer (Thermo Finnigan EA-1112, Thermo Fisher Scientific Inc., MA, USA). Microbial respiration was measured in 10-ml capped tubes containing 1 g of soil, as described elsewhere (Bastida *et al.,* 2015).

The urease activity in the soil was determined by the buffered method of Kandeler & Gerber (1988). Phosphomonoesterase and β-glucosidase activities were analyzed following the methods described by Tabatabai & Bremmer (1969) and a modification of Tabatabai's method (1982), respectively. Polyphenol oxidase was determined by the method of Allison (2006). N-acetyl-glucosaminidase and cellobiohydrolase activities were performed as described by Allison & Jastrow (2006).

Phospholipids were extracted from 6 g of soil with a mixture containing 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 (Guckert *et al*., 1985) and designated as described by Frostegard *et al*. (1993). The samples were analyzed with a Trace Ultra Thermo Scientific gas chromatograph fitted with a 60-m capillary column (ThermoTR-FAME 60 m x 0.25 mm ID x 0.25 μm film), using helium as the carrier gas. The assignation of fatty acids to microbial groups was carried out as described in Bastida *et al*. (2017).

2.4. Plant water status and gas exchange parameters

The stem water potential (ψ_{stem}) was analyzed monthly at midday using a pressure chamber (model3000; Soil Moisture Equipment Corp., Santa Barbara, USA) and following the recommendations of Turner (1988). Two mature leaves from the canopy were selected. At least 2 h before the measurement, the leaves were covered with aluminum foil and enclosed within polyethylene bags (McCutchan & Shackel, 1992). Instantaneous measurements of net

photosynthesis (A) and stomatal conductance (g_s) were carried out with a portable photosynthesis system (LI-6400 Li-Cor, Lincoln, NE, USA). Leaf gas exchange was analyzed monthly on 16 young, fully expanded leaves per treatment, placed in a 2-cm^2 leaf cuvette. Measurements were performed at a saturating light intensity of 1200 µmol m^{-2} s^{-1} and at ambient temperature and relative humidity.

2.5. Data analysis

The normality and homogeneity of the variables were checked by the Kolmogorov-Smirnov and Levene tests, respectively. The variables were transformed logarithmically when necessary. Data were analyzed using two-way ANOVA with irrigation treatment and crop as main factors. Differences were considered significant at P <0.05. The structure of the microbial community was determined using principal component analysis of the relative abundances of single fatty acids. The loading scores of each variable were represented by vectors. The statistical analyses were carried out using IBM-SPSS Statistics (version 23.0) software.

3. RESULTS

3.1. Water and soil chemical analysis

Significant differences between TW and RW water were observed: RW had greater salinity and sodicity, with average values of EC_w around 3.21 dS m^{-1} and of SAR_w around 9.45 [meq l^{-1}]$^{0.5}$, whereas TW had lower values of EC_w, 1.00 dS m^{-1}, and of SAR_w, 1.39 [meq l^{-1}]$^{0.5}$. The RW water also had higher concentrations of NO_3^-, PO_4^{3-}, SO_4^{2-}, Cl^-, K, B, and Na than TW (Table 1). The concentrations of organic C and N in the reclaimed water were 18 and 7 mg l^{-1}, respectively.

The soil TOC content was influenced significantly by treatment and crop, and by their interaction (Table 2). Total N was influenced significantly by treatment and by the interaction between irrigation treatment and crop. The soil EC was influenced significantly by treatment.

The greatest soil TOC value was recorded for TW_c (Table 3). The soil TOC content did not differ in the soils that received reclaimed water (RW, TW_c, or RW_c), in comparison to TW (Table 3). This trend was observed in both mandarin and grapefruit crops. Total N was lower in RW_c soil

than in the rest of the soils under grapefruit. In the mandarin, the soil N content was greatest in TW_c soil, with no significant diffferences among the other treatments. The EC followed the same trend in both crops: the RW treatment gave the greatest values, followed by TW_c and RW_c (Table 3).

3.2. Microbial biomass and community structure estimated by PLFAs

The bacterial PLFA content was influenced significantly by irrigation treatment, crop, and their interaction, while the fungal PLFA content was influenced exclusively by crop (Table 2). Overall, the bacterial and fungal soil PLFA contents were higher under grapefruit than under mandarin trees. In the case of grapefruit, TW produced a lower bacterial PLFA content than RW, RW_c, or TWc. In contrast, treatment RW showed the lowest values in the mandarin trees (Fig. 1). The content of Gram-positive bacterial fatty acids was lowest with TW in the grapefruit soil and with RW in the mandarin. A similar pattern was observed for the Gram-negative PLFA contents (Fig. 1). The fungal PLFA content of the grapefruit soil was greatest with RW_c. In the mandarin soil samples, the fungal biomass was lower in RW soil in comparison to the other treatments. The ratio of the bacterial to fungal fatty acid contents was lowest for TW in the grapefruit soil (Table 3).

To estimate the structure of the microbial community, a factor analysis of the relative abundances of fatty acids was performed together for the grapefruit and mandarin soil samples (Fig. 2A). Factor 1 explained 22.16% of the variance of the results and Factor 2 explained 14.63% (Fig. 2A). According to Factor 1, the structure of the microbial community of TW under mandarin, RW under grapefruit and the other treatments differed. Two-way ANOVA analysis of the factors revealed that Factor 1 was influenced significantly by crop ($F = 8.06$, $P = 0.012$), treatment ($F = 11.73$, $P < 0.001$), and their interaction ($F = 26.57$, $P < 0.001$). Factor 2 was influenced significantly by crop ($F = 41.40$, $P < 0.001$) and treatment ($F = 3.56$, $P = 0.038$), but not by their interaction ($F = 0.49$, $P = 0.69$). According to Factor 2, the structure of the microbial communities of grapefruit soil samples (positive side of Factor 2) was different from that of mandarin samples (negative side of Factor 2). Gram-negative (18:1ω9c and 18:1ω9t), actinobacterial (10Me16:0), and fungal (18:2ω6t) fatty acids received a high loading score in Factor 1. Gram-positive (16:1ω9) and fungal (18:2ω6c) fatty acids received a high loading score

209 in Factor 2. Phosphatase activity and the total organic C and N contents were the only variables
210 that correlated (negatively) with Factor 1 in the factorial analysis ($P < 0.05$) (Table 4). Urease
211 activity correlated positively with Factor 2 and EC correlated negatively ($P < 0.05$).

212 Furthermore, a factor analysis of the relative abundances of fatty acids was performed,
213 separately, for the grapefruit and mandarin soil samples. In the case of grapefruit crop, Factor 1
214 explained 33.34% of the variance of the results and Factor 2 explained 27.16% (Fig. 2B).
215 According to Factor 1, the structure of the soil microbial community of TW differed from that of
216 the soils receiving reclaimed water (RW, TW_c, RW_c). According to Factor 2, the structure of the
217 microbial community of RW soil differed from that of the other soils.

