



Effects of irrigation management on arid soils enzyme activities

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ABSTRACT

Unconventional water resources such as treated wastewater (TWW) are being increasingly used for irrigation in regions with limited freshwater availability. Likewise, subsurface drip irrigation (SSDI) has been proposed for improving water use efficiency in arid lands. The combined effects of both factors (SSDI/TWW) on soil health have received little attention. This study assessed the mid-term (6 years) impact of irrigation with TWW apply by SSDI and surface drip irrigation (SDI) on soil chemical properties and enzyme activities under arid climate. The calculation of a numerical index (integrated biological response version 2; IBRV2) for enzymatic activities showed a significant deviation in soils irrigated with TWW with regard to control soils (irrigated with desalinated brackish water–DBW), particularly after SSDI. That combination TWW/SSDI inhibited most of assessed enzyme activities, save for catalase, with respect to the reference treatment (for example in a clay-loamy soil, decrease varied between 69% for esterase and 12% for dehydrogenase). Increased soil salinity (up to $EC \sim 15 \text{ dS m}^{-1}$) due to SSDI/TWW appears as one of the potential factors involved. These results suggest that current irrigation strategies in arid lands might accelerate soil degradation processes in regions where desertification is of particular concern.

1. Introduction

In a context of global climate change leading to severe limitations of freshwater resources for agriculture, irrigation with unconventional water sources such as treated wastewater (TWW) represents a key factor for sustaining agricultural production in arid and semi-arid regions (Díaz et al., 2013). This water source has considerable potential for irrigation and it has been increasingly applied in many arid and semi-arid regions worldwide, including China, the Middle East, Mediterranean countries, Australia, North and South America, and Africa (Grattan et al., 2015). Nevertheless, the quality of reclaimed water is often poorer than that of fresh groundwater or other conventional water resources, to the extent of threatening both soil conservation and the sustainability of agricultural systems, if not properly managed (Bedbabis et al., 2014). Numerous studies have linked soil physical and chemical degradation upon irrigation with TWW in arid regions (e.g., Lado and Ben-Hur, 2009). Salinization, sodification-alkalization and related changes in soil structure, build-up of trace elements, and nutrients imbalances can be mentioned among the most relevant negative effects. All of them are known to be unfavourable towards crop productivity, and have also been identified as responsible for accelerated desertification

processes (Dorta-Santos et al., 2014). However, impacts on soil biological properties such as enzyme activities have been studied to a much lesser extent (Chen et al., 2008; Alguacil et al., 2012; Adrover et al., 2017; Bastida et al., 2018).

Surface (SDI) and subsurface (SSDI) drip irrigation systems have been extensively used in arid and semiarid lands for improving water use efficiency (Al-Ghobari and Dewidar, 2018). SSDI is becoming increasingly prevalent in drought-prone irrigated agroecosystems due to greater yields and lesser weed growth (Schmidt et al., 2018). Additionally, the application of TWW via SSDI prevents the above-ground dispersion of pathogenic microorganisms, odors, and animal and human contact (Ayars, 2015). Drip irrigation systems usually extend their influence over a small soil volume, usually greater in the case of SSDI (Ayars, 2015). Since many soil biological and physicochemical properties are closely linked to soil moisture (Austin et al., 2004), altered distribution of nutrients and salinity as a function of shifts in soil moisture could represent potential negative tradeoffs for soil health at the field scale, including microbial communities and biogeochemical cycles (Schmidt et al., 2018). These issues, to the knowledge of the authors, have received little attention in the literature.

Enzymes respond to soil changes long before other soil quality

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indicators do, which explains the widespread use of soil enzyme activities as indicators of soil management-induced (e.g., irrigation, fertilization, land use or contamination) changes at short term (Burns et al., 2013). They represent a reliable measurement of soil performance, because of their significant role in biogeochemical cycling of nutrients and soil organic matter decomposition (Wallenstein and Burns, 2011). Although the measurement of soil enzyme activities is relatively easy and quick compared with other soil variables, the interpretation of the corresponding data is not always straightforward, provided their dependence on multiple abiotic and biotic fluctuating variables (Nannipieri et al., 2018). Moreover, the activity of a given enzyme may reflect the contribution of multiple locations where the protein occurs in soil, and a specific substrate may be decomposed by different proteins in a given enzymatic assay. To partially solve these drawbacks, some researchers have suggested alternative strategies such as: (i) using a set of enzyme activities linked to more than one biogeochemical cycle (Nannipieri et al., 2018), integrating their values by means of numerical indexes (Paz-Ferreiro and Fu, 2016), or (ii) combining enzyme activities with other soil physicochemical properties (Trasar-Cepeda et al., 2000), for the sake of a better understanding of soil biochemical processes.

Enzymatic indexes have proven to be a suitable tool for assessing short-term responses of soil biological activity to organic amendments (Shi et al., 2018), contaminants (Sanchez-Hernandez et al., 2017), the effectiveness of remediation actions (Garaiyurrebaso et al., 2017), or changes in land use (Schmidt et al., 2018). However, there is a limited knowledge on the biochemical processes of arid agricultural soils under different land management such as irrigation with marginal quality waters (Alguacil et al., 2012).

The scope of this study was to assess the mid-term effects (~6 years) of drip irrigation management with TWW, as compared with desalinated brackish water (DBW) irrigation used as a control treatment, on soil enzyme activities in top-soil samples under arid climate. The specific aims were: (i) to identify what variable among soil type, water quality and drip irrigation system has a higher impact on the response of soil enzymes; and (ii) to evaluate the suitability of the integrated biological response index (IBRv2) – a common index in environmental toxicology (Sanchez et al., 2013) – for assessing environmental stressors on enzymatic response of soil. It is expected that our results will lead to a better understanding of the effects of drip irrigation with marginal quality waters on soil microbial activities, allowing a deeper evaluation of the sustainability of these agricultural systems in terms of soil quality.

2. Materials and methods

2.1. Study area and experimental design

The study was carried out at the volcanic island of Fuerteventura (Canary Islands, Spain), located between 28°45' and 28°02' N and 13°49' and 14°20' W, and 115 km off the NW coast of Africa. Fuerteventura represents one of the most arid territories of the European Union, with a high percentage of its surface under intense desertification (Díaz et al., 2011). Most areas of the island receive on average 150 mm of rainfall per year, with no more than 300 mm in any case. The rainfall is seasonal, from October to March, with high inter-annual variability. Mean annual temperature is approximately 20 °C, average annual relative humidity ~ 64%, average wind speed ~ 3.4 m s⁻¹, average radiation ~ 19 M J m⁻² day⁻¹, and an average of 10.6 h sunshine per day. The net combination of these climatic factors leads to a reference evapotranspiration (ET_o) of ~1700–1800 mm year⁻¹.

