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Microbial Activity and Organic Matter Dynamics During 4 Years of Irrigation with Treated Wastewater

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Abstract The global changes in rainfall frequency and quantity have subjected arid and semi-arid regions to long periods of drought. As this phenomenon corresponds to increasing trend of water shortage, the use of treated wastewater (TWW) has been suggested as an alternative for irrigation of agricultural crops in these areas. The aim of the study was to investigate the short- and middle-term effects of TWW irrigation on the soil microbial activities and organic carbon content. The microbial community activity was measured every 1–3 months for 4 years in a persimmon (*Diospyros kaki*) orchard. These activities were used here as an indicator for the soil health. The hydrolysis activity (detected by fluorescein diacetate hydrolysis (FDA) assay) increased during the irrigation season and was significantly higher in soils irrigated with TWW compared to those irrigated with freshwater (FW). This activity was also negatively correlated with dissolved organic carbon (DOC) concentrations during the irrigation season, suggesting that the community degraded the DOC in the soils regardless of its origin. The irrigation season was also characterized by an increase in nitrification potential in both TWW- and FW-irrigated soils, which coincided with high concentrations of nitrate (50 mg kg^{-1} soil). Overall, there was an increase in all measured activities during the irrigation season, and they were higher in the TWW soils. However, it appears that after each irrigation season, the potential activity of the community returned to levels similar to or even slightly lower than those of FW-irrigated soil during the wet season, suggesting that the periodic irrigation did not significantly change the soil microbial activity.

Introduction

Irrigation of agricultural land with treated or untreated wastewater (WW) has been a widespread practice used for either WW disposal [1, 2] or as a renewal water source in response to global change in rainfall quantities and distribution [3–5]. However, this practice may introduce various potential risks that could hamper soil health and quality and as a consequence its fertility and suitability for food production [6, 7]. These risks include addition of high levels of minerals [8], dissolved organic carbon (DOC) [9, 10], detergents, and pharmaceuticals [11, 12]. This practice may also introduce pollutants such as heavy metals [10, 13] and xenobiotic compounds [2].

One of the potential outcomes of using treated wastewater (TWW) for irrigation is the long-term addition of external organic carbon (C) and nitrogen (N) to the soils. The increase in organic C and N due to irrigation with TWW has been documented. Total C and N were found to be higher in long-term (almost 100 years) WW-irrigated soils in Germany compared to

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soil that was irrigated with freshwater (FW) or not irrigated at all [1]. Similarly, 90-year application of untreated WW in cropland in Mexico caused an increase of 1.4-fold and 3-fold in organic C and N, respectively [10]. The organic C content of hazelnut orchards is higher in those flooded periodically with urban and industrial WW compared to fields that had never been flooded [14]. In Israel, organic C content in the topsoil of TWW-irrigated soils was demonstrated to be, in general, higher or comparable to that in FW-irrigated soils, but a priming effect and a lower organic C content were found in the subsoil of the TWW-irrigated soils [4]. In other studies, a higher organic C content was observed in WW-irrigated soils [6, 14]. This increase in soil organic C and N content may affect the characteristic of the soil, and as a consequence, its suitability for agricultural crops [15].

Enzyme activities and respiration of the total microbial community are common indicators of soil quality [7, 14]. The general view is that if the level of microbial activities is unchanged or higher than the baseline, the microbial community is handling the change [15]. Friedel et al. [9] showed that increased organic matter resulted in an increase in respiration when total organic C is elevated in Eutric Vertisols soil. A similar increase in respiration was observed in orchard Vertic Xerocrept soils irrigated with TWW [6]. In contrast, Eutric Histosol soils irrigated with municipal WW varying in their chemical composition exhibited lower respiration potential compared to FW-irrigated soils [16]. Various enzymes are used routinely to evaluate the potential activity of the microbial community such as dehydrogenase, catalase, urease, phosphatase, proteinase, and glucosidase. The activity of dehydrogenases is usually higher in soils irrigated with TWW or untreated WW compared to soils that are irrigated with FW [9, 14, 15, 17]. However, irrigation with different concentrations of WW results in different dehydrogenase activity [16]. An increase in the organic load in the soil is also accompanied by an increase in the activity of β-glucosidases [1, 14, 17, 18] and various enzymes involved in the phosphate cycle [1, 15, 18].