218 In the grapefruit soil, positive correlation coefficients were found between Factor 1 and different
219 variables (EC, respiration, cellobiohydrolase, and N-acetyl glucosaminidase) ($P < 0.05$) (Table
220 4). Negative correlation coefficients were observed between Factor 2 and pH and the β-
221 glucosidase, urease, and p-phenol oxidase activities ($P < 0.05$).

222 In the mandarin soil, Factor 1 (31.10% of the variance of the results) separated TW samples
223 from the rest. Factor 2 (20.78%) discriminated between RW and the other treatments (Fig. 2C).
224 Negative correlation coefficients were found between Factor 1 of mandarin soil and the
225 phosphatase, urease, and p-phenol oxidase activities ($P < 0.05$). A positive correlation
226 coefficient was observed between Factor 2 and soil respiration ($P < 0.05$) (Table 4).

227

228 ***3.3. Soil respiration and enzyme activities***

229 Respiration and all the enzyme activities were influenced significantly by irrigation treatment,
230 crop, and their interaction (Table 2). With the exception of RW, soil respiration was higher in
231 grapefruit soil samples than in mandarin ones. Respiration was greatest in TW_c soil in the case
232 of grapefruit, and in RW in the case of mandarin (Table 3). The ratio of respiration to TOC was
233 greatest in TW_c soil samples for grapefruit. In contrast, for mandarin, this ratio was lowest in
234 TW_c samples.

235 The enzyme activities were higher in grapefruit soil than in mandarin soil, with the exception of
236 β-glucosidase, cellobiohydrolase, and p-phenol oxidase for TW_c. The enzyme activites of soils
237 irrigated with reclaimed water were never lower than for TW (Fig. 3).

In grapefruit soil, the β-glucosidase and cellobiohydrolase activities were greater in RW and TW_c than in TW and RW_c. In mandarin soil, the activity of these enzymes was greatest for TWc and no differences were observed between TW and RW_c. The p-phenol oxidase activity was greatest for RW soil in the case of grapefruit and for RW_c in the case of mandarin (Fig. 3).

Urease activity was higher for RW than for the rest of the treatments, for both crops (Fig. 4). For both crops, the N-acetyl glucosaminidase activity was greatest for RW and TW_c,. Phosphatase activity was greatest for TW_c, under both crops

3.4. Plant physiology and crop yield

The treatment and crop, and their interaction, influenced significantly the net photosynthesis (*A*) and stomatal conductance (g_s). The stem water potential (Ψ_s) was affected significantly by crop (Table 2). Lower annual average values of *A* and g_s were observed in plants irrigated with RW, RW_c or TW_c, in comparison to TW (Table 5). The average annual values of *A* ranged between 12.92 and 9.76 μmol m^{-2} s^{-1}, in TW and RW_c, respectively, in the case of grapefruit; in mandarin trees, the values were 8.77 and 6.81 μmol m^{-2} s^{-1} in TW and RW, respectively. Similar behavior was observed in the average annual values of g_s - which ranged between 0.161 and 0.105 mol m^{-2} s^{-1} in TW and RW_c, respectively, for grapefruit; in mandarin trees, the values were 0.086 and 0.062 mol m^{-2} s^{-1} in TW and RW, respectively (Table 5). Regarding plant water status, both species reached similar ψ_{stem} values in the different irrigation treatments, although grapefruit had a lower average annual value of -1.11 MPa across the treatments, with respect to -0.81 MPa in mandarin trees. The yield was influenced by crop but not by irrigation treatment (Table 2). Grapefruit yield was similar for all irrigation treatments and reached about 100 t ha^{-1} (Table 5). In the case of mandarin, TW, RW and RW_c showed similar yields, while TW_c, reached higher values of 45 t ha^{-1}.

4. DISCUSSION

4.1. The biomass and structure of the soil microbial community

The impacts of irrigation with water from different sources on soil microbial communities are still obscure (Rietz & Haynes, 2003). It is known that reclaimed water has elevated content of salts

267 which can impact soil microbiota (García & Hernández, 1996; Becerra-Castro *et al.,* 2015).
268 However, the negative impacts of salts in reclaimed waters can be buffered by the high content
269 of Ca and Mg in soils of the south-east of Spain (Adrover *et al.,* 2012). Despite the negative
270 aspect of its high salt content, reclaimed water has elevated content of soluble organic matter
271 (Adrover et al., 2012) - that may promote the microbial biomass in soils (Friedel *et al.,* 2000;
272 Chen *et al.,* 2015). In consequence, the equilibria between these positive and negative factors
273 can determine the final impact of reclaimed water in soils.

274 Interestingly, the analysis of PLFAs revealed different sensitivities of the soil microbial
275 communities from grapefruit and mandarin crops to the irrigation treatments. These results
276 highlight the importance of evaluating the impact of irrigation systems on the soil microbial
277 community of different crops. The greater microbial biomass in grapefruit soil was linked to the
278 greater plant activity in comparison to mandarin, as revealed by the higher net photosynthesis
279 and stomatal conductance in grapefruit. The bacterial biomass was stimulated by irrigation with
280 regenerated water (RW, TW_c, RW_c) in grapefruit soil, but not in mandarin soil. Moreover, the
281 use of RW during the whole year decreased the microbial biomass in mandarin soil, while the
282 seasonal combinations (TW_c and RW_c) mantained it at the control level (TW). As hypothesized,
283 the combined water treatments influenced positively the biomass of the soil microbial
284 community, but this effect was dependent on the crop. Nevetheless, the crop yield of grapefruit
285 and mandarin was not negatively affected by any of the treatments in comparison to TW.

286 The distinct responses of the two species could be due to the fact that rosstocks had different
287 physiological resilience to salinity (Pedrero *et al.,* 2015; Nicolás *et al.,* 2016) comparing *Citrus*
288 *macrophylla* (tolerant to salinity) with Carrizo citrange (sensitive). Indeed, a significant decrease
289 in mandarin net photosynthesis and stomatal conductance was observed in treatment RW,
290 which coincided with the lower soil microbial biomass, in comparison to TW. However, no
291 negative effects of irrigation with reclaimed water were observed in grapefruit. Previous
292 research has highlighted that plant-water relations may be affected by water origin
293 (Paranychianakis *et al.,* 2004) and ψ_{stem} may regulate processes such leaf physiological
294 parameters (*A* and g_s) (Gomes *et al.,* 2004). Thus, the distinct physiological nature and water
295 relations of each crop could have a critical influence on the structure of belowground microbial
296 communities. Various studies, particularly in agroecosystems, have detected that plant species

297 may alter the structure of the microbial community (Kowalchuk *et al.,* 2002; Haichar *et al.,*
298 2008). It is noteworthy that the selection of a particular microbial community under each of the
299 two crop species studied here occurred even though both crops were located in the same soil
300 and received the same irrigation treatments.

