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Reclaimed Water Use in Agriculture: Effects on Soil Chemical and Biological Properties in a Long-Term Irrigated Citrus Farm

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Abstract: In Mediterranean regions, the scarcity of freshwater for agricultural purposes is leading to the use of alternative water sources. This study aimed to evaluate the effects of long-term irrigation with reclaimed water on chemical and biological soil properties. On a mandarin tree orchard (*Citrus clementina*, cv. Orogrande), freshwater (FW) and tertiary reclaimed water (RW) were supplied for irrigation. In spring 2017, a soil sampling was carried out, collecting from each experimental plot four samples at 0–0.20 m depth. Chemical and biochemical soil properties were determined on air dried and sieved soil and on fresh and field-moist soil, respectively. The irrigation with reclaimed water significantly increased the soil water extractable organic carbon (WEOC), available P, Mg, and Na content, and the electrical conductivity (EC). Although not significant, the respiration rates and enzymatic activities were higher in RW treatment. The results of this research highlighted that the irrigation with reclaimed water, providing organic carbon and other nutrients, could have, in the long-term, beneficial effects on soil microorganism and their activities. In any case, especially in arid and semi-arid environments, a proper management of wastewater should be recommended to avoid soil degradation due to salt accumulation in the rootzone.

Keywords: tertiary reclaimed water; soil sampling; WEOC; soil respiration; soil enzymatic activities



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1. Introduction

The re-use in agriculture of reclaimed water originating from wastewater treatment plants is becoming an opportunity to increase the water resources for irrigation and a way to protect the environment from direct disposal of wastewater to the water bodies.

Globally, agriculture is a major consumer of wastewater. The FAO reported that approximately 10% of the total global irrigated land area receives untreated or partially treated wastewater, encompassing 20 million hectares in 50 countries [1].

Depending on the quality level of the wastewater reclamation treatment and wastewater origin, the irrigation with reclaimed water could differently affect soil properties and, consequently, crop production [2–6]. The impact of the irrigation with this alternative water resource depends, in fact, on the quality and quantity of wastewater applied, soil type, duration of irrigation, and local climate [6,7].

Considering the growing competition between agriculture and domestic use of fresh water, especially in arid and semiarid environments, and the consequent increasing use of reclaimed water for irrigation, in order to meet crop water needs, research on long-term effects of application of reclaimed water on soil properties is of great relevance and interest.

Usually, soil physical and chemical variables (such as *pH*, texture, bulk density, water retention capacity, soil organic matter, and nutrient availability) alter very slowly or only when the soils undergo a drastic change. Therefore, investigations focused only on these soil properties could not yield conclusive evidence of the impacts of irrigation with reclaimed water, whereas more appropriate variables should be considered in order to assess the effects of reclaimed water on soil quality and health and on the ecosystem functions.

Several studies highlighted that irrigation with reclaimed water could affect the organic matter and essential crop nutrient content in soils [2,4,8,9] and, consequently, could promote soil aggregate stability, water retention capacity, cation exchangeable capacity, and the microorganism activities and structure. Tarchouna et al. [7] observed that, although wastewater contained organic carbon (OC), the supply of wastewater diminished OC content in soil, probably related to an intensification of microbial activity due to labile C and N supplied by the wastewater. Labile and biodegradable fraction of soil total organic matter (estimated as water extractable organic C, WEOC) is highly related to the plant nutrient cycling and to soil microbial activity, since it is an immediate energy source for microorganisms [10,11]. In addition, this fraction is highly sensitive to tillage, manuring, fertilization, crop rotation, and other agronomic management in comparison with total organic matter [12–15]. It is consequently considered, along with soil respiration and enzyme activity, a sensitive indicator of management-induced changes in soil quality [16–21].

Soil respiration and enzymatic activities are good estimators of overall biological activity, and they are closely related to the processes of organic matter decomposition and mineralization [22–26]. Previous studies showed that numerous factors such as climate, type of amendment, agricultural management, crop type, and edaphic properties can affect these sensitive soil indicators [27–33]. Consequently, wastewater, being a potential source of chemical substances applied to the soil, may change gradually the microbial dynamics.

Since the soils are living systems containing vast assemblages of soil organisms which perform critical functions to terrestrial life, sensitive soil variables such as WEOC, respiration, and enzymatic activities should be considered when investigating the effect of management practices on soil health.

In light of the issues reported, a study was carried out to assess the effects of long-term irrigation with reclaimed water on main chemical and biochemical soil properties, with a particular focus on labile and biodegradable fractions of soil organic matter, soil respiration, and enzymatic activities as sensitive indicators of management-induced modifications of soil quality status. To this aim, in a mandarin (*Citrus clementina*, cv. Orogrande) orchard in Murcia region (Spain), irrigated over ten consecutive years with tertiary reclaimed water, a soil sampling was carried out in spring 2017 when soil moisture and temperature conditions should be favorable for microbial activities.