While some studies evaluated the accumulative effects on organic C content and microbial activity in soils irrigated with wastewater for long periods of time [1, 9, 17], no information is available regarding the changes that occur during the irrigation period and the following rainy season on an annual basis. The aim of the current study was to describe the short- and middle-term effects of TWW irrigation on the soil microbial activities and organic C content. In order to do so, an experimental plot was set up in a persimmon (*Diospyros kaki*) orchard in the central coastal region of Israel, irrigated with either FW or TWW, and the organic carbon and microbial activities were measured periodically for 4 years.

Materials and Methods

Experimental Setup and Environmental Parameters

A field experiment was conducted in a persimmon (grafted on Vergiana persimmon rootstock) orchard in Atlit located in the central coastal region of Israel (a Mediterranean climate with an average rainfall of 550 mm from mid-November to mid-April) for six successive years (2002–2008). Trees were 10 years old, planted 6×4 m (420 trees per hectare) on a fine clayey, thermic, smectitic, chromic Haploxerert soil with a high stone content, which improves soil aeration and water permeability. Soil texture was: 57.7% clay, 28.6% silt, 13.8% sand, with 3.1% $CaCO_3$, and 38.6 available NO_3-N mg kg^{-1}.

The experiment consisted of two irrigation treatments with two water types: FW and TWW. The TWW were received from the nearby secondary treatment Nir-Etzion WW facility treating rural-domestic WW. The main chemical properties of the two water qualities are presented in Table 1. Treatments were set up randomly in five blocks, and each block consisted of one plot of each treatment (total of 10 plots). In each plot, there were three rows of trees (16–18 trees), with two rows serving as border rows. Soils samples were taken only from the central rows. Irrigation was controlled by an irrigation computer, and applied 2–3 times per week, from April to November. The water dose was calculated according to the evaporation from a class "A" pan and a crop coefficient varying during the growth season. The annual rainfall and actual irrigation amounts are presented in Table 2. Nutrient solution was added to the FW treatment, at a concentration adjusted to provide the same level of N found in the TWW, i.e., approximately 28 mg L^{-1} in an ammonium fertilizer. The ratio of N:P:K in the nutrient solution was: 1:0.3:1 (weight basis). Fertigation was proportional for the whole period of irrigation and was at the recommended concentrations for this crop. The water quality in the TWW was monitored monthly. In the beginning of 2007, the wastewater treatment plant was upgraded, and as a result, the composition of the TWW used for irrigation during the dry season (April to November) was changed, and the average N concentration declined to a level similar to that in FW (Table 1). Following this change, a nutrient solution similar to the FW treatment was also added to the TWW treatment.

Sample Collection

Samples were collected during the years 2004–2008. For microbial activity and organic matter content, five individual soil samples of 200 g were collected from the upper, most active, 5-cm layer under five individual

Table 1 Chemical characteristics of the freshwater, treated wastewater, and upgraded treated wastewater used for irrigation in the current study

Parameter	Units	Freshwater	Treated wastewater	Upgraded treated wastewater
BOD	mg L^{-1}	–	25–80	5–10
COD	mg L^{-1}	0	173–300	52
pH		7.4–7.6	7.1–7.5	7.4
EC	dS m^{-1}	0.7–0.9	1.8–2.2	1.5
SAR	(mEq L^{-1})$^{1/2}$	3.2–3.9	4.6–5.4	3.3
P	mg L^{-1}	0	5.0–11.0	3.5
K	mg L^{-1}	0	40–60	31.7
Cl	mg L^{-1}	165–300	300–365	308
Na	mg L^{-1}	126–150	207–252	184
Ca	mg L^{-1}	120–160	106–212	144
Mg	mg L^{-1}	36–48	42–85	53
N-NO$_3$	mg L^{-1}	5–10	0.1–1.7	0.1–1.0
N-NH$_4$	mg L^{-1}	0	25.0–40.0	5.0–7.5
Total N	mg L^{-1}	5–10	30–48	7.0–10.0
B	mg L^{-1}	0.09	0.16	0.16

BOD biological oxygen demand, *COD* chemical oxygen demand, *EC* electric conductivity, *SAR* sodium adsorption ratio

For the treated wastewater, a range is given as measurements were taken over the first 3 years. For the upgraded treated wastewater, most parameters were measured once, and therefore, a single value is given

drippers from each plot, mixed, and transferred to the lab at ambient temperature. For analysis of organic carbon, samples were frozen and kept in −20°C until further analysis was carried out. Microbial enzyme activity was analyzed on the fresh samples shortly after collection. During the first year (2004), only four sampling were performed as a preliminary step to estimate feasibility of the methodology. In the following years (2005–2007), samples were collected every 1–3 months, and on some occasions, twice a month. The mineral nitrogen concentration of the top soil layer (0–5 cm depth) was measured twice a year, in the spring (April–May) after the rainy season and in the fall (October–November) at the end of the irrigation season. Samples were collected from under the drips in three locations in each plot, and were mixed together to yield a composite sample.