301 Furthermore, we found clear impacts of the irrigation treatments on the structure of the soil
302 microbial communities under both crops, as demonstrated in other studies (Gatta *et al.,* 2015;
303 García-Orenes *et al.,* 2015). The structures of the communities from TW and RW soil were
304 clearly different from those of the combined treatments. Indeed, the application of RW only in
305 summer (TW_c) increased the ratio of Gram-positive to Gram-negative bacteria, in comparison to
306 irrigation during the whole year with reclaimed water (RW). The fact that the Gram-positive
307 bacteria include groups with endosporulation capacity could mean that the TW_c treatment
308 promoted a reservoir of biological activity in soil. Indeed, the increase of Gram-positive in
309 comparison to Gram-negative bacteria has been suggested as an indicator of soil resistance
310 against harsh conditions (de Vries & Shade, 2013). However, TW_c and RW_c clustered closely
311 for both crops. Overall, these findings suggest that: i) the structure of the microbial community is
312 controlled predominantly by the plant species rather than by the irrigation treatments studied
313 here; ii) irrigation treatment impacted the structure of the microbial community of each crop and
314 may explain further changes in microbial activity, as discussed below; and iii) combined dual-
315 irrigation (TW_c and RW_c) treatments led to the evolution of a similar community structure. Since
316 the microbiota of wastewater has short duration in soil (García-Orenes *et al.,* 2007; Becerra-
317 Castro *et al.,* 2015), it is probable that the changes in community structure are due to the
318 indirect chemical effects of irrigation with different waters and plant-specific interactions with soil
319 microbial communities (Becerra-Castro *et al.,* 2015).

320

321 ***4.2. The activity of the soil microbial community***

322 The respiration of organic matter by soil microbial communities produces CO_2. In the present
323 work, soil respiration and enzyme activities were higher, overall, under grapefruit trees than
324 under mandarin. Probably, these results derive from the greater plant activity of grapefruit and
325 the selection of a more abundant microbial community under grapefruit, as revealed by the
326 analysis of PLFAs. The soil under grapefruit exhibited greater activity - in terms of soil

327 respiration and enzymes related to the cycles of C, N and P - with treatments RW, RW_c, and
328 TW_c than with TW. Several studies observed an increased extracellular enzyme activity in soils
329 treated with reclaimed water and explained that the soluble organic matter contained in such
330 water can act as a substrate for these enzymes and for microbial growth (Adrover et al., 2012;
331 Chen *et al.,* 2015; García-Orenes *et al.,* 2015). Nevertheless, changes in enzyme activities and
332 respiration can be also explained by variations in community structure, as observed in this
333 study.

334 Despite the efforts to evaluate these hydrolytic enzymes involved in the extracellular cycling of
335 nutrients, little has been done to understand the responses of polyphenol oxidase in
336 agroecosystems. This enzyme is involved in the formation of soil humic substances
337 (Sinsabaugh *et al.,* 2010; Lucas-Borja *et al.,* 2012). Interestingly, the activity of this enzyme was
338 greatest with treatment RW for grapefruit soil and with RW_c for mandarin. This result not only
339 reinforces the differing impact of reclaimed water on the soil biogeochemistry under the two
340 crops, but also highlights potential differences in the stabilization of organic matter in the soil
341 under each crop.

342 Although some authors have argued that elevated salinity could produce a less efficient
343 microbial community in terms of using C sources (Rietz & Haynes, 2003), we found evidence
344 that supports the selection of a resistant and well adapted microbial community which maintains
345 nutrient cycling in soil. For instance, the microbial biomass of mandarin soil was low for RW, in
346 comparison to TW_c, but its high microbial efficiency is manifested by the higher MB/TOC and
347 Resp/TOC ratios for RW, in comparison to TW_c. Thus, it seems that a less abundant but more
348 efficient microbial community was selected under mandarin trees receiving RW irrigation and
349 that it made use of the soluble organic matter contained in the reclaimed water.

350

351 **5. CONCLUSION**

352 Irrigation with reclaimed water did not negatively impact the soil microbial community of
353 semiarid soils under grapefruit and mandarin crops. Indeed, the annual use of reclaimed water
354 or the dual irrigation with TW_c influenced positively the microbial biomass and biogeochemical
355 activities of soil microbial communities under grapefruit. In contrast, the mandarin community
356 seemed more sensitive to the annual irrigation with RW but, overall, responded positively to

dual irrigation, particularly TW_c. Changes in biomass and activity were coupled to variations in the structure of the microbial community and, overall, the microbial responses were probably shaped by the specific plant physiology and water relations of the crop. This finding supports the specific selection of a given microbial community by each crop. Moreover, crop yield was not negatively affected by the irrigation with reclaimed water. Overall, our results support the use of reclaimed water when crop demands cannot be satisfied.

6. ACKNOWLEDGEMENTS

Felipe Bastida is grateful the Spanish Government for his "Ramón y Cajal" contract (RYC-2012-10666) and FEDER funds. The authors thank the Spanish Ministry for the projects AGL2014-54636-R, AGL2010-16707, AGL2010-17553 and AGL2013-49047-C2-2-R. The authors are grateful to the Fundación Séneca (19896/GERM/15 and 19903/GERM/15). Authors declare that there is not conflict of interest.

7. REFERENCES

Acosta-Martínez, V., Zobeck, T.M., Gill, T.E., Kennedy, A.C., 2003. Enzyme activities and microbial community structure in semiarid agricultural soils. *Biolog. Fertil. Soils*. 38, 216-227. DOI:10.1007/s00374-003-0626-1

Adrover, M., Farrús, E., Moyà, G., Vadell, J., 2012. Chemical properties and biological activity in soils of Mallorca following twenty years of treated wastewater irrigation. J. Environ.Manage. 95, 188-S192. DOI: 10.1016/j.jenvman.2010.08.017

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. *Crop evapotranspiration guidelines for computing crop water requirements*. FAO Irrigation and Drainage paper no 56. Rome, Italy , pp 15-27

Allison, S.D., 2006. Soil minerals and humic acids alter enzyme stability: implications for ecosystem processes. Biogeochemistry. 81, 361-373. DOI: 10.1007/s10533-006-9046-2

384 Allison, S.D., Jastrow, J.D., 2006. Activities of extracellular enzymes in physically isolated
385 fractions of restored grasslands soils. Soil Biol. and Biochem. 38, 3245-3256. DOI:
386 10.1016/j.soilbio.2006.04.011

387 Bastida, F., Zsolnay, A., Hernández, T., García, C., 2008a. Past, present and future of soil
388 quality indices: a biological perspective. Geoderma 147, 159-171. DOI:
389 10.1016/j.geoderma.2008.08.007

390 Bastida, F., Kandeler, E., Moreno, J.L., Ros, M., García, C., Hernández, T., 2008b. Application
391 of fresh and composted organic wastes modifies structure, size and activity of soil
392 microbial community under semiarid climate. Appl. Soil Ecol. 40, 318-329. DOI:
393 10.1016/j.apsoil.2008.05.007