The study site was located in the Pozo Negro experimental farm owned by the local government Cabildo Insular de Fuerteventura. In this farm, two agricultural fields cultivated with *Jatropha curcas* L. (JCL) from January 2010 until February 2016 were selected. Two different soil types, not used for agricultural purposes during the last two decades before this experiment, occurred in them: Typic Torrifluvents (TT) and Typic Haplocambids (TH) (Soil Survey Staff, 2014). The former is 60–80

cm thick on average, and sandy-loam in texture at the top layer (0–20 cm; clay 143 g kg⁻¹, silt 179 g kg⁻¹, sand 678 g kg⁻¹), whereas the latter is thicker (90–100 cm) and clay-loamy at 0–20 cm (clay 293 g kg⁻¹, silt 506 g kg⁻¹, sand 201 g kg⁻¹). Prior to the treatment the reaction of both soils was alkaline (pH 8.5–8.9), and salinity levels were significantly higher in soil TH (average EC_es ~ 2.5 vs. 1.2 dS m⁻¹ at 0–20 cm depth, respectively). Both soils contained carbonates, and soil organic carbon and nitrogen levels were generally larger in soil TH, although low in absolute terms (<6 g kg⁻¹ C and <0.6 g kg⁻¹ N), as usual in soils of arid zones (Dorta-Santos et al., 2014). Twelve experimental plots (20 m² in size; 10 JCL plants per plot) were established in each field in a complete randomized block design with three replicates per treatment. Treatments consisted of two contrasting irrigation water qualities (treated wastewater – TWW, and desalinated brackish water – DBW, commonly applied all over the island to irrigate horticultural crops with high added value, as a control), under two irrigation systems (surface drip irrigation – SDI and subsurface drip irrigation – SSDI).

The TWW was obtained from a nearby treatment plant, where the incoming urban wastewater is subjected to filtration for removal of solids, decantation and biological digestion. The DBW was generated from saline groundwater and treated by reverse osmosis at a desalination plant in the experimental farm. Table 1 shows the physicochemical characterization of the irrigation waters, sampled every month along the cropping period. The analyses performed followed the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). As expectable, total suspended solids (TSS) were much higher in TWW than DBW (24 mg L⁻¹ vs. 1 mg L⁻¹), as well as pH (6.9–9.9 vs. 6.4–7.3, respectively), and EC values (1800 vs. 300 µS cm⁻¹, respectively). Sodium Adsorption Ratios ranged from 3.1 to 22.6 in the DBW and from 9.6 to 17.2 in the TWW. Chloride ion contents reached maximum values of 127 mg L⁻¹ (DBW) and 651 mg L⁻¹ (TWW). Boron concentrations varied from 0.7 to 1.4 mg L⁻¹ and 1.4–2.4 mg L⁻¹ in the TWW and DBW, respectively. Ammonium, nitrate and potassium levels were approximately 12, 1.5 and 31 times greater, respectively, in TWW than in DBW. Finally, COD and BOD values in TWW and DBW ranged from 25 to 110 mg L⁻¹ and from 0.4 to 33 mg L⁻¹, respectively.

The experimental fields were equipped with localized automatic irrigation systems with lines spaced 1 m apart, either at the soil surface (SDI) or at 20 cm below the soil surface (SSDI). Pressure-compensating and non-leakage emitters with delivery rates at 2.2 L h⁻¹ were spaced 0.5 m in the irrigation lines. The irrigation rates applied varied monthly during the crop period, adjusted to match approximately 100% of ET_o,

Table 1

General characterization of desalinated brackish water (DBW) and treated wastewater (TWW) used for irrigation during the study period; n = 55.

Parameter	DBW	TWW
TSS mg L ⁻¹	1.4 ± 3.0	21.8 ± 23.6 *
pH	6.8 ± 0.4	7.7 ± 0.8 *
EC dS m ⁻¹	0.3 ± 0.1	1.8 ± 0.3 *
SAR (meq L ⁻¹) ^{0.5}	12.7 ± 4.8	11.7 ± 1.6
Ca ²⁺ mg L ⁻¹	0.8 ± 0.4	22.0 ± 5.0 *
Mg ²⁺ mg L ⁻¹	1.0 ± 1.6	14.6 ± 6.7 *
K ⁺ mg L ⁻¹	1.1 ± 0.9	34.5 ± 3.7 *
Na ⁺ mg L ⁻¹	61.3 ± 27.0	286.7 ± 74.2 *
Cl ⁻ mg L ⁻¹	74.3 ± 28.0	442.1 ± 115.6 *
B mg L ⁻¹	2.0 ± 0.4	0.9 ± 0.2 *
N-NH ₄ ⁺ mg L ⁻¹	0.3 ± 0.4	3.5 ± 6.3
N-NO ₃ ⁻ mg L ⁻¹	1.0 ± 0.2	5.6 ± 6.2
S-SO ₄ ²⁻ mg L ⁻¹	3.4 ± 1.2	25.6 ± 8.0*
P-PO ₄ ³⁻ mg L ⁻¹	0.2 ± 0.3	4.9 ± 2.8
COD mg L ⁻¹	4.6 ± 6.4	50.5 ± 25.1*
BOD mg L ⁻¹	2.7 ± 5.2	10.7 ± 10.1*
<i>E. coli</i> (cfu 100 ml ⁻¹)	nd	0–12000 ^a

Asterisks denote significant differences (p < 0.05) between water qualities.

TSS, total suspended solids; SAR, sodium absorption ratio; COD, chemical oxygen demand; BOD, biological oxygen demand.

^a Range of colony forming units.

from 2.7 mm day⁻¹ (December) to 9.2 mm day⁻¹ (August). ETo was calculated from the Penman-Monteith-FAO model using data obtained from an on-site weather station. Fertilization consisted of an initial 250 g plant⁻¹ application of NPK granulated fertilizer (15:15:15) at the plant base, and two additional fertigation applications equivalent to 38 g of 20:5:5 NPK, and 75 g of 15:15:15 NPK per plant along the cultivation period.

2.2. Soil sampling and analysis

A total of 48 topsoil samples (0–20 cm depth; below a drip emitter in SDI; above a drip emitter in SSDI) were collected in February of 2016 (one week after ending of water application and immediately before cropping removal). In each experimental plot of both fields (TT and TH) two soil samples (each of them combination of three subsamples) were taken at random per experimental plot (n = 48).