Carbon and Nitrogen Species Content

Organic carbon fractions were determined according to Tarchitzky et al. [19]. Humic substances were extracted from the soil according to the procedure recommended by the International Humic Substances Society [20]. For DOC, water extracts were prepared by shaking dry soil with deionized water in a 1:10 soil:water weight ratio for 2 h. The suspension was then centrifuged at 10,000×*g* for 30 min, filtered through Whatman 42 paper and subsequently through a 0.45-μm membrane filter (Supor-450, Gelman Sciences, Ann Arbor, MI). The concentration of DOC was determined, after acidification to pH 5 on a Carlo Erba Total Carbon analyzer. In order to determine the mineral N, soil samples were air-dried and extracted with 1 M KCl (1:5, *w/w*) for NH$_4^+$ and NO$_3^-$ [21] following [22]. The extracts were analyzed using an autoanalyzer (Quickchem 8000, Lachat Instruments, Milwaukee, WI).

Table 2 Amounts of rainfall and irrigation (in millimeter) during a long-term experiment in a persimmon orchard

Year	2004	2005	2006	2007
Rainfall[a]	738	458	595	702
Irrigation with freshwater	894	838	769	641
Irrigation with TWW	906	812	590	610

The wet season in Israel is between November and March. Irrigation was used between March–April and November

[a] Rainfall data were received from the Israeli Meteorological Service

Potential Microbial Activity Assays

Respiration was measured by the titration technique (Schinner et al., 1996). Soils were incubated at 30°C for 24 h with 1 N NaOH trap, followed by acid titration. Soil oxidative potential was estimated by dehydrogenase (DEH) activity with the substrate 2,3,5-triphenyltetrazolium chloride (TTC) [23]. Six grams of soil was added to 2.5 mL of 8% CaCO$_3$ and 1 mL of 3% TTC, and the samples were incubated in the dark, for 24 h, at 37°C. The resulted formazan was measured by a spectrophotometer at 485 nm. Fluorescein diacetate hydrolysis (FDA) assay was used to estimate the hydrolytic activity by adding 10 μg/ml FDA in a 60-mM sodium phosphate buffer (pH 7.6) and incubating at 27°C for 30 min. The amount of the hydrolyzed FDA was measured by a spectrophotometer at 494 nm [24]. Nitrification potential was evaluated by potential NH$_4^+$ oxidation using the shaken slurry method [25]. Twenty grams of soil was added to 90 mL of phosphate buffer (pH 7.2). $(NH4)_2SO_4$ and $KClO_4$ were added to a final concentration of 0.25 and 1 M, respectively. The mixture was shaken for 8 h and sampled every 2 h. Nitrate concentrations were determined using an autoanalyzer (details given above).

Data Analysis

Each of the activities was measured in five separate subsamples for each treatment. For each activity, the results were subjected to analysis of variance (ANOVA) with JMP 7.0 software (SAS Institute, 2005), to obtain an F value for significance ($p<0.005$) in the two-way linear model, with irrigation type, month, and season and their interactions as main effects.

Results

Four years of TWW irrigation of persimmon orchard did not affect tree development and fruit yield compared to trees irrigated with FW (data not shown). However, some influences on the organic C content and microbial activity were recorded. Four microbial activity assays were used to assess the level of activity of the total microbial community throughout the experiment. The dehydrogenase (DEH) assay and potential respiration provide an indication of the oxidative potential and overall respiration, respectively. Activity by FDA indicates the degree of degradation of complex organic compounds, and nitrification potential evaluates the oxidation of NH_4^+ which is common in fertilized soils as well as in soils irrigated with TWW [10]. In each activity assay, the microbial community followed a similar pattern in the two irrigation treatments (Figs. 1 and 2a). However, according to a two-way ANOVA test, the levels of the activities were significantly different ($p<0.005$, probability $F<0.0001$) from each other in the two water qualities in relation to the date of sampling.