394 Bastida, F., Selevsek, N., Torres, I.F., Hernández, T., García, C., 2015. Soil restoration with
395 organic amendments: linking cellular functionality and ecosystem processes. *Sci. Rep.*
396 5, 15550. DOI: 10.1038/srep15550

397 Bastida, F., Torres, I.F., Romero-Trigueros, C., Baldrian, P., Vetrovsky, T., Bayona, J.M.,
398 Alarcón, J.J., Hernández, T., García, C., Nicolás, E.. 2017. Combined effects of
399 reduced irrigation and water quality on the soil microbial community of a citrus orchard
400 under semi-arid conditions. Soil Biol. Biochem. 104, 226-237. DOI:
401 10.1016/j.soilbio.2016.10.024

402 Becerra-Castro, C., Lopes, A.R., Vaz-moreira, I., Silva, E.F., Manaia, C.M., Nunes, O.C., 2015.
403 Wastewater reuse in irrigation: a microbiological perspective on implications in soil
404 fertility and human and environmental health. Environ. Int. 75, 117-135. DOI:
405 10.1016/j.envint.2014.11.001

406 Bligh, E.G., Dyer, W.J., 1959. A rapid method for total lipid extraction and purification. Can. J. of
407 Biochem. .Phys. 37, 911-917.

408 Chen, W., Wu, L., Frankerberger, W.T., Chang, Jr. A.C., 2008. Soil enzyme activities of long-
409 term reclaimed wastewater-irrigated soils. J. Environ. Qual. 37, 36-42. DOI:
410 10.2134/jeq2007.0315

411 Chen, W., Lu, S., Pan, N., Wang, Y., Wu, L.. 2015. Impact of reclaimed water irrigation on soil
412 health in urban green areas. Chemosphere. 119, 654-661. DOI:
413 10.1016/j.chemosphere.2014.07.035

414 de Vries, F.T., Shade, A., 2013. Controls on soil microbial community stability under climate
415 change. Front. Microbiol. 4, 1-16. DOI: 10.3389/fmicb.2013.00265

416 Frostegard, A., Tunlid, A., Baath, E., 1993. Shifts in the structure of soil microbial communities
417 in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol.Biochem. 26,
418 723-730. DOI: 10.1016/0038-0717(93)90113-P

419 García, C., Hernández, T., 1996. Influence of salinity on the biological and biochemical activity
420 of a calciorthird soil. Plant Soil. 178, 255-263. DOI: 10.1007/BF00011591

421 García-Orenes, F., Roldán, A., Guerrero, C., Mataix-Solera, J., Navarro-Predeño, J., Gómez, I.,
422 Mataix-Beneyto, J., 2007. Effect of irrigation on the survival of total coliforms in three
423 semiarid soils after amendment with sewage sludge. Waste Manage. 27, 1815-1819.
424 DOI: 10.1016/j.wasman.2006.08.012

425 García-Orenes, F., Caravaca, F., Morugán-Coronado, A., Roldán, A., 2015. Prolonged irrigation
426 with municipal wastewater promotes a persistent and active soil microbial community in
427 a semiarid agroecosystem. Agr. Water Manage. 149, 115-122. DOI:
428 10.1016/j.agwat.2014.10.030

429 Gatta, C., Libutti, A., Gagliardi, A., Beneduce, L., Brusetti, L., Borruso, L., Disciglio, G.,
430 Tarantino, E., 2015. Treated agro-industrial wastewater irrigation of tomato crop: effects
431 on qualitative/quantitative characteristics of production and microbiological properties of
432 the soil. Agr. Water Manage.149, 33-43. DOI: 10.1016/j.agwat.2014.10.016

433 Gomes, M.M., Lagoa, A.M.M.A., Medina, C.L., Machado, E.C., Machado, M.A., 2004.
434 Interactions between leaf water potential, stomatal conductance and abscisic acid
435 content of orange trees submitted to drought stress. BJPP 13, 155–161. DOI:
436 10.1590/S1677-04202004000300005.

437 Haichar, F.Z., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., Heulin, T.,
438 Achouak, W., 2008. Plant host habitat and root exudates chape soil bacterial community
439 structure. ISME J. 2, 1221-1230. DOI: 10.1038/ismej.2008.80.

440 Ibekwe. A.M., Poss, J.A., Grattan, S.R., Grieve, C.M., Suarez, D., 2010. Bacterial diversity in
441 cucumber (Cucumis sativus) rhizosphere in response to salinity, soil pH, and boron. Soil
442 Biol. Biochem. 42, 567-575. DOI: 10.1016/j.soilbio.2009.11.033

443 Ibor, C.S., Mollá, M.G., Reus, L.A., Genovés, J.C., 2011. Reaching the limits of water resources
444 mobilization: irrigation development in the Segura river basin, Spain. Water Altern. 4,
445 259-278.

446 Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric
447 determination of ammonium. *Biol. Fertil. Soils* 6, 68-72. DOI: 10.1007/BF00257924

448 Kowalchuk, G.A., Buma, D.S., de Boer, W., Klinkhamer, P.G.L., van Veen, J.A., 2002. Effects of
449 above-ground plant species composition and diversity on the diversity of soil-borne
450 microorganisms. *Anton. Leeuw. Int.J. G.* 81, 509-520. DOI: 10.1023/A:1020565523615

451 Lucas-Borja, M.E., Candel, D., Jindo, K., Moreno, J.L., Andrés, M., Bastida, F., 2012. Soil
452 microbial community structure and activity in monospecific and mixed forest stands,
453 under Mediterranean humid conditions. Plant Soil. 354, 359-370. DOI:
454 10.1007/s11104-011-1072-8

455 McCutchan, H., Shackel, K.A., 1992. Stem-water potential as a sensitive indicator of water
456 stress in prune trees (Prunus domestica L. cv French). *J. Am. Soc. Hortic. Sci.* 117,
457 607–611.

458 Mounzer, O., Pedrero-Salcedo, F., Nortes, P.A., Bayona, J.M., Nicolás-Nicolás, E., Alarcón,
459 J.J., 2013. Transient soil salinity under the combined effect of reclaimed water and
460 regulated deficit drip irrigation of Mandarin trees. Agr. Water Manage. 120, 23-29. DOI:
461 10.1016/j.agwat.2012.10.014

462 Nannipieri, P., Grego, S., Ceccanti, B., 1990. Ecological significance of the biological activity in
463 soils, in: Soil Biochemistry (Eds.) Bollag, J.M., Stotzky, G. Marcel Dekker, New York,
464 pp. 293-355.