Prior to physicochemical and biochemical analysis, all soil samples were air-dried and passed through a 2 mm sieve. In this regard, air-drying of soil samples has been found to be adequate for soil under Mediterranean climate (and other environments), as reported by Zornoza et al. (2007), Moreira et al. (2017) and Bueis et al. (2018), who found that microbial biomass content and enzyme activities determined on air-dried samples were representative of those detected using field-moist soil samples. Furthermore, in areas undergoing drought and extremely high evaporation, such as Fuerteventura Island, microorganisms may be physiologically adapted to seasonal dryness (Zornoza et al., 2007). For this study, soil samples were taken one week after the end of irrigation, a period long enough to allow the drying of the topsoil, under the particular climatic conditions in Fuerteventura (see above).

Some physicochemical parameters were measured in soil samples, following the Standard Methods (Soil Survey Staff, 1996) including soil particle size distribution, electrical conductivity and pH in saturated paste extract (ECes; pHes), oxidizable soil organic carbon (SOC), soil labile organic carbon (SLOC), total nitrogen (TN), available phosphorus (Av. P), and equivalent calcium carbonate (CaCO₃).

The potential activities of esterase, catalase, alkaline phosphatase, β-glucosidase, urease, protease and dehydrogenase were determined in aqueous soil suspensions (1:25 soil: distilled water, w:v) using an orbital shaker for 30 min at room temperature (~20 °C) (Sanchez-Hernandez et al., 2017). All enzyme activities were measured by discontinuous kinetic assays, and the products of the respective reactions were read in an Asys HiTech UVM340 plate reader (Asys HiTech GmbH, Eugendorf, Austria). Blanks (sample-free reaction media) were included in each batch to discount non-enzymatic generation of the reaction products. Calibration curves were made including soil-water suspensions to correct the potential sorption of substrate or product to soil particles.

Esterase (EC 3.1.1.1) activity was determined following the method by Sanchez-Hernandez et al. (2017). Catalase activity (EC 1.11.1.6) was measured following the method described by Trasar-Cepeda et al. (1999). Alkaline phosphatase (EC 3.1.3.1) and β-glucosidase (EC 3.2.1.21) activities were determined according to the spectrophotometric methods by Deng et al. (2013), with slight modifications described in Sanchez-Hernandez et al. (2017). Protease activity (EC 3.4.21.92) was measured according to Schinner et al. (1996), but the enzyme assay was run in 1.5-ml microfuge tubes. Urease activity (EC 3.5.1.5) was measured following the unbuffered method by Schinner et al. (1996). The activity of dehydrogenase was measured according to von Mersi and Schinner (1991), and using iodinitrotetrazolium chloride (INT) as the substrate.

2.3. Data analysis

The properties of both water sources were compared by means of a Student t-test, or a Mann-Whitney U test in case of non-normal distribution (Kolmogorov–Smirnov test) and/or heteroscedasticity (Levene test). Soil variables were transformed if necessary, in order to fit a

normal distribution and a general linear model (GLM) univariate analysis was used to determine the effect of soil type (TT vs. TH), water quality (DBW vs. TWW) and irrigation system (SDI vs. SSDI). Pearson's correlation coefficients were calculated to check for significant relationships between enzyme activities and soil chemical parameters. Principle components analysis (PCA) was carried out on normalized variables to examine ordination and distribution of enzyme activities and chemical parameters with regard to applied treatments. Statistical methods were implemented using SPSS (version 25.0), with the significance level set at $p \leq 0.05$.

The numerical index IBRv2, proposed by Sanchez et al. (2013), was applied to elucidate the impact of the irrigation on the global response of enzymes. This index was originally developed to assess toxic effects of polluted environments on organisms, and has recently been used in the assessment of pesticide impact on soil enzyme activities (Sanchez-Hernandez et al., 2017). The IBRv2 index was calculated using all enzyme activities as follows:

$$IBRv2 = \sum_{i=1}^n |A_i|$$

where A_i represents a deviation value for each enzyme activity respect to that measured in the control soil (soils irrigated with DBW using SDI; mean value of enzyme activity set to 0). The A_i values were calculated using the equation:

$$A_i = \left[\frac{\text{Log} \left(\frac{X_i}{X_0} \right) - \mu}{\sigma} \right] - Z_0$$

for each soil enzyme activity, single data (X_i) were divided by the corresponding reference value (X_0) and logarithmically transformed to reduce the variance (Sanchez et al., 2013). The log-transformed data are standardized considering the general mean (μ) and the standard deviation (σ). Then, the mean of the standardized enzyme activity is subtracted from the mean of the reference standardized data (Z_0), to obtain the enzyme activity deviation index (A_i), whose values can be plotted in a radar graph to visually check for inhibition (negative A_i or area in the radar plot below the reference level) or induction (vice versa) of any given enzymatic activity. Finally, the IBRv2 value is calculated as the sum of the absolute values of the deviation indexes ($|A_i|$). The results can range between 0 and 24, with higher IBRv2 scores addressing greater deviations in global soil enzymatic activity with regard to the chosen reference treatment.

3. Results

3.1. Soil chemical parameters

Average and standard deviation values of the assessed soil chemical properties from TT and TH soils under different irrigation treatments are shown in Fig. 1, whereas Table 2 displays the results of the GLM analysis. Both soil salinity and SLOC contents were significantly higher in the TH soil. Conversely, pH levels were higher in the TT soil (Table 2). Water quality had a significant effect in EC and available P, that were higher in soils irrigated with TWW (average EC: 6.8 dS m⁻¹ under irrigation with TWW vs. 2.8 dS m⁻¹ with DBW; average P Olsen: 47.0 mg kg⁻¹ under irrigation with TWW vs. 23.4 mg kg⁻¹ with DBW). Save for the contents in CaCO₃, all soil properties were significantly affected by the irrigation system, being SOC, SLOC, TN and available P levels higher under SDI (Table 2). For example, average SOC content was 7.3 g kg⁻¹ in soils with SDI, whereas in soils with SSDI was 4.5 g kg⁻¹. The opposite happened for EC, where highest values were found under SSDI system (EC average: 5.8 and 3.7 dS m⁻¹ for SSDI and SDI soils respectively). The salinity levels reached in soils irrigated with TWW using SSDI were particularly high, up to 15 dS m⁻¹. Similarly, soils with SSDI showed higher pH