2. Materials and Methods

2.1. Experimental Site and Treatments

The long-term field trial was established in 2008 on an orchard, located in Campotéjar-Murcia, Spain (38°07'18" N; 1°13'15" W), characterized by a Mediterranean semiarid climate. The orchard was cultivated with fully-grown mandarin trees (*Citrus clementina*, cv. Orogrande) with tree spacing of 5 m × 3.5 m. The historical annual reference evapotranspiration (*E_{T0}*) and rainfall (2008–2017) are, on average, 1365 and 284 mm, respectively (Figure 1). 2017 was a very dry year in comparison with historical data, with a total rainfall of 204 mm [34]. The soil was classified as a sandy clay loam (USDA classification), with average contents in sand, silt, and clay of 42%, 32%, and 26%, respectively. In 2007, before the beginning of applying reclaimed water, the soil electrical conductivity of the saturated paste extract (*EC_e*) was, on average, 2.1 dS m⁻¹ [35].

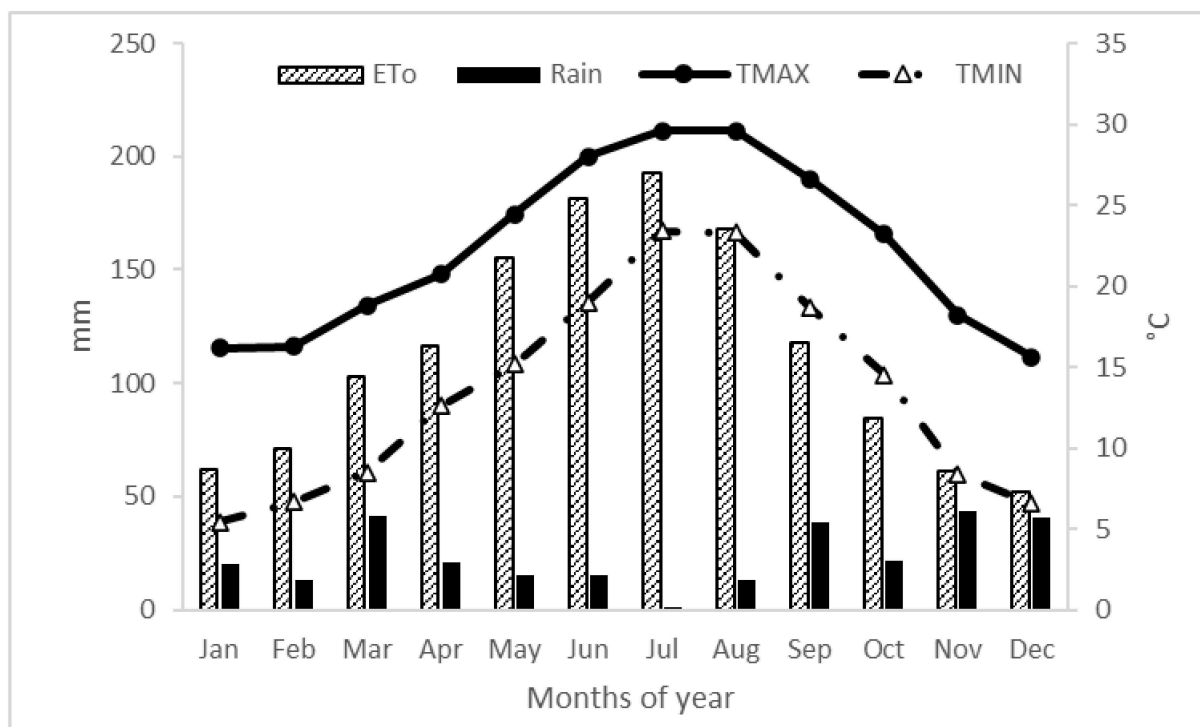


Figure 1. Monthly average reference evapotranspiration (ETo), rainfall, and maximum and minimum temperatures (TMAX, TMIN) recorded during 10 years of experiment (2008–2017).

The long-term field experiment compared two water sources and two irrigation treatments. The two water sources were: water transferred from the channel (FW), with an average electrical conductivity of 1.2 dS m^{-1} , and tertiary reclaimed water (RW) pumped from the Molina de Segura tertiary wastewater treatment plant with an average electrical conductivity of 3.4 dS m^{-1} [34]. The average chemical composition of the two water sources used for irrigation is reported in Table 1.

Table 1. Average chemical composition of freshwater (FW) and reclaimed water (RW) used in experimental trial (EC_w , electrical conductivity; SAR_w , sodium adsorption ratio; pH and the content of cations and anions).

Parameters	Units	FW	RW
EC_w	dS m^{-1}	1.2	3.4
SAR_w		2.2	6.4
pH		8.2	7.8
Ca^{2+}	meq L^{-1}	4.1	6.8
Mg^{2+}	meq L^{-1}	4.0	8.2
K^+	mg L^{-1}	9.1	36.1
Na^+	meq L^{-1}	4.6	16.8
Cl^-	meq L^{-1}	3.9	15.4
NO_3^-	mg L^{-1}	3.8	12.2
PO_4^{3-}	mg L^{-1}	1.8	2.2
SO_4^{2-}	meq L^{-1}	5.4	12.4

Modified from Abadia et al. [34].