Nicolás, E., Alarcón, J.J., Mounzer, O., Pedrero, F., Nortes, P.A., Alcobendas, R., Romero-Trigueros, C., Bayona, J.M., Maestre-Valero, J.F., 2016. Long-term physiological and agronomic responses of mandarin trees to irrigation with saline reclaimed water. Agr. Water Manage. 166, 1-8. DOI: 10.1016/j.agwat.2015.11.017

Paranychianakis, N.V., Chartzoulakis, K.S., Angelakis, A.N., 2004. Influence of rootstock, irrigation level and recycled water on water relations and leaf gas exchange of Soultanina grapevines. *Environ. Exp. Bot.* 52, 185–198. DOI: 10.1016/j.envexpbot.2004.02.002

Pedrero, F., Maestre-Valero, J.F., Mounzer, O., Nortes, P.A., Alcobendas, R., Romero-Trigueros, R., Bayona, J.M., Alarcón, J.J., Nicolás, E., 2015. Response of Young ´Star Ruby` grapefruit trees to regulated deficit irrigation with saline reclaimed water. Agr. Water Manage. 158, 51-60. DOI: 10.1016/j.agwat.2015.04.012

Rietz, D.N., Haynes, R.J., 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biol. Biochem. 35**,** 845-854. DOI: 10.1016/S0038-0717(03)00125-1

Rodríguez-Morgado, B., Gómez, I., Parrado, J., García-Martínez, A.M., Aragón, C., Tejada, M.,2015. Obtaining edaphic biostimulants/biofertilizers from different sewage sludges. Effects on soil biological properties. Environ. Technol. 36, 2217-2226. DOI: 10.1080/09593330.2015.1024760

Romero, P., Navarro, J.M., Pérez-Pérez, J., García-Sánchez, F., Gómez-Gómez, A., Porras, I., Martinez, V., Botía, P., 2006. Deficit irrigation and rootstock: their effectson water relations, vegetative development, yield, fruit quality and mineralnutrition of Clemenules mandarin. Tree Physiol. 26, 1537–1548.

Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42, 391-404. DOI: 10.1016/j.soilbio.2009.10.014

Soil Survey Staff. 2014. *Keys to soil taxonomym* 12th ed. USDA-natural Resources Conservation Service, Washington, D.C.

492 Tabatabai, M.A., Bremmer, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil
493 phosphatase activity. Soil Biol. Biochem. 1, 301-307. DOI: 10.1016/0038-
494 0717(69)90012-1

495 Tabatabai, M.A., (1982) Soil enzymes. In: *Methods of soil analysis, Part 2. Chemical and*
496 *Microbiological Properties, 2nd*.(Eds.) Page, A.L., Miller, R.H., Keeney, D.R., American
497 Society of Agronomy-Soil Science Society of America, Madison., pp 903-947.

498 Tejada, M., Benítez, C., 2014. Effects of crushed maize straw residues on soil biological
499 properties and soil restoration. Land Degrad. Dev . 25, 501-509. DOI: 10.1002/ldr.2316

500 Torres, I.F., Bastida, F., Hernández, T., García, C., 2015. The effects of fresh and stabilized
501 pruning wastes on the biomass, structure and activity of the soil microbial community in
502 a semiarid climate. *Appl. Soil Ecol.* 89, 1-9. DOI: 10.1016/j.apsoil.2014.12.009

503 Turner, N.C., 1988. Measurements of plant water status by pressure chamber technique.
504 *Irrigation Sci.* 9, 289–308. DOI: 10.1007/BF00296704

505 Zornoza, R., Acosta, J.A., Bastida, F., Domínguez, S.G., Toledo, D.M., Faz, A., 2015.
506 Identification of sensitive indicators to assess the interrelationship between soil
507 quality, management practices and human health. Soil. 1, 173-185. DOI:
508 10.5194/soil-1-173-2015.

509

510

511 **Figure Captions**

512 **Figure 1**. The PLFA content of microbial groups in soils under different irrigation treatments and
513 crops. TW (transfer water); RW (reclaimed water); TWc (annual transfer water except
514 summer irrigation with RW); RWc (annual RW irrigation except summer transfer water
515 irrigation).

516 **Figure 2**. Factor analysis of phospholipid fatty acids in soils under different irrigation treatments
517 and crops: A) grapefruit and mandarin soils; B) grapefruit soil and C) mandarin soil. TW
518 (transfer water); RW (reclaimed water); TWc (annual transfer water except summer
519 irrigation with RW); RWc (annual RW irrigation except summer transfer water irrigation).

520 **Figure 3**. The activity of β-glucosidase, cellobiohydrolase, and p-phenol oxidase in soils under
521 different irrigation treatments and crops. TW (transfer water); RW (reclaimed water);
522 TWc (annual transfer water except summer irrigation with RW); RWc (annual RW
523 irrigation except summer transfer water irrigation). PNP (p-nitro phenol).

524 **Figure 4**. The activity of urease, N-acetyl-glucosaminidase, and phosphatase in soils under
525 different irrigation treatments and crops. TW (transfer water); RW (reclaimed water);
526 TWc (annual transfer water except summer irrigation with RW); RWc (annual RW
527 irrigation except summer transfer water irrigation). PNP (p-nitro phenol).

Tables

Click here to download Tables: Tables_Bastida et al.docx

Table 1. Physical and chemical analyses (electrical conductivity; EC_w, sodium absorption ratio; SAR_w, pH, cations; Na, K, Ca, and Mg, and anions; Cl^-, NO_3^-, PO_4^{---} and SO_4^{--}) in 2015, for both transfer water (TW) and reclaimed water (RW). Values are annual averages ± SE with N = 12.

		TW	RW
EC_w	**dS m^{-1}**	1.00±0.01	3.21±0.20
SAR_w	**(meq L^{-1})$^{0.5}$**	1.39±0.10	9.45±0.30
pH		8.41±0.09	7.70±0.10
Ca	**meq L^{-1}**	1.99±0.10	3.58±0.20
Mg	**meq L^{-1}**	1.58±0.10	3.92±0.30
K	**mg L^{-1}**	3.65±1.40	38.94±1.40
Na	**meq L^{-1}**	1.86±0.20	18.30±1.2
B	**mg L^{-1}**	0.10±0.01	0.66±0.04
Cl^-	**meq L^{-1}**	3.15±0.40	20.10±3.01
NO_3^-	**mg L^{-1}**	7.7±3.60	25.42±10.6
PO_4^{-3}	**mg L^{-1}**	0.31±0.02	1.73±0.70
SO_4^{-2}	**meq L^{-1}**	5.90±0.50	17.20±3.4

Table 2. Two-way ANOVA of chemical, biochemical, microbial, and plant physiology variables, including irrigation treatment and crop as factors.