Several observations can be noted for each of the tested microbial activities, regardless of the water type used for irrigation. High DEH activity was detected in the fall of 2005, summer 2006, and winter 2007 (Fig. 1a). However, there was no seasonal effect on DEH activity throughout the entire 4 years of sampling. In addition, respiration rates did not exhibit clear seasonal trend, although a spring peak was observed in 2005, as well as peaks in the falls of 2006 and 2007 and winter of 2007 (Fig. 1b). In contrast, the hydrolytic activity and nitrification potential exhibited more distinctive seasonal patterns (Figs. 1c and 2a, respectively). FDA activity was usually higher in the summer–fall and lower during winter time (Fig. 1c). In couple of cases, the peak in FDA activity in the TWW-irrigated soil was delayed compared to the FW-irrigated soil (in 2004 and 2006). From the second year of the experiment, a pattern was observed for the nitrification potential as well. In 2005, 2006, and 2007, peaks were observed in both summer and fall seasons (Fig. 2a).

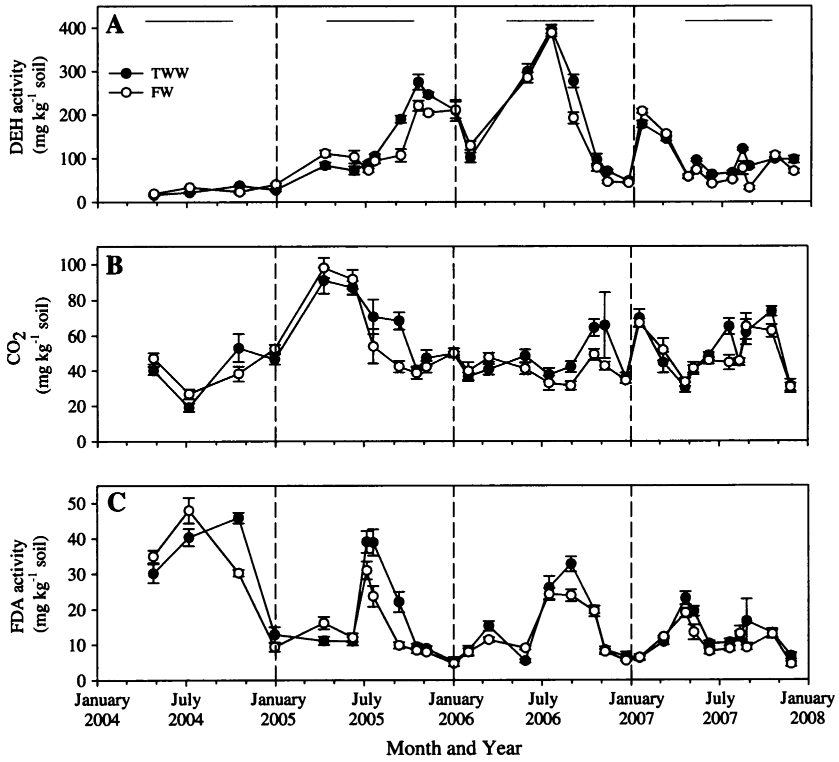


Figure 1 Potential activity of the microbial community in soil irrigated with either freshwater (*FW*) or treated wastewater (*TWW*). **a** Dehydrogenase. **b** Respiration by CO_2 emission. **c** Hydrolytic activity by fluorescein diacetate assay. Soil was collected from an experimental plot of a persimmon orchard in the north of Israel over a period of 4 years of sampling. The *error bars* of five replicates are indicated. *Lines* in **a** indicate the irrigation season