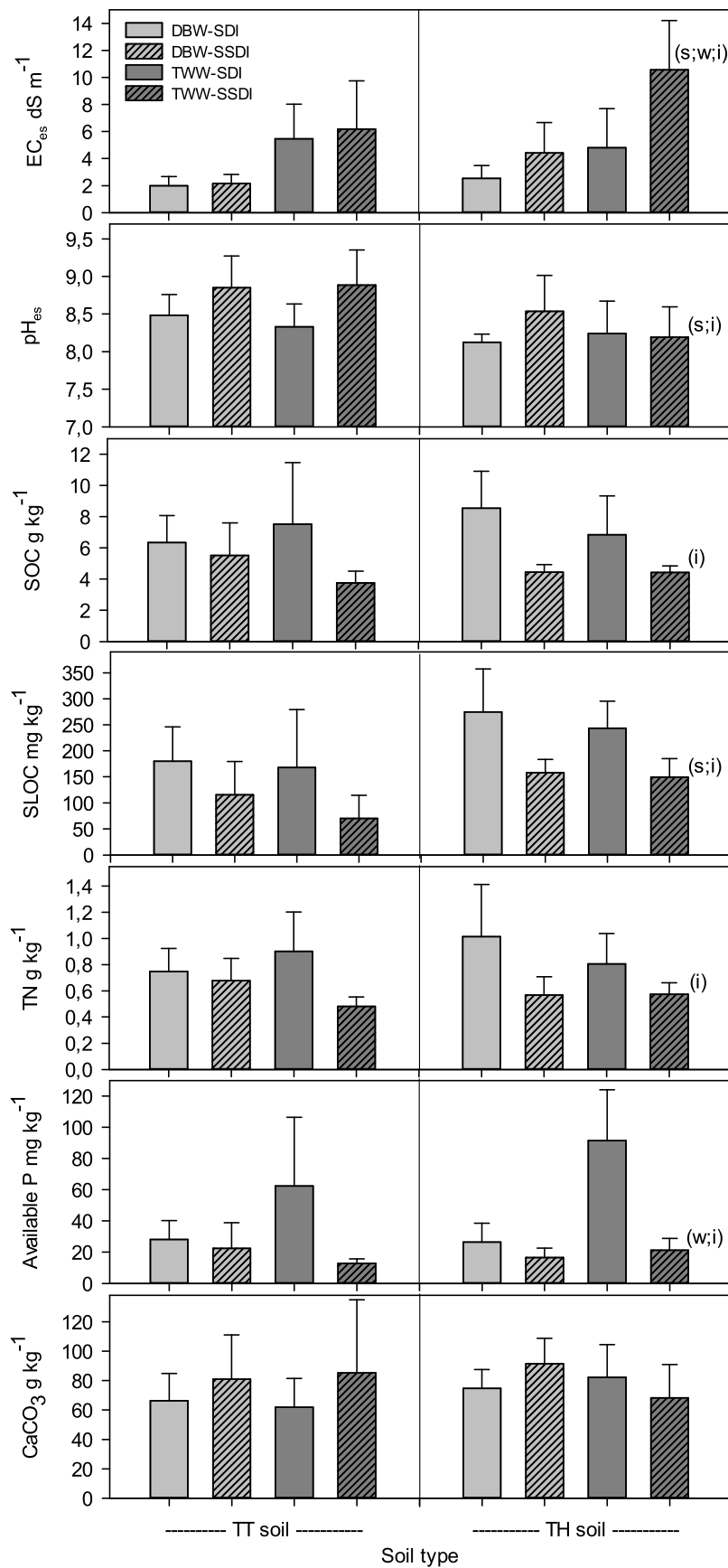


Fig. 1. Top soil (0–20 cm depth) chemical properties in soils after six years under different irrigation treatments (DBW, desalinated brackish water; TWW, treated waste water; SDI, surface drip irrigation; SSDI, subsurface drip irrigation); average ± standard deviation; n = 6; letters s, w and i, denote significant effects of soil type, water quality and irrigation system, respectively, according to a GLM univariate analysis (p < 0.05).

EC_{es}, electrical conductivity in saturated extract; pH_{es}, pH in saturated extract; SOC, soil organic carbon; SLOC, soil labile organic carbon; TN, total nitrogen; Available P, available phosphorous.

Table 2

Results of the GLM univariate analysis comparing chemical properties and enzymes activity in cultivated soils after six years under different irrigation treatments (DBW, desalinated brackish water; TWW, treated wastewater; SDI, surface drip irrigation; SSDI, subsurface drip irrigation); n = 24.

Parameter/ treatment	Soil Type	Water Quality	Irrigation System
Log EC	TH > TT (F = 4.79, p < 0.05)	TWW > DBW (F = 33.23, p < 0.001)	SSDI > SDI (F = 7.19, p < 0.05)
pH	TT > TH (F = 10.22, p < 0.005)	NS	SSDI > SDI (F = 8.56, p < 0.01)
Log SOC	NS	NS	SDI > SSDI (F = 27.84, p < 0.001)
SLOC	TH > TT (F = 14.95, p < 0.001)	NS	SDI > SSDI (F = 24.37, p < 0.001)
Log TN	NS	NS	SDI > SSDI (F = 26.11, p < 0.001)
Log P Olsen	NS	TWW > DBW (F = 10.45, p < 0.005)	SDI > SSDI (F = 40.80, p < 0.001)
CaCO ₃	NS	NS	NS
Esterase	NS	DBW > TWW (F = 21.00, p < 0.001)	SDI > SSDI (F = 30.69, p < 0.001)
Phosphatase	NS	NS	SDI > SSDI (F = 26.39, p < 0.001)
Log Glucosidase	NS	DBW > TWW (F = 11.83, p < 0.01)	SDI > SSDI (F = 22.07, p < 0.001)
Urease	NS	NS	SDI > SSDI (F = 8.71, p < 0.05)
Protease	TH > TT (F = 14.99, p < 0.001)	NS	SDI > SSDI (F = 4.28, p < 0.05)
Catalase	TH > TT (F = 24.77, p < 0.001)	NS	SSDI > SDI (F = 15.24, p < 0.001)
Dehydrogenase	TT > TH (F = 4.27, p < 0.05)	NS	SDI > SSDI (F = 6.18, p < 0.05)

GLM: General Linear Model; TT: Typic Torrifluent; TH: Typic Haplocambid; TWW: Treated wastewater; DBW: Desalinated brackish water; NS: Non-significant.

values than soils with SDI (average pH: 8.6 vs. 8.3 respectively) (Table 2).

3.2. Soil enzyme activities

Fig. 2 displays the individual enzyme concentrations in soils under different irrigation treatments. The results of GLM analysis are shown in Table 2. Both protease and catalase activities were higher in the TH soil, whereas dehydrogenase activity was higher in TT soil. No significant differences between both soil types could be found for the other enzymes (Table 2). The effect of water quality proved to be significant only on esterase and glucosidase activities, so that irrigation with TWW led to lower levels as compared with soils irrigated with DBW (thus, average esterase was 1.9 and 2.9 $\mu\text{mol h}^{-1} \text{g}$ dry soil in soils irrigated with TWW and DBW respectively). All enzyme activities were significantly affected by the irrigation system (Table 2), in such a way that, with the sole exception of catalase activity (3.3 vs. 2.5 $\mu\text{mol h}^{-1} \text{g}$ dry soil with SSDI and SDI respectively), the rest of enzymes showed higher levels in soils under SDI.