	TOC		**TN**		**EC**		**pH**		**Resp**		**Resp/TOC**		**Bacteria**	
	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value
Treatment (T)	5.10	0.011	23.84	<0.001	8.46	< 0.001	7.02	0.003	3.96	0.027	8.37	<0.001	7.02	0.003
Crop (C)	10.19	0.006	3.27	0.09	1.22	0.28	101.1	<0.001	24.55	<0.001	10.84	0.005	101.1	<0.001
T x C	8.34	< 0.001	12.67	<0.001	0.63	0.66	9.80	0.001	1.93	0.17	4.39	0.020	9.80	0.001
	Fungi		**G+**		**G-**		**B/F**		**G+/G-**		**β-glucosidase**		**Cellobiohydrolase**	
	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value
Treatment (T)	1.49	0.25	10.23	0.001	6.17	0.005	4.62	0.016	13.51	<0.001	68.19	<0.001	170.1	<0.001
Crop (C)	46.83	<0.001	110.55	<0.001	75.63	<0.001	16.47	0.001	7.98	0.012	162.4	<0.001	287.2	<0.001
T x C	0.99	0.42	18.38	<0.001	4.30	0.021	4.49	0.018	10.75	<0.001	32.07	<0.001	28.31	<0.001
	PPO		**Urease**		**NAG**		**Phos**		**A**		**g_s**		**Ψ_s**		**Y**	
	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P* value	F ratio	*P value*
Treatment (T)	7.75	0.002	43.44	<0.001	118.4	<0.001	74.73	<0.001	9.10	<0.001	16.39	<0.001	0.49	0.692	0.98	0.14
Crop (C)	3.07	0.099	228.7	<0.001	294.6	<0.001	82.83	<0.001	133.17	<0.001	148.02	<0.001	109.64	<0.001	25.97	< 0.001
T x C	14.23	<0.001	17.17	<0.001	17.26	<0.001	43.04	<0.001	4.37	<0.005	3.06	0.028	0.15	0.926	3.34	0.09

Abbreviations: TOC (total organic C), TN (total nitrogen), EC (electrical conductivity), Resp (basal soil respiration), Bacterial (bacterial PLFA content), Fungi (fungal PLFA content), B/F (bacterial to fungal PLFA ratio), G+/G- (Gram+ to Gram- bacterial ratio), PPO (poly-phenol oxidase), NAG (N-acetyl glucosaminidase), A (net photosynthesis), g_s (stomatal conductance), stem water potential (Ψ_s), crop yield (Y).

Table 3. Soil chemical parameters, basal respiration, and microbial ratios in grapefruit and mandarin irrigation treatments.

	Grapefruit				Mandarin			
Parameter	**TW**	**RW**	**TW_c**	**RW_c**	**TW**	**RW**	**TW_c**	**RW_c**
TOC	0.99±0.11	0.92±0.01	1.10±0.06	0.91±0.07	0.75±0.04	0.81±0.07	1.24±0.07	0.77±0.09
Total N	0.19±0.01	0.14±0.01	0.19±0.01	0.12±0.02	0.13±0.01	0.14±0.01	0.26±0.05	0.16±0.01
EC	616±187	2486±221	1786±98	2070±143	839±91	2316±173	1326±84	1770±95
pH	8.01±0.24	8.43±0.27	8.11±0.15	8.09±0.19	8.18±0.22	8.30±0.17	8.30±0.24	8.41±0.20
Respiration	25.21±1.39	29.98±2.14	47.08±4.98	28.09±2.01	13.88±0.87	31.40±2.54	20.85±3.27	13.86±0.55
Resp/TOC	15.21±1.19	19.77±2.21	23.52±0.97	18.98±2.73	12.59±2.35	25.53±2.47	8.55±1.48	11.73±1.14
B/F	2.96±0.26	3.44±0.13	3.43±0.17	3.26b±0.21	3.50±0.14	3.76±0.16	3.35±0.01	3.70±0.20
G+/G-	0.93±0.078	0.86±0.11	1.15±0.094	0.83±0.10	1.21±0.10	0.77±0.12	1.04±0.097	1.13±0.083

TOC (total organic C, ln %); Total N (ln %); EC (electrical conductivity, µS cm^{-1}); Respiration (basal respiration, mg CO_2-C kg^{-1} soil day^{-1}); Resp/TOC (the ratio between basal respiration and TOC); B/F (ln bacterial to fungal fatty acid ratio); G+/G- (the ratio between Gram-positive and Gram-negative bacterial fatty acids). TW (transfer water); RW (reclaimed water); TW_c (annual transfer water except summer irrigation with RW); RW_c (annual RW irrigation except summer transfer water irrigation). Data are averages ± standard deviation with N = 12.

Table 4. Correlation coefficients between factors obtained from the factor analysis of PLFAs and chemical and microbial variables. This table shows correlations for the PLFA analysis of the grapefruit and mandarin soils together, as well as for each soil separately.

	EC	pH	TOC	TN	Respiration	β-glucosidase	Phosphatase	Urease	PPO	CBH	NAG
Grapefruit and Mandarin											
Factor 1	-0.001	0.219	-0.580	-0.498	-0.375	-0.269	-0.749	0.099	-0.102	-0.285	-0.244
	(0.995)	(0.304)	(0.003)	(0.013)	(0.071)	(0.203)	(<0.001)	(0.644)	(0.635)	(0.176)	(0.250)
Factor 2	0.070	-0.450	-0.081	-0.337	0.314	0.160	0.027	0.568	-0.275	0.134	0.232
	(0.744)	(0.027)	(0.708)	(0.107)	(0.136)	(0.455)	(0.900)	(0.004)	(0.193)	(0.534)	(0.275)
Grapefruit											
Factor 1	**0.586**	0.348	0.302	-0.146	0.672	0.390	0.155	-0.070	0.159	0.651	0.686
	(0.045)	(0.268)	(0.340)	(0.650)	(0.017)	(0.210)	(0.630)	(0.83)	(0.622)	(0.022)	(0.014)
Factor 2	-0.502	-0.834	0.337	0.237	0.254	-0.663	0.424	-0.878	-0.703	-0.439	-0.470
	(0.097)	(0.001)	(0.284)	(0.459)	(0.426)	(0.019)	(0.169)	(<0.001)	(0.011)	(0.153)	(0.123)
Mandarin											
Factor 1	-0.446	-0.485	-0.443	-0.531	-0.473	-0.465	-0.680	-0.744	-0.742	-0.481	-0.542
	(0.146)	(0.110)	(0.150)	(0.076)	(0.121)	(0.127)	(0.015)	(0.006)	(0.006)	(0.113)	(0.069)
Factor 2	0.172	-0.573	-0.114	-0.249	0.697	-0.159	-0.092	0.496	-0.530	-0.018	0.022
	(0.594)	(0.052)	(0.725)	(0.435)	(0.012)	(0.622)	(0.776)	(0.101)	(0.077)	(0.955)	(0.947)

Abbreviations: EC (electrical conductivity), TOC (total organic C), TN (total nitrogen), PPO (poly-phenol oxidase), NAG (N-acetyl glucosaminidase). *P* values are in parentheses.