The two irrigation treatments were: irrigation to fully satisfy the crop water requirements (100% of actual crop evapotranspiration, ETc) and regulated deficit irrigation (RDI), restoring 100% of ETc during the whole year, except during the second stage of fruit development, phenological periods less sensitive to water stress, when only the 50% of ETc was restored [34]. Irrigation was scheduled on the basis of weekly ETc estimated as crop reference evapotranspiration (ETo), calculated using the Penman–Monteith equation [36],

and month-specific crop factors [37], as described by Pedrero et al. [35]. The meteorological data were collected from an automated weather station situated in the experimental field. The average amounts of water applied in the full irrigation and in RDI treatments were 7500 and 6200 m³ ha⁻¹, respectively. Hence, RDI treatment allowed average reductions of about 17% of water per year. Both treatments included application of the same amounts of fertilizer (N—P₂O₅—K₂O) applied through the drip irrigation system.

The experimental trial was arranged in a randomized complete block design (RCBD) with four replications. The single plot size was of 210 m² with twelve mandarin trees. In this study, only the regulated deficit irrigation treatments with and without reclaimed water were considered.

2.2. Soil Sampling and Laboratory Analysis

In spring 2017, after ten years of irrigation with reclaimed water, soil characterization was performed, collecting from each plot four soil samples at 0–0.20 m depth, on 35 m² test area placed in the middle row of the plot. Each sample was divided into two subsamples: one was immediately stored at 4 °C in plastic bags until assaying of biochemical analysis, the other was air-dried and sieved for chemical one.

On air-dried and sieved soil sampling, total organic carbon content (TOC) was measured by dry combustion [38] with a TOC Vario Select analyzer (Elementar, Hanau, Germany), total nitrogen (N) was analyzed according to the Kjeldahl procedure, available phosphorus (P) was determined according to Olsen method; exchangeable potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) were determined by extraction in a barium chloride–triethanolamine buffered solution (*pH* = 8.2), followed by Inductively Coupled Plasma–Optical Emission spectrometry (ICP-OES) quantification [39]; *pH* and electrical conductivity (EC) were measured in a 1/5 (*w/v*) aqueous/soil extract, *pH* was quantified with a CRISON microTT 2050 pHmeter and EC_{1.5} with a CRISON GPL32 conductivimeter. To convert the EC_{1.5} values to *ECe*, the following equation was applied [40]:

$$ECe = EC_{1.5} \times 7.5 \quad (1)$$

Exchangeable sodium percentage (*ESP*) was calculated as follows [41]:

$$ESP (\%) = \frac{Na^+}{CEC} \times 100 \quad (2)$$

where *Na*⁺ is the ion concentration on exchange complex (meq 100 g⁻¹) and *CEC* is the soil cation exchangeable capacity (meq 100 g⁻¹), calculated as the sum of the exchangeable cation concentrations (*Ca*²⁺, *Mg*²⁺, *K*⁺, and *Na*⁺) [41].

On fresh and field-moist soil samples, water extractable organic carbon (WEOC) was measured. Extraction of water-soluble carbon from the soil was carried out according to a protocol obtained by combining procedures reported in Haynes [42] and Rees and Parker [43]: 30 g of fresh-weight soil and 60 mL of distilled water (1:2 solid/liquid ratio) was placed in an Erlenmeyer flask, capped, and shaken for 30 min. The extracts were centrifuged at 10,000 rpm for 10 min and the supernatant was filtered through 45 µm Millex filter. Total organic carbon in water extracts was analyzed with TOC Vario Select analyzer (Elementar, Germany) [44].

On two out of the four fresh samples per plot, soil respiration and enzymatic activities (dehydrogenase, urease, alkaline phosphatase, and β-glucosidase) were quantified. Soil respiration was measured by the titrimetric method [45]. This method is based on the determination of CO₂ evolution from incubated soil. Approximately 30 g of soil was placed in vials, and deionized water was added to obtain a gravimetric water content of 30% [46]. Each vial containing soil was placed in a 1.0 L glass jar containing a vial with 4 mL of 1-M NaOH; moreover, 4 mL of acidified water was added into the base of the jar to maintain the humidity and reduce soil drying. Each jar was sealed tightly and placed in darkness in an incubation chamber with constant temperature of 28 °C. During incubation the CO₂

produced was trapped into NaOH solution, forming Na_2CO_3 , and the excess NaOH, which did not react, was titrated with HCl. Soil respiration was determined after 1, 3, 7, 14, 21, and 28 days of incubation: the glass jar was opened and the reaction of hydroxide with carbon dioxide was immediately stopped by adding 8 mL of 0.75-N BaCl_2 , then the NaOH vial was removed from the jar, and the excess NaOH was titrated out with 0.1 M HCl, setting the titration *pH* of 8.8. Soil respiration as $\mu\text{g C-CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ was computed from the titration value according to the equation:

$$C - \text{CO}_2 = \frac{(V_0 - V) \times M \times EW \times 1000}{dw \times h_{inc}} \quad (3)$$

where V_0 is the volume (mL) of acid (HCl) used to titrate the alkali (NaOH) of a blank solution (incubated without soil), V is the volume (mL) of acid used to titrate the alkali solution incubated with the soil sample, M is the molarity (mol L^{-1}) of the acid (HCl) and EW is the equivalent weight (g mol^{-1}) of C-CO_2 , 1000 is the converting factor from mg to μg , dw is the dry weight of soil (g), and h_{inc} is the incubation duration expressed in hours [47].