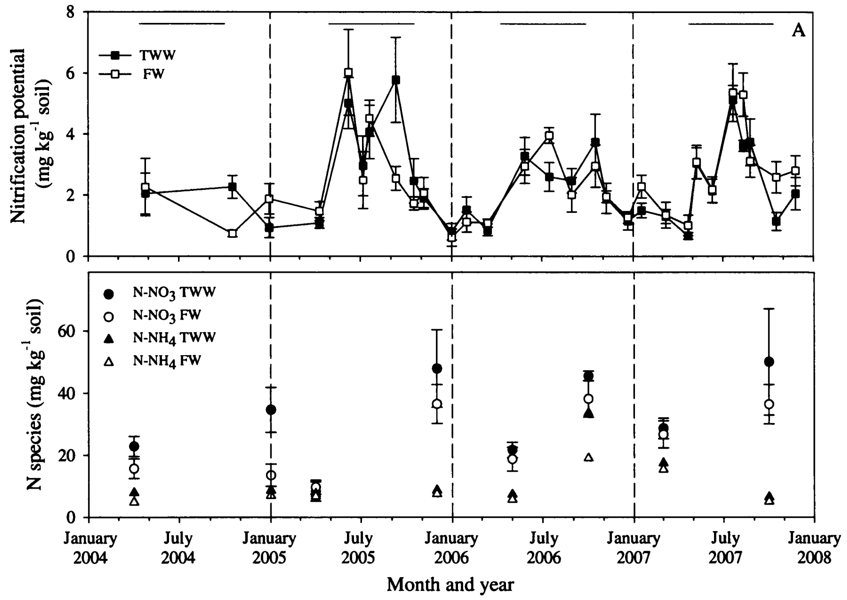


Figure 2 Nitrification potential (**a**) and nitrate and ammonium concentration (**b**) in soil. The nitrification potential activity was measured continuously throughout the 4-year experiment. The nitrate and ammonium were measured at the beginning (April–May) and end (November–December) of each irrigation season of each year. The *error bars* of five replicates are indicated. *Lines* in **a** indicate the irrigation season

The concentrations of NH_4^+ and NO_3^- were measured at the beginning and at the end of each irrigation season. While NH_4^+ concentrations usually did not change during the irrigation season, the NO_3^- concentration at the end of irrigation season (of 35–50 mg N kg^{-1} soil) was higher than at the beginning (10–30 mg N kg^{-1} soil) (Fig. 2b). As with the microbial activities, the overall trends of NO_3^- concentrations were similar for soils irrigated with either TWW or FW, but usually, the concentration of NO_3^- was higher in the TWW-irrigated soil. These general patterns of the NO_3^- concentrations were in accordance with the nitrification potential activity; the increase in nitrification activity during the summer and fall, as indicated by the high levels of NO_3^- measured at the end of these periods, matched the higher soil temperatures during this time of the year (Fig. 2).

The respiration and hydrolytic activities of the microbial community depend on the supply of organic material (OM). Therefore, the concentrations of three OM fractions—(1) dissolved organic carbon (DOC), (2) fulvic acids (FA), and (3) humic acids (HA)—were determined in the soils irrigated with either TWW or FW. These three fractions represent the labile, semi-labile, and refractory parts of C in the OM, respectively. Over the 4 years of the experiment, DOC concentrations ranged from 50 to 480 mg C kg^{-1} soil (Fig. 3a). An increase in the levels of DOC was observed from April to August 2005, which corresponded to the irrigation period. This was followed by a decrease until October 2005, and then an overall increase in DOC until the end of January 2006. In the following year (2006), the winter season was characterized by an overall decrease in DOC concentration, while the summer to fall period showed an increase. Except for a few sampling dates in 2004 and 2005, the DOC content of the TWW was higher than in the FW irrigation (Fig. 3a). During the irrigation period in 2007, only a small fluctuation was observed in the DOC concentration, probably due to the upgraded quality of the TWW.

Unlike the DOC, fluctuations of FA and HA concentrations were smaller in amplitude. FA concentration depended on season, while HA was less affected (Fig. 3b, c, respectively), regardless of irrigation type. The fall season was characterized by a peak of FA and HA in both treatment regimes, with the exception of HA in 2006 and 2007. During these years, the peak in HA occurred earlier, during the summer. Similar to the DOC fraction, the first 2 years of the experiment were characterized by higher FA and HA concentrations in the TWW-irrigated soil than in the FW-irrigated soil (Fig. 3b, c). While DOC was almost always higher in the soil irrigated with TWW; from 2005 onward, the difference was less significant for the FA and HA fractions.

DOC concentration in the soil had some correlation to the hydrolysis activity (Fig. 4). As environmental conditions are quite different between seasons, the trends for each season were considered separately. During winter time (no irrigation), the FDA activity was low regardless of the original irrigation water quality, and the DOC concentration was in the range of 100–500 mg C kg^{-1} soil (Fig. 4a). A weak positive correlation was observed between DOC and FDA in both treatments (r=0.40 and r=0.32; p<0.05; in FW and TWW, respectively). During the irrigation period in the summer, a weak negative correlation was observed between DOC and FDA in both treatments (r=−0.41 and r=−0.53, in FW and TWW, respectively; p<0.05). In both seasons, the DOC concentration was higher in the TWW-irrigated soils (Fig. 4).