Table 5. The physiology, water status and yield of evaluated crops

	Grapefruit				Mandarin			
Parameter*	**TW**	**RW**	**TW_c**	**RW_c**	**TW**	**RW**	**TW_c**	**RW_c**
A	12.91±0.33	12.31±0.37	11.55±0.89	9.76±0.94	8.77±0.29	6.81±0.32	8.31±0.32	6.95±0.56
g_s	0.16±0.01	0.13±0.01	0.12±0.01	0.10±0.01	0.086±0.01	0.062±0.01	0.070±0.01	0.065±0.01
Ψ_s	1.083±0.84	1.13±0.94	1.11±0.97	1.13±0.94	0.80±0.37	0.81±0.38	0.81±0.47	0.85±0.45
Y	96.67±12.25	101.67±14.72	91.25±11.58	103.33±15.97	35.86 ± 3.47	31.21 ± 2.14	45.26 ± 3.51	36.54 ± 2.87

Average values of gas exchange parameters (*net photosynthesis* –A, *µmol·m^{-2}·s^{-1}- and stomatal conductance –g_s, mol·m^{-2}·s^{-1})*, plant water status *(stem water potential* -Ψ_s, -MPa) and crop yield *(Y, t ha^{-1}*). TW (transfer water); RW (reclaimed water); TW_c (annual transfer water except summer irrigation with RW); RW_c (annual RW irrigation except summer transfer water irrigation). Data are averages ± standard deviation with N =12

Figure

Figure 1

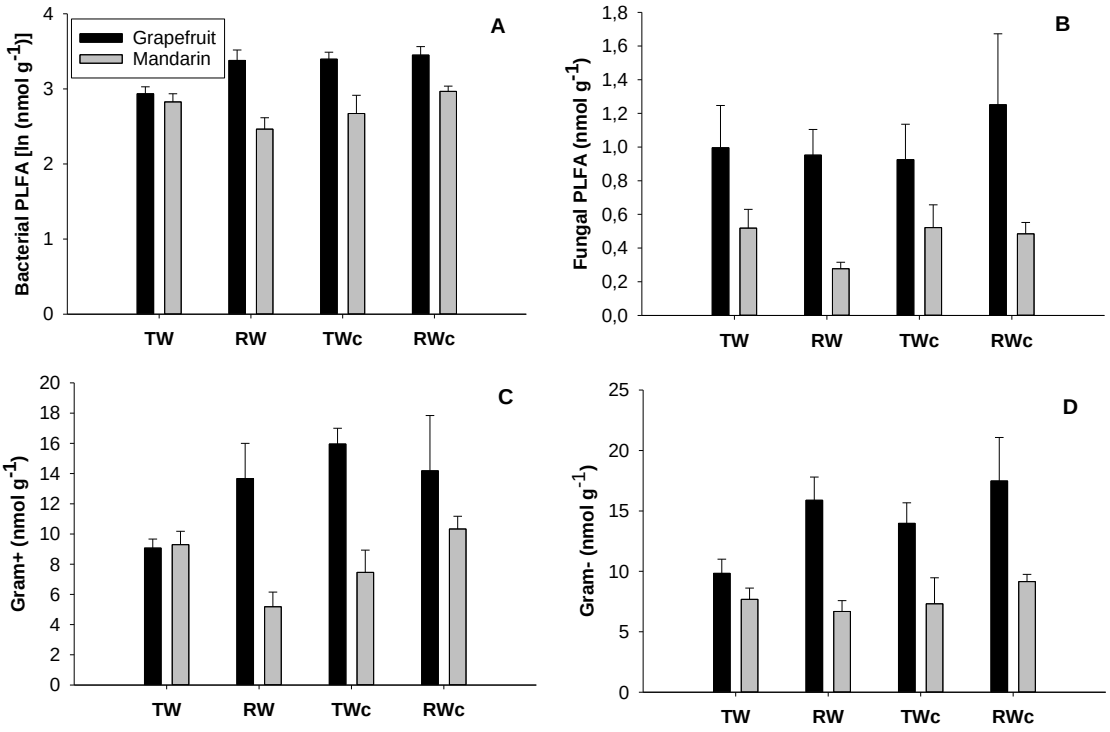


Figure 2

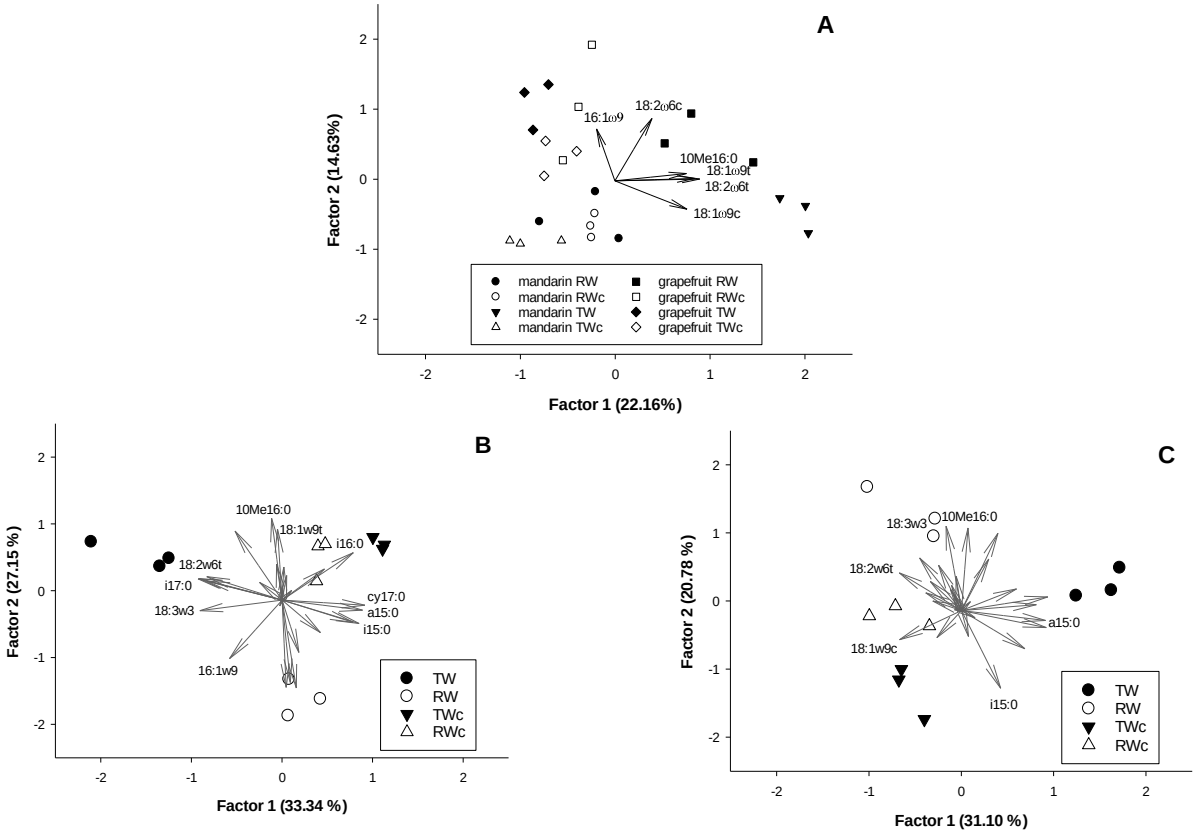

A
Factor 2 (14.63%)
Factor 1 (22.16%)
16:1ω9
18:2ω6c
10Me16:0
18:1ω9t
18:2ω6t
18:1ω9c
mandarin RW
mandarin RWc
mandarin TW
mandarin TWc
grapefruit RW
grapefruit RWc
grapefruit TW
grapefruit TWc
B
Factor 2 (27.15 %)
Factor 1 (33.34 %)
10Me16:0
18:1w9t
i16:0
18:2w6t
i17:0
18:3w3
cy17:0
a15:0
i15:0
16:1w9
TW
RW
TWc
RWc
C
Factor 2 (20.78 %)
Factor 1 (31.10 %)
18:3w3
10Me16:0
18:2w6t
a15:0
18:1w9c
i15:0
TW
RW
TWc
RWc