Dehydrogenase activity was determined by the method of Masciandaro et al. [48]. In this procedure, 1 g of soil was mixed with 0.2 mL of 4% INT (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride) in distilled water. The control was the soil treated with distilled water (0.2 mL), instead of INT. Soils were incubated for 20 h at 22 °C in darkness. The INTF (1-(4-Iodophenyl)-5-(4-nitrophenyl)-3-phenylformazan) formed by the reduction of INT was extracted by adding 10 mL of a mixture of 1:1.5 tetrachloroethylene and acetone; next, the soil mixture was vigorously shaken by hand for 1 min and finally filtered through a Whatman no. 41 filter paper. The INTF concentration was measured spectrophotometrically at 490 nm, and the results were expressed as $\mu\text{g INTF g}^{-1} \text{ soil h}^{-1}$.

Urease activity was determined by the buffered method of Kandeler and Gerber [49]. In this procedure, 2.5 mL of a solution of urea (0.72 M) and 20 mL of borate buffer (*pH* 10) were added to 5 g of soil in hermetically sealed flasks, and then incubated for 2 h at 37 °C. Then, the reaction was stopped by adding 30 mL of a mix solution of KCl/HCl (1 N/0.01 N) and the ammonium content was determined according to the modified Berthelot's method [50]. Controls were prepared without substrate to determine the ammonium produced in the absence of added urea. The intensity of green color that forms upon treatment of an aliquot of the extract with salicylate and hypochlorite at high *pH* was determined at 667 nm in a spectrometer, and the enzymatic activity was expressed as $\mu\text{g NH}_3 \text{ g}^{-1} \text{ soil h}^{-1}$.

The alkaline phosphatase and β -glucosidase determination follow similar protocols. Alkaline phosphatase (Alkaline phosphomonoesterase) activity was measured by the method of Tabatabai and Bremner [51], adding 0.2 mL of toluene, 4 mL of MUB (modified universal buffer, *pH* 11), and 1 mL of substrate 0.025 M *p*-nitrophenyl phosphate (*p*-NPP) to 1 g of soil. β -glucosidase activity was determined according to a modification of Tabatabai's method [52], adding 0.2 mL of toluene, 4 mL of MUB, and 1 mL of substrate 0.025 M of *p*-nitrophenyl- β -D-glucopyranoside (*p*NG) at 1 g of soil. The mixture obtained is then incubated at 37 °C for one hour. Following incubation, 1 mL 0.5 M of CaCl_2 and 4 mL 0.5 M of NaOH were added for the alkaline phosphomonoesterase activity assay, and 4 mL of tris(hydroxymethyl)aminomethane-sodium hydroxide (THAM-NaOH) was added for the β -glucosidase assay. For the control test, the respective substrate was added before the addition of CaCl_2 and NaOH (i.e., immediately before the soil suspension is filtered). The absorbance of the supernatant from the reaction (*p*-nitrophenol) was determined at 400 nm in a spectrometer and the enzymatic activities were expressed as $\mu\text{g p-nitrophenol g}^{-1} \text{ soil h}^{-1}$.

The results of enzymatic activities are expressed on soil dry weight.

2.3. Data Analysis

Soil data were preliminary analyzed in order to investigate the distribution of the variables under study for the whole data set. Data were then tested for normality using the Kolmogorov–Smirnov test. This preliminary evaluation showed that, for most of the variables considered, data distribution did not deviate from Gaussianity according to the normality test.

Then, data were subjected to a nested analysis of variance for a randomized complete block design (RCBD), considering the sub-replicates within each plot as pseudo-replicates [47]; means were compared using SNK post hoc test at $p = 0.05$ level. Moreover, Person linear correlation analysis was performed to investigate relationships between chemical and biological soil variables. Data analysis was carried out using the SAS/STAT 9.2 software [53].

3. Results and Discussion

The irrigation over ten consecutive years with reclaimed water affected soil chemical properties (Table 2). In particular, although not significant, total organic carbon (TOC) content was higher in the treatment RW (+28%) than FW. Previous studies reported conflicting results related to the TOC increase in soil irrigated with reclaimed water. In fact, Rusan et al. [2] observed, in a soil characterized by fine texture, increases of organic matter mostly in the topsoil after two years of application, and Bedbabis et al. [4] found that in a sandy soil the organic matter content significantly increased in the long-term application. Conversely, other authors highlighted decreases of organic matter, especially in coarse sandy soil, probably due to supplied nutrients by irrigation with wastewater that stimulated microbial activity in the soil, promoting mineralization of organic matter [7,54,55]. These different results can depend on several factors such as wastewater composition, period of application, soil texture and management, etc. In our study, the irrigation with reclaimed water significantly increased the soil water extractable organic carbon (WEOC) content (Table 2); these results agreed with previous research [7]. Therefore, the application of reclaimed water can be a strategy to save freshwater resources and increase soil organic matter content, mostly its labile fraction, that represents an immediate energy source for microorganisms involved in several soil biological processes (organic matter decomposition, nutrients cycling, etc.) [12].