Fig. 3 shows the IBRv2 values and associated radar plots for TT and TH soils under different irrigation treatments. In both soil types, the combination TWW-SSDI led to the highest deviation in soil enzyme activities with regard to control (IBRv2 scores \sim 9.7 and 8.3 for TT and TH soil respectively). Under that treatment an inhibition in all the assessed enzymes, with the exception of catalase, was observed (Fig. 3). Conversely, irrigation with TWW under SDI induced the lowest deviation with respect to control (IBRv2 scores \sim 2.8 and 4.9 for TT and TH soil respectively) in both soils, being more evident in the TH soil, where most enzymes were inhibited, with the exception of urease and

dehydrogenase (Fig. 3).

Multivariate analyses (PCA) were performed to check correlations among all the properties under study in both soil types (Fig. 4). In this analysis the percentages of variance explained by the first two components (axes I and II) were 71.5 and 63.4% for TT and TH soil respectively, indicating that the conclusions derived from the 2-dimensional figures are representative of the true ordination of the soil properties. For TT soil the variables with the greatest positive weight in component I were SOC and SLOC (score = 0.936 and 0.933, respectively), whereas those with the greatest negative weight were pH and catalase activity (scores = -0.583 and -0.304 , respectively). For TH soil the variables with the greatest positive weight in component I were also SOC and SLOC (score = 0.963 and 0.947, respectively), whereas those with the greatest negative weight were catalase activity and CaCO₃ (scores = -0.430 and -0.243 , respectively). Fig. 4 shows positive correlation between SOC, SLOC and TN with most of the enzyme activities, excluding catalase that appears to be positively correlated with soil salinity.

4. Discussion

In extremely arid territories, such as Fuerteventura Island, soils can be naturally affected by several degradation processes as salinization, sodification/alkalization, and low organic matter and nutrients content, all of them related to the lack of water and high evaporation rates. In these circumstances, an appropriate selection of alternative, non-freshwater sources and irrigation technology is needed, so as to guarantee the long-term sustainability of agricultural production and soil quality. Under Mediterranean conditions, SSDI has been shown to be quite efficient towards water use, root system development, yield and product quality (Schmidt et al., 2018). In their comprehensive review, Ayars et al. (2015) provide additional evidence for the goodness of SSDI systems for different crops in central California. However, few of these studies have focused on the effects in top-soil properties under extremely arid conditions and limited marginal water quality supply (\sim 100% ET_{Po}). In our case, no advantage whatsoever was detected when using SSDI, as compared to SDI, in any of the assessed chemical properties (Fig. 1; Table 2). Conversely, soils irrigated with buried drip lines reached the highest salinity values, as well as the lowest contents in soil organic carbon (either total or labile) and nutrients. It therefore seems that, in spite of its efficiency towards water use and uptake, SSDI may be unfavourable towards some soil properties of concern in arid regions, at least at the soil surface. Possible reasons for this could come from the restricted movement of water in SSDI systems (Ayars et al., 2015), capable to induce higher salinity levels along the edge of the wetting bulb (Dorta Santos et al., 2016), that could eventually extend its influence close to the soil surface. Hanson et al. (2008) reported salt accumulations above the drip lines in SSDI, to the extent of making periodic additional leaching treatments necessary to remove the excess of salt. The combined effect of an increasing, evaporation-induced saline concentration and the lack of leaching above the drip lines accounts for the accumulation of salts close to the soil surface under this irrigation system. Such effects become dramatic when salinity in the irrigation water exceeds a certain threshold (Table 1). The lower content in soil organic matter observed under SSDI could also be linked to a heterogeneous root distribution observed in the field, as roots are homogeneously distributed along the entire soil profile under SDI, whereas under SSDI roots are driven to grow below the irrigation line (>20 cm depth) (Dorta-Santos et al., 2016).

Noticeably, no significant increases in SOC, SLOC or TN were found after irrigation with TWW under SDI, as expectable from its chemical composition (Table 1). Similar findings have been reported by other authors after variable irrigation periods with TWW (Morugán-Coronado et al., 2011). A BOD/COD ratio in TWW higher than 0.1 (\sim 0.21 in our study) means that its organic load could be readily decomposed by soil microorganisms (Alguacil et al., 2012). The incorporation of easily