Figure 3

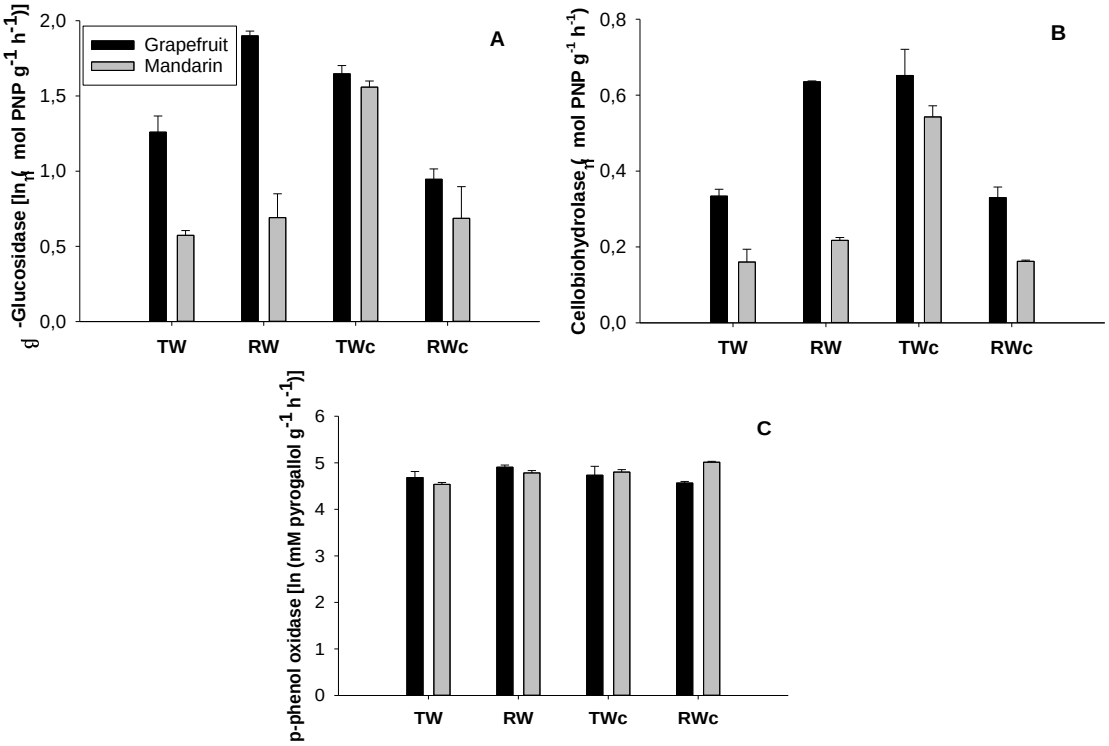
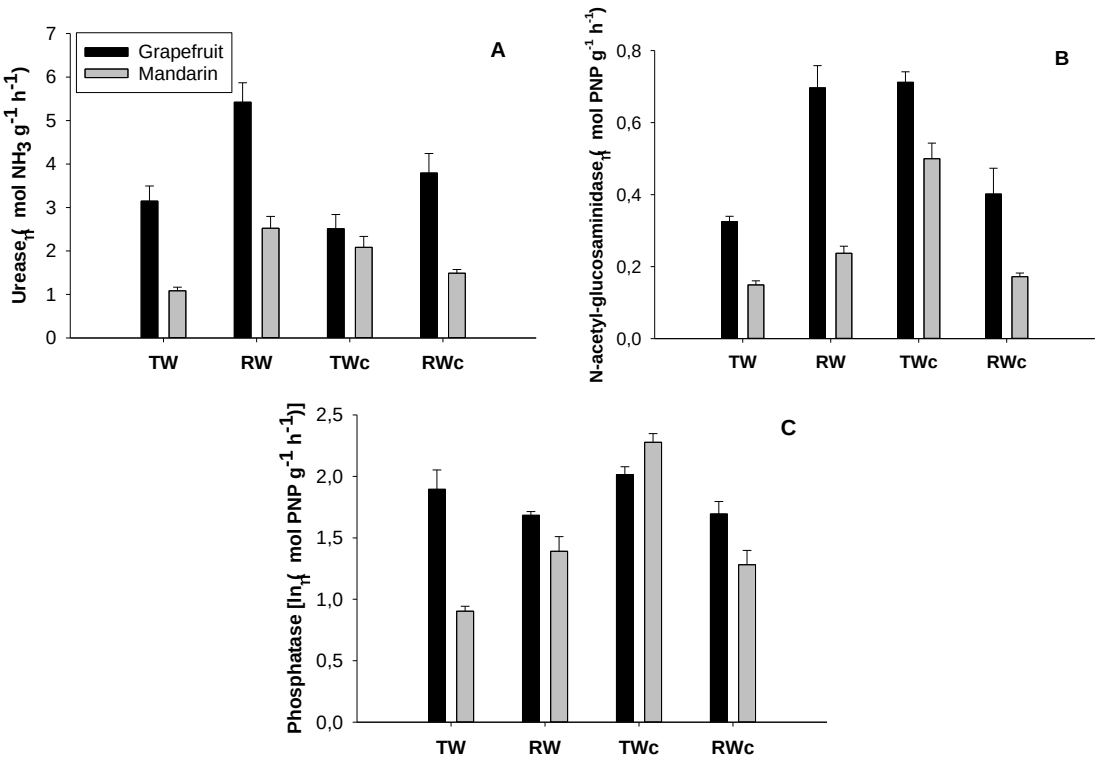

Grapefruit
Mandarin
A
B
C
TW
RW
TWc
RWc
-Glucosidase [In mol PNP g-1 h-1)]
Cellobiohydrolase mol PNP g-1 h-1)
p-phenol oxidase [In (mM pyrogallol g-1 h-1)]

Figure 4


A
Grapefruit
Mandarin
Urease (μ mol NH3 g-1 h-1)
0
1
2
3
4
5
6
7
TW
RW
TWc
RWc
B
N-acetyl-glucosaminidase (μ mol PNP g-1 h-1)
0,0
0,2
0,4
0,6
0,8
TW
RW
TWc
RWc
C
Phosphatase [ln (μ mol PNP g-1 h-1)]
0,0
0,5
1,0
1,5
2,0
2,5
TW
RW
TWc
RWc