Table 2. Effects of different water quality on soil chemical properties (FW = freshwater; RW = reclaimed water).

Treatments	TOC g kg ⁻¹	WEOC mg kg ⁻¹	pH	EC _{1:5} dS m ⁻¹	Total N g kg ⁻¹	Available P mg kg ⁻¹	Ca ²⁺ mg kg ⁻¹	K ⁺ mg kg ⁻¹	Mg ²⁺ mg kg ⁻¹	Na ⁺ mg kg ⁻¹	ESP %
FW	17.004	14.302	8.462	0.481	0.904	154.9	2345.9	268.1	341.9	65.8	1.857
RW	21.770	22.514	8.486	0.717	1.024	241.5	2515.8	235.2	518.4	358.3	8.356
	n.s.	*	n.s.	**	n.s.	**	n.s.	n.s.	*	***	***

*, **, *** = Significant at the $p \leq 0.05$, 0.01 and 0.001 levels, respectively; n.s. = not significant.

The pH and N, Ca and K content were not affected by treatments under study, even though a slight increase was observed for N and Ca contents (+13 and +7%, respectively) in the soil irrigated with reclaimed water. This finding disagrees with other previous studies that showed significant increases of soil pH, attributed to the high content of cations such as Ca, Mg, and Na [4,7].

In our study, soil EC, available P, Mg²⁺, Na⁺, and ESP were significantly higher in RW treatment (Table 2). In particular, EC_{1:5}, after ten years of irrigation, was significantly higher (+49%) in RW treatment compared with FW (Table 2). This behavior was predictable considering the chemical characteristics of the reclaimed water (Table 1). In any case, the irrigation practice increased the soil salinity, regardless of the type of water used. In fact, compared with the soil EC value at the beginning of the experimental trial ($EC_e = 2.1$ dS m⁻¹), average increases of 72 and 156% were observed, respectively, for FW and RW treatments. After ten years of irrigation with reclaimed water, the soil electrical conductivity (5.38 dS m⁻¹),

expressed as EC of the saturated paste extract ($ECe = EC_{1:5} \times 7.5$, [40]), exceeded the salinity threshold value (4 dS m^{-1}), whereas the soil ECe in FW treatment was slightly below the threshold value (3.6 dS m^{-1}). Similar results were observed on the same experiment previously, where it was shown that irrigation with reclaimed water tends to accumulate salts in the root zone, therefore, the use of these irrigation waters can cause problems of salinity and accumulation of B in the plant and soil in the long-term [56–58]. This finding is also in agreement with other long-term studies, highlighting that salts supplied with reclaimed water could accumulate in the root zone and increase soil salinity [2,4,59] with adverse consequences for soil quality and crop yield. In RW treatment, the soil Na content and ESP value were 5.4 and 4.5 times higher than FW. However, due to high exchangeable Ca and Mg soil content, after ten years of wastewater irrigation the soil cannot be defined sodic because the ESP value remained lower than the soil sodicity threshold (15%). Furthermore, the high soil concentrations of Ca and Mg, due to the wastewater application (Table 1), could counteract the negative effects of the Na on the soil particle dispersion. In other studies, it was observed that long-term irrigation with reclaimed water caused important increases of soil ESP values [5,59], also far above the soil sodicity threshold. High levels of soil sodicity obtained after irrigation with wastewater could lead to clay particles dispersion, aggregate destabilization, impaired water movement in the soil profile, aeration problems, and salt accumulation [59]. The composition of the water, particularly the cation ratios, can affect soil aggregate stability, which in turn affects soil physical conditions and water infiltration [6]. The negative impact of sodicity is more evident in soils with high clay contents [60,61]. Physical and chemical properties, particularly of high clay soils, have been found to be degraded following long-term irrigation with reclaimed water [62–64]. In our experiment, the soil could be probably considered with low sensitivity to sodicity effect because clay content was, on average, 26%. In any case, based on these results, it is very important to adopt proper management of reclaimed water and periodic monitoring of soil properties to avoid soil degradation.

Soil respiration, estimated as the CO_2 produced and released during an incubation period of 28 days, did not show significant differences between treatments (Figure 2). The results indicated that the highest C– CO_2 emission rates were found at the beginning of the experiment, then they tended to decline with incubation time. The rate of C– CO_2 emission from soil reached a maximum after one day of incubation, with values of 14.8 and $9.8 \mu\text{g g}^{-1} \text{ h}^{-1}$ in RW and FW treatments, respectively. These values dropped to 4.5 and $3.0 \mu\text{g g}^{-1} \text{ h}^{-1}$, in RW and FW, respectively, at the end of incubation time (after 28 days). In both treatments, higher availability of the readily labile organic C fraction to soil microorganisms at the beginning of the incubation period increased soil respiration rate which gradually decreased during the experiment, probably due to the gradual depletion of the available pool of C [65,66].