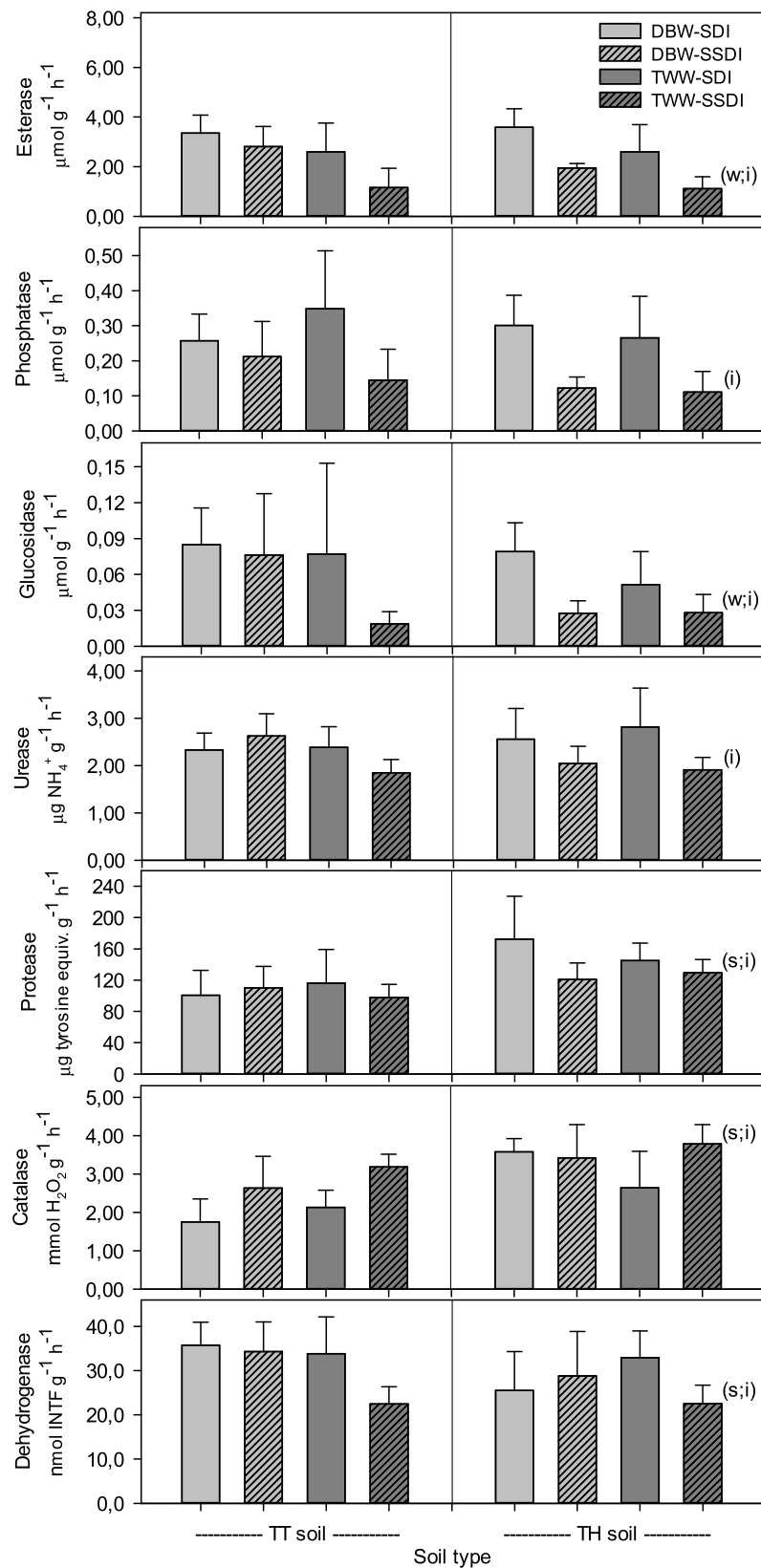


Fig. 2. Top soil (0–20 cm depth) enzymes activity in soils after six years under different irrigation treatments (DBW, desalinated brackish water; TWW, treated waste water; SDI, surface drip irrigation; SSDI, subsurface drip irrigation); average \pm standard deviation; n = 6; letters s, w and i, denote significant effects of soil type, water quality and irrigation system, respectively, according to a GLM univariant analysis ($p < 0.05$).

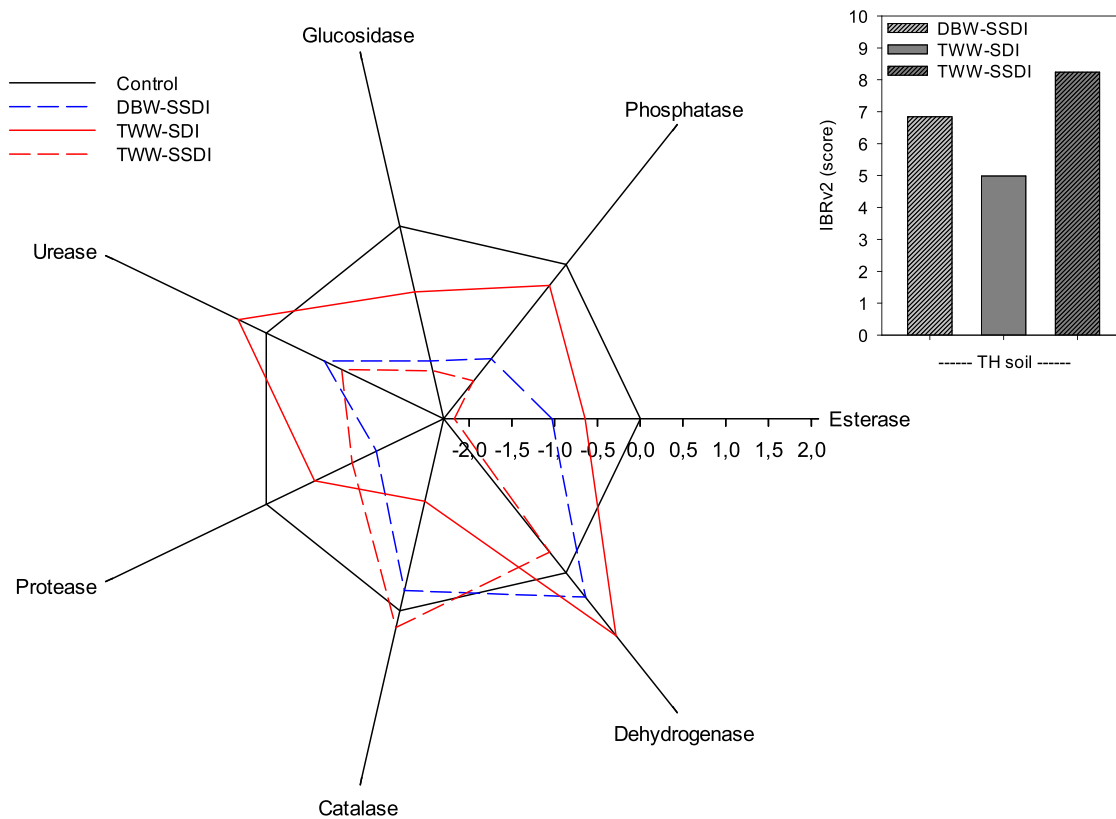
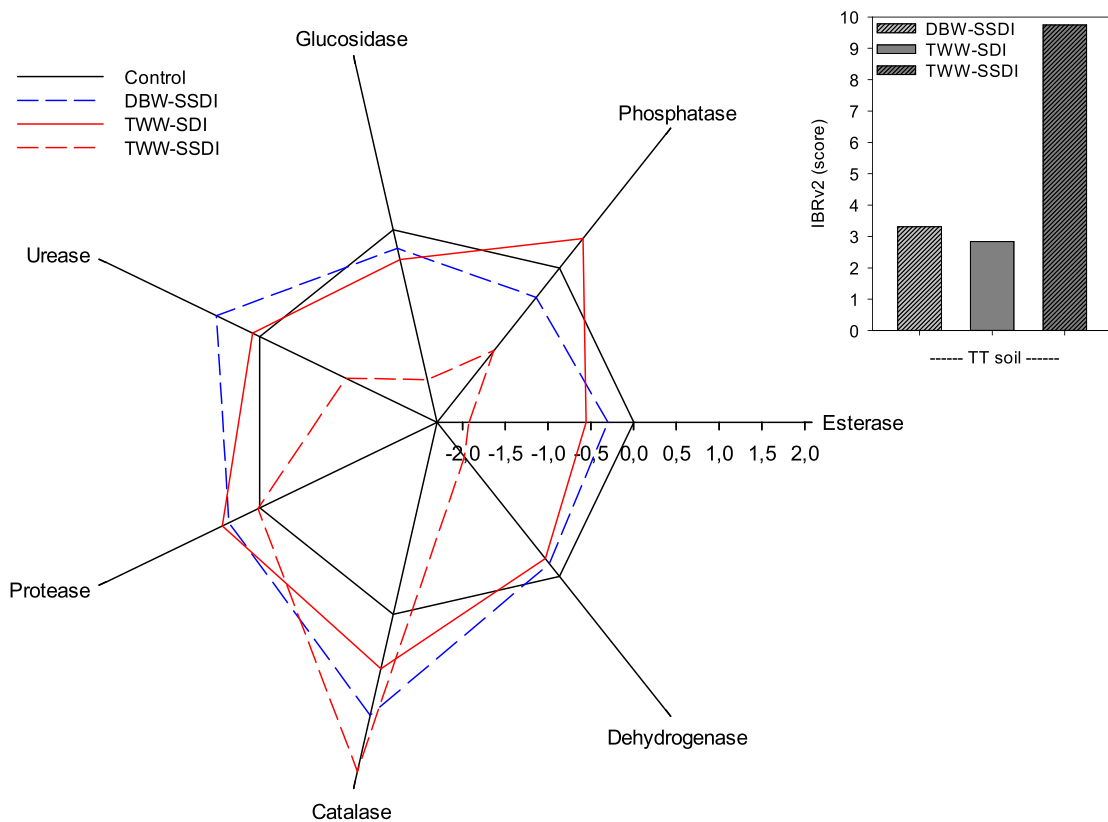


Fig. 3. IBRv2 values and associated radar plots from soils after six years under different irrigation treatments (TT, Typic Torrifluent; TH, Typic Haplocambid; DBW, desalinated brackish water; TWW, treated wastewater; SDI, surface drip irrigation; SSDI, subsurface drip irrigation).

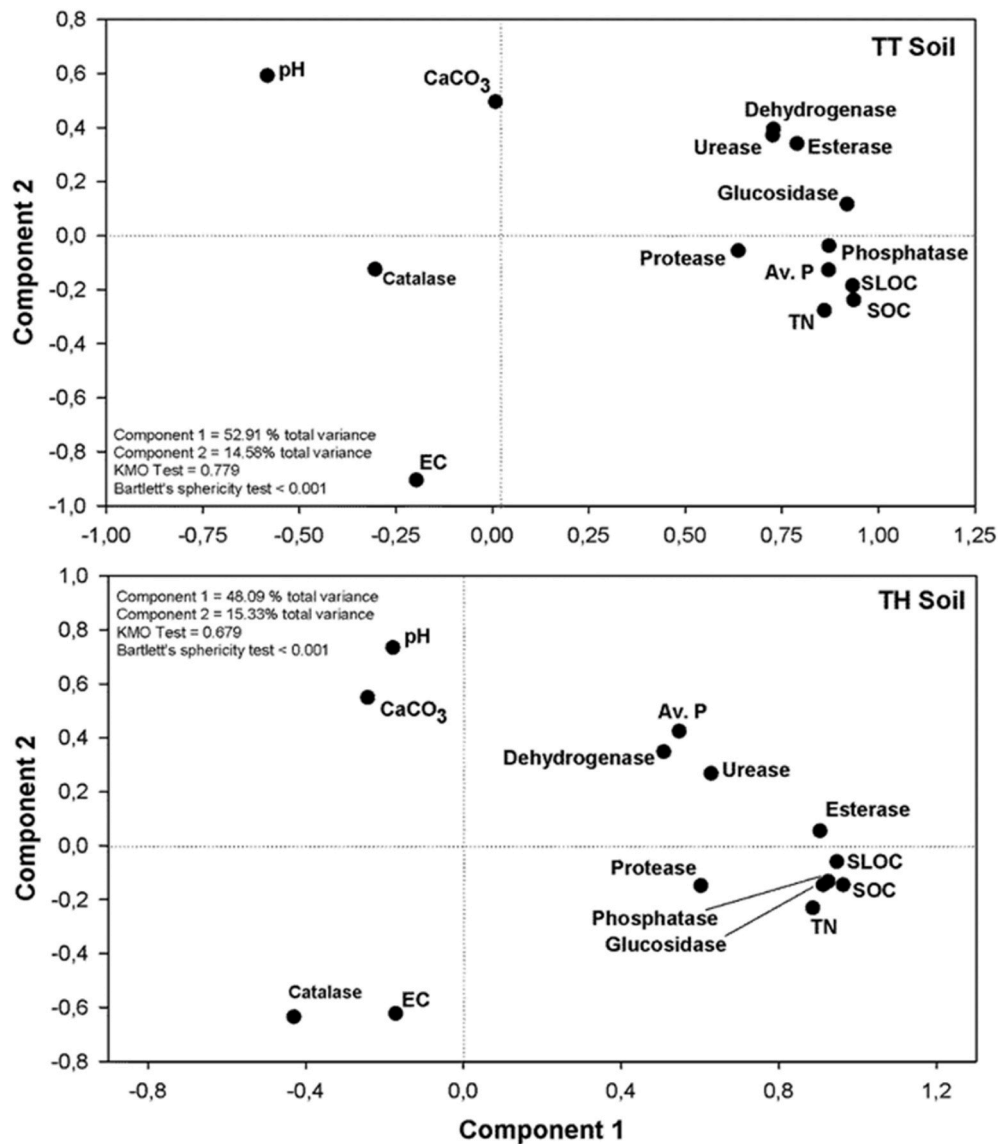


Fig. 4. Soil chemical parameters and enzyme activities ordination from top 0–20 cm layer in TT and TH soils under different irrigation treatments using PCA.

degradable organic substances via TWW may therefore increase both the microbial population and microbial activity and, consequently, organic-matter mineralisation (Chen et al., 2011). In the case of TN, the addition represented by TWW use (Table 1) can be offset by nitrogen leaching below the root zone, losses to volatilisation and crop removal (Bar Tal, 2011). Conversely, soil available phosphorous increased significantly in both soil types after irrigation with TWW under SDI (Fig. 1). Other authors have found similar results in carbonate-bearing soils, such as those in this study, attributing that increase to the mobilization of phosphorous linked to soil carbonates as a result of the combined action of microbial biomass and the dissolving power of TWW (Díaz et al., 2013).