In RW treatment, characterized by the highest values of WEOC and TOC, higher soil respiration rates were observed during the whole period of incubation. Likely, the soil microbial activity in the RW treatment was stimulated by the greater organic matter availability, which created favorable conditions for soil microorganisms and consequently soil respiration and C– CO_2 emissions increased [66]. Linear correlation analysis showed that soil TOC and WEOC content were significantly and positively correlated with respiration rate in the whole period of soil incubation (Table 3). In particular, a stronger relationship was observed between respiration rates in the whole incubation period and WEOC, the labile fraction of TOC (significance of Pearson's correlation ranged from 0.0002 to <0.0001), whereas it was weaker with total organic carbon. Similar results were found in previous studies, highlighting that the quality of organic matter affects CO_2 fluxes more than the quantity [67,68]. In addition, the soil total nitrogen content was positively and significantly correlated with soil respiration rate in the whole incubation period (Table 3), as observed also by Fan et al. [69], who found that the respiration rate mainly depended on organic soil C and N content. Consequently, in our research, the higher soil N content in the RW

treatment could have contributed to increase the respiration rate in this treatment compared to FW one.

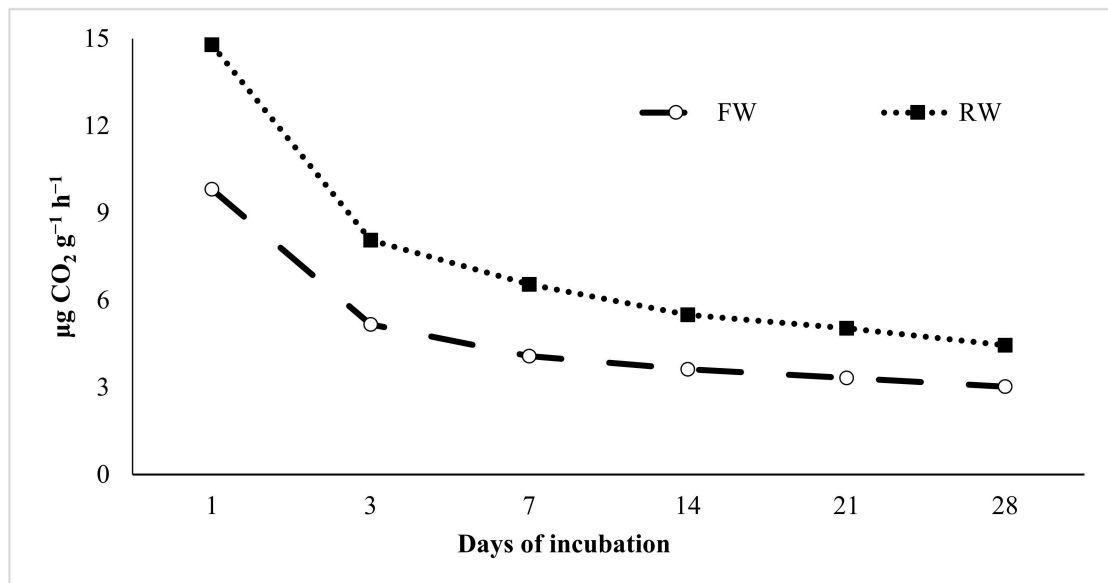


Figure 2. Soil respiration rate during 28 days of incubation in the two different quality water treatments (FW, freshwater; RW, reclaimed water).

Table 3. Pearson’s linear correlation coefficients between the main soil chemical properties and respiration rate and enzymatic activities: in brackets the number of samples used is reported.

	Soil Respiration						Enzyme Activities				
	1 Day (34)	3 Days (36)	7 Days (36)	10 Days (36)	14 Days (36)	21 Days (36)	28 Days (36)	Urease (36)	Dehydr (36)	βGluc (36)	Phosph (36)
TOC	0.45978 <i>0.063</i>	0.59437 <i>0.0119</i>	0.61466 <i>0.0086</i>	0.64113 <i>0.0055</i>	0.61961 <i>0.008</i>	0.62516 <i>0.0073</i>	0.67392 <i>0.003</i>	0.23013 <i>0.3742</i>	0.51759 <i>0.0333</i>	0.41802 <i>0.095</i>	−0.01207 <i>0.9633</i>
WEOC	0.69787 <i>0.0018</i>	0.78623 <i>0.0002</i>	0.85926 <i><0.0001</i>	0.83792 <i><0.0001</i>	0.89064 <i><0.0001</i>	0.9052 <i><0.0001</i>	0.88855 <i><0.0001</i>	0.69849 <i>0.0018</i>	0.77609 <i>0.0002</i>	0.4382 <i>0.0785</i>	0.4159 <i>0.0968</i>
pH	−0.3959 <i>0.1157</i>	−0.32745 <i>0.1995</i>	−0.30456 <i>0.2346</i>	−0.26701 <i>0.3002</i>	−0.23229 <i>0.3696</i>	−0.27723 <i>0.2814</i>	−0.31231 <i>0.2223</i>	−0.39116 <i>0.1205</i>	0.08072 <i>0.7581</i>	−0.13862 <i>0.5957</i>	−0.14404 <i>0.5813</i>
EC _{1:5}	0.48219 <i>0.05</i>	0.56918 <i>0.0171</i>	0.55966 <i>0.0195</i>	0.59199 <i>0.0123</i>	0.53791 <i>0.0259</i>	0.57089 <i>0.0167</i>	0.58783 <i>0.0131</i>	0.43388 <i>0.818</i>	0.31287 <i>0.2214</i>	0.2756 <i>0.2843</i>	0.31576 <i>0.217</i>
Available P	0.1986 <i>0.4448</i>	0.24264 <i>0.3481</i>	0.34859 <i>0.1703</i>	0.38744 <i>0.1244</i>	0.35139 <i>0.1666</i>	0.35608 <i>0.1607</i>	0.38532 <i>0.1267</i>	0.32302 <i>0.206</i>	0.47847 <i>0.052</i>	0.20872 <i>0.4214</i>	0.06369 <i>0.8081</i>
Total N	0.71939 <i>0.0011</i>	0.79253 <i>0.0001</i>	0.8092 <i><0.0001</i>	0.8236 <i><0.0001</i>	0.76879 <i>0.0003</i>	0.75451 <i>0.0005</i>	0.78736 <i>0.0002</i>	0.3378 <i>0.1848</i>	0.67907 <i>0.0027</i>	0.80982 <i><0.0001</i>	0.16781 <i>0.5197</i>
Ca ²⁺	0.3419 <i>0.1792</i>	0.44895 <i>0.0706</i>	0.47784 <i>0.0524</i>	0.4816 <i>0.0503</i>	0.46576 <i>0.0595</i>	0.48694 <i>0.0474</i>	0.54279 <i>0.0244</i>	0.17266 <i>0.5075</i>	0.31619 <i>0.2163</i>	0.23773 <i>0.3582</i>	−0.09645 <i>0.7127</i>
K ⁺	0.1597 <i>0.5404</i>	0.10658 <i>0.6839</i>	0.0958 <i>0.7145</i>	0.03214 <i>0.9025</i>	0.02285 <i>0.9306</i>	0.06141 <i>0.8149</i>	0.11375 <i>0.6638</i>	0.07441 <i>0.7765</i>	−0.18581 <i>0.4752</i>	−0.06115 <i>0.8157</i>	−0.10484 <i>0.6888</i>
Mg ²⁺	0.59715 <i>0.0114</i>	0.70762 <i>0.0015</i>	0.65355 <i>0.0044</i>	0.68948 <i>0.0022</i>	0.65998 <i>0.0039</i>	0.6585 <i>0.004</i>	0.64061 <i>0.0056</i>	0.29127 <i>0.2567</i>	0.50528 <i>0.0386</i>	0.36482 <i>0.1499</i>	0.29656 <i>0.2477</i>
Na ⁺	0.48417 <i>0.0489</i>	0.59072 <i>0.0125</i>	0.55174 <i>0.0217</i>	0.61125 <i>0.0091</i>	0.57504 <i>0.0157</i>	0.5743 <i>0.0159</i>	0.53763 <i>0.026</i>	0.29294 <i>0.2538</i>	0.53597 <i>0.0266</i>	0.30568 <i>0.2328</i>	0.37555 <i>0.1374</i>
ESP	0.3802 <i>0.1322</i>	0.46019 <i>0.0631</i>	0.42087 <i>0.0925</i>	0.4885 <i>0.0466</i>	0.45315 <i>0.0677</i>	0.44308 <i>0.0749</i>	0.38901 <i>0.1228</i>	0.26508 <i>0.3038</i>	0.47294 <i>0.0552</i>	0.25072 <i>0.3317</i>	0.4113 <i>0.101</i>

The *p* values are reported in italics.

All soil enzymes measured exhibited higher activity at the RW treatment than FW (Figure 3). Chen et al. [70] demonstrated that long-term irrigation with reclaimed water did not adversely affect soil enzyme activities; on the contrary, in particular conditions, it stimulated them. The increases of enzymatic activities, especially that involving nutrient

and C cycle, were probably due to the accumulation in the soil of biodegradable organic C with the application of wastewater. In our study, significant and positive correlations were found between WEOC values and urease and dehydrogenase activities (Table 3), proving that soil microbial activities were strongly linked to the quality of organic matter. In addition, the total N content was also significantly and positively correlated with dehydrogenase and β glucosidase activities, as reported by other authors [71].