The values for the activities of extracellular enzymes measured in this work are comparable to those reported by other researchers in semiarid/arid regions worldwide. For example, alkaline phosphatase and β -glucosidase activities have been found to be as low as 0.08–0.33 $\mu\text{mol PNP g}^{-1} \text{ soil h}^{-1}$ in soil rhizosphere in SE Spain (Rodríguez-Caballero et al., 2017), whereas Akhtar et al. (2018) reported activity values for alkaline phosphatase ranging between 0.12 and 0.36 $\mu\text{mol PNP g}^{-1} \text{ soil h}^{-1}$ in soils of NW China. As far as urease activity is concerned, values in soils of arid regions have been commonly reported to be lower than 1 $\mu\text{mol NH}_3/\text{NH}_4^+ \text{ g}^{-1} \text{ soil h}^{-1}$, as can be seen in García

et al. (2005) and Rodríguez-Caballero et al. (2017). Unlike hydrolases, which are mostly released to soil solution, dehydrogenase activity is mostly intracellular and related to oxidative phosphorylation reactions, and hence it has been regarded as an excellent surrogate for soil microbial activity in semiarid Mediterranean ecosystems (García et al., 2005; Bastida et al., 2008; Rodríguez-Caballero et al., 2017). The values reported elsewhere for dehydrogenase activity in soils from arid regions usually exceed 100 $\mu\text{g INTF g}^{-1} \text{ soil h}^{-1}$, whereas in this study they are invariably below 50 $\mu\text{g INTF g}^{-1} \text{ soil h}^{-1}$ (Fig. 2), thus suggesting a limited soil microbial activity. Differences observed between soil TT and TH in some enzyme activities (e.g. protease, catalase, dehydrogenase), and discrepancies in treatment effects among them can be due to soil characteristics such as texture, water holding capacity, salinity, soil reaction and/or nutrients content.

Conversely to our findings, several authors have reported enhancements in soil enzyme activities after a long period (10–43 years) of irrigation with TWW (Chen et al., 2008; Alguacil et al., 2012; Adrover et al., 2017), linking this beneficial effect to the addition of nutrients and easily-decomposable organic matter. Our results show a tight relationship between soil organic matter and nutrients and the activity of most soil enzymes, thus indicating that organic matter enhanced and prompted the microbial activity. When comparing TWW-SDI with the

control treatment (DBW-SDI), an increase in some enzyme activities (e.g. urease) can be observed, whereas the rest of enzymes are inhibited, which suggest that other factors can counteract the positive effect associated to the organic load in TWW to a different extent in both soil types. After joining soil enzymatic activities in a single value (i.e., enzymatic index), it becomes clear that irrigation with TWW, and the use of SSDI have led to a generalized decrease in the top-soil biological activity, with regard to the control treatment (DBW-SDI). Three main factors could contribute to this. The first one is EC, as increases in soil salinity have been shown to be detrimental for dehydrogenase, β -glucosidase, urease, protease and alkaline phosphatase activities (Singh, 2016), due to changes in soil osmotic potential and specific ion toxicity. Garcia et al. (2000) stated that such decreases could be due to a 'salting-out' effect, involving a decrease in enzyme solubility through dehydration, thus altering the enzyme 'catalytic site', as well as salinity-induced proteolysis. In the present study, increases in soil salinity were observed under SSDI, even if DBW was applied, although only in the clayey (TH) soil, where an upward, evaporation-induced movement of water and salts may be favoured by soil capillarity. The second reason may be the soil water content, because topsoil gets dry under extremely arid conditions shortly after water application ceases, especially under intended irrigation deficit and SSDI. Many soil microbial activities are negatively affected by these changes in water potential (Setia and Marschner, 2013), whereas osmotic stress limits the microbial growth and activity, causes dehydration and prompts cell lysis in the less tolerant microorganism groups (Singh, 2016). Soil drying would have even a more intense negative effect in saline soils than in non-saline ones, suggesting that limited microbial biomass in saline soils cannot tolerate low osmotic potential (Setia and Marschner, 2013). Finally, we should also consider soil pH, as long as it directly affects the activity of extracellular soil enzymes (Sinsabaugh et al., 2008). High pH values usually inhibit microbial growth and correlate negatively with some enzymatic activities such as protease (Singh, 2016). Our results show lower pH values under SDI, more closely reflecting the pH of irrigation water than soils under SSDI. Additionally, although not afforded in this study, adverse effects on soil microbial activities can be associated to the presence of harmful trace elements and organic constituents, such as pesticides and polycyclic aromatic hydrocarbons in the reclaimed wastewater (Chen et al., 2008).

The particular case of catalase activity deserves special attention. This enzyme is released when high levels of reactive oxygen species occur, so as to prevent damages to DNA, proteins and lipids (Kaushal et al., 2018). Therefore, a high catalase activity is usually interpreted as a response to oxidative stress. In some instances, catalase activity has also been related to a high metabolic rate of soil microbiota that leads to high respiration, whereby it correlates with extracellular, hydrolytic enzymes, as observed by Akhtar et al. (2018). In this study, catalase activity correlated negatively with the other measured enzymatic activities, and hence its relative placement in both PCA plots, opposite to them, as well as to organic carbon and nutrients content (Fig. 4). Conversely, catalase activity displayed a positive relationship with soil pH and EC, which suggests the possibility of enhanced oxidative stress associated with alkaline pH and high soil salinity. The response of catalase activity towards soil salinity has been suggested to vary according to factors such as soil type, chemical composition of the irrigation water, and soil microbiota, being either positive (Huang et al., 2019) or negative (Shi et al., 2019) in different scenarios.

5. Conclusions

The agricultural development in extremely arid environments requires alternative practices based on the use of marginal water sources such as urban reclaimed water. Since these resources usually have a lower quality than freshwater, appropriate selection of irrigation technology should be assessed in order to guarantee the long-term sustainability of agricultural production in terms of soil quality. Under the

experimental conditions of this study (high evaporative demand/drip irrigation with TWW/and water dose applied \sim 100% ETPo) it could be concluded that SSDI system represents no advantage as compared to SDI in terms of top-soil quality. Conversely, it threatens the sustainability of the agrosystem, prompting mid-term soil degradation. The global inhibition of soil enzyme activity observed in this study cannot be attributed to a unique factor; instead, it is more likely to be due to a combination of them, mainly build-up of salts and soil drying. Although the effects of soil use and management on soil enzymes have been widely afforded in the literature, little attention has been paid to how microbial activities and soil enzymes are influenced by salinity and moisture content. Due to the relevance of soil salinization processes in arid and semiarid lands that should be a priority research line in future works.

In this study soil enzymatic indexes have been proved to be quite sensitive to changes in arid soils, detecting soil mild changes before the onset of an advanced environmental degradation. Therefore, enzymatic indexes can be used as indicators, combined with other physicochemical soil properties, to measure degree of irrigated arid soil stresses and for a better understanding of soil biochemical processes. Nevertheless, soil properties spatial heterogeneity linked to drip irrigation systems, e.g. water and dissolved nutrients concentrated in a wetting bulb that affects a relatively small soil volume, while displacing salts only to its periphery, required a more detailed soil sampling than that carried out in this study, in order to globally assess the impact of this irrigation technologies in the long-term sustainability of arid soil health.

CRedit authorship contribution statement

Francisco J. Díaz: Conceptualization, Investigation, Writing - original draft. **Juan C. Sanchez-Hernandez:** Investigation, Writing - review & editing. **Jesús S. Notario:** Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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