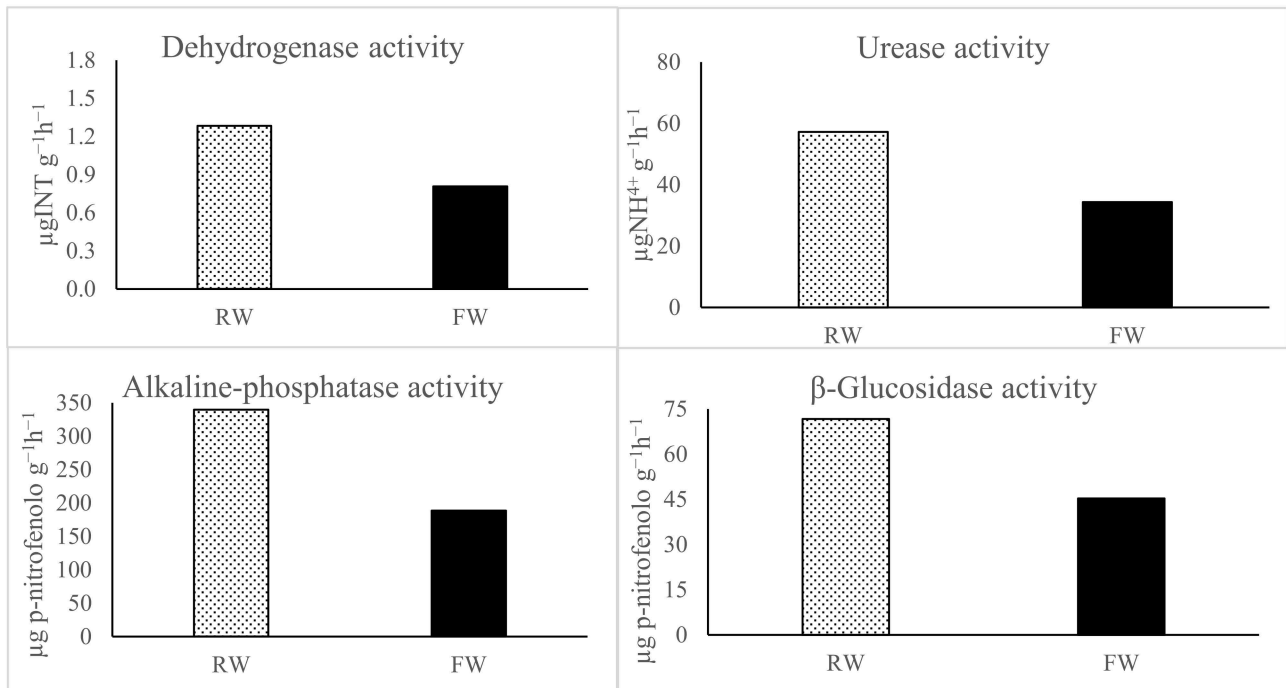


Figure 3. Soil enzymatic activity in the two different quality water treatments (FW, freshwater; RW, reclaimed water).

If the soil enzyme activities remain unchanged or increase under irrigation with reclaimed water, the biogeochemical processes governing C, N, and P cycling are not interrupted, and ecosystem functions should be saved. In this manner, the application of reclaimed water would be environmentally sustainable. The inhibition of the activities of one or more key enzymes could have significant consequences in the rate of soil nutrients cycling and, consequently, in the long-term, soils irrigated with wastewater could be exposed to degradation risk. Furthermore, irrigation with high quality reclaimed water (tertiary treatment) may minimize the potential adverse effects from toxic and persistent organic compounds [70].

Results from this field investigation indicated the beneficial effects that, in the long-term period, irrigation with reclaimed water had on soil biological health related to the biodegradable organic matter and nutrients supplied with reclaimed water, which served as a source of energy for microorganisms and enzymes. Then, agricultural reuse of reclaimed water can be considered an ecologically viable method to disposal of these waters. In any case, careful management of wastewater is always recommended to avoid an excessive accumulation of salts and other undesirable compounds that can contribute to soil degradation. The risks of wastewater reuse in agriculture are extensive, ranging from changes to physico-chemical and biological properties of soils to impacts on human health. The irrigation with treated wastewater could affect soils differently, depending on intrinsic soil characteristics, environment conditions, wastewater origin, and quality of reclamation process. In particular, in arid and semi-arid environments, characterized by uneven and scarce precipitation, rains could not be sufficient to leach the excess salts. Consequently, the salinization in the root zone could have negative effects on soil properties and crops.

Previous studies highlighted that soil respiration and enzymatic activities could be inhibited by soil enrichment, with toxic compounds applied with reclaimed water [70,72,73]. In recent decades, the innovative technologies developed to reclaim wastewater and rigorous controls on treated water should ensure a safer use of this alternative water resource. Furthermore, continuous monitoring of soil health is recommended to guarantee that the application of reclaimed water does not negatively impact on soil chemical, physical, and biological properties.

4. Conclusions

Irrigation with tertiary reclaimed water (RW) can be an important strategy to save limited freshwater resources, especially in arid and semi-arid areas. The safe reuse of wastewater could reduce the amount of the freshwater used in agriculture and prevent potential contamination of both surface water and groundwater. With regard to the soil chemical properties, the RW irrigation, in the long-term, increased the labile and biodegradable fraction of organic carbon (WEOC), and this in turn affected soil respiration and enzymatic activities, being a source of readily available energy for soil microorganisms. After 10 years of irrigation with two types of water, significantly higher values of electrical conductivity (EC_e) and Na content were observed in the soil receiving reclaimed water (5.4 dS m^{-1} and 358 mg kg^{-1} , respectively) in comparison to fresh water (3.6 dS m^{-1} and 66 mg kg^{-1} , respectively). Soil irrigated with reclaimed water could be defined as saline ($EC_e > 4 \text{ dS m}^{-1}$) but not sodic ($ESP < 15\%$), although the Na concentration was about 5-fold higher in RW treatment soils than FW ones. The results of this study highlighted that the supply of reclaimed water could be a viable strategy, in a view of sustainable use and conservation of natural resources. In any case, when using this water source, monitoring of the main soil chemical and biological properties is always recommended to avoid soil degradation and, consequently, productivity losses.

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