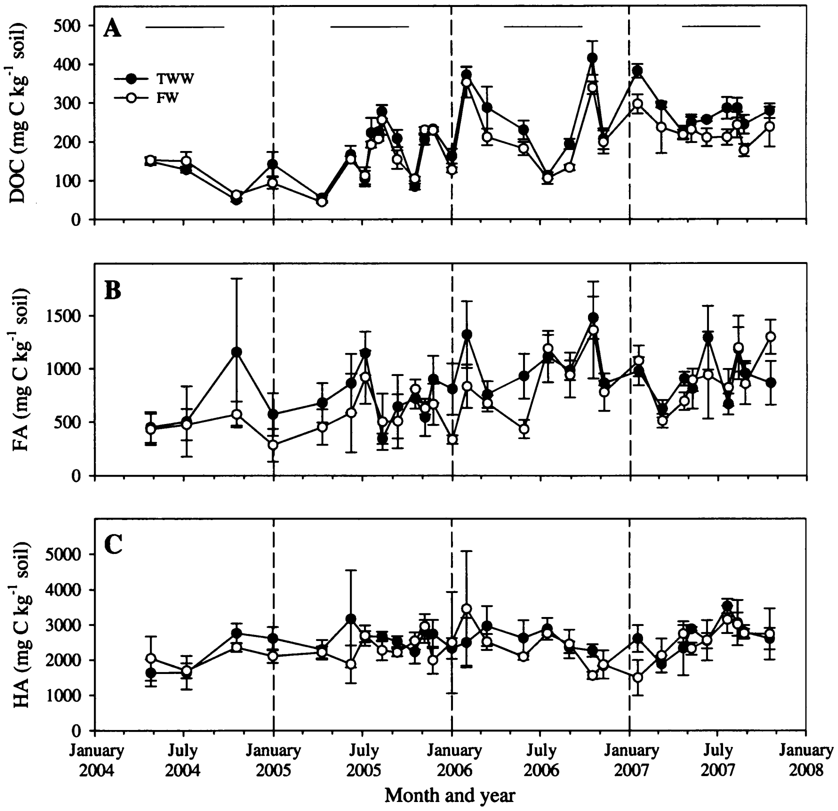


Figure 3 Organic carbon types (DOC, FA, and HA) concentrations (mg C kg^{-1} soil) in soils irrigated with either freshwater (*FW*) or treated wastewater (*TWW*). The *error bars* of three replicates are indicated. *Lines* in **a** indicate the irrigation season

In order to simplify the evaluation of whether the irrigation with TWW had an overall effect on the microbial activities, the ratios between TWW and FW of each activity were calculated (Fig. 5). During winter and early spring, the potential activities were higher or the same in the FW-irrigated plots (values below or equal to 1). During the period of irrigation from May to November of each year, the potential activities were higher in the TWW-irrigated plots (values above 1). A decrease in the ratio occurred after the end of the irrigation season in the winter.

Discussion

The global shortage in freshwater has led to increased use of TWW and untreated municipal wastewater for irrigation of cropland [26]. One parameter to consider in this application is the addition of organic carbon to the soil via the TWW, which may have immediate and long-term effects on soil characteristics and, as a consequence, its productivity and fertility [9]. An increase in DOC, FA, and HA with time was observed regardless of treatment. The absolute increase in DOC concentration was the smallest (from ca. 160 to ca. 270 mg C kg^{-1} soil); the increase in HA was the highest (from ca. 2,000 to ca. 3,000 mg C kg^{-1} soil); and the increase in the FA fraction was intermediate (ca. 700 to 1,300 mg C kg^{-1} soil). This suggests that the more biologically labile organic compounds, represented by DOC and to some extent also the FA, end up in a sink of non-labile fractions, represented by HA.

The fact that TWW added more DOC to the soil, with no apparent increase in FA and HA in TWW-irrigated soils after years of irrigation, may suggest that the TWW TOC is highly bioavailable. This finding is in contrast with the observed increase in humified materials when compost was applied to a sandy soil, and it was suggested that the conditions were favorable for the transformation between labile organic carbon and the refractory fraction [27]. However, the organic material concentrations in compost are much higher, and therefore, the transformations occur in that media may not be comparable to TWW.

The slight increase in DOC concentrations in the soil was observed in both irrigation regimes. However, the DOC concentrations were almost always higher in the TWW-irrigated soils. The increase in DOC with time in both treatments is probably due to natural processes, typical in agricultural land, where the high activity of the soil flora, fauna, and microorganisms (roots, micro-, and macrofauna) produces readily available DOC [28], tree litter [29], and the degradation products of these materials. The differences in DOC between the two irrigation regimes were most likely due to the additional organic C in the TWW. It is a well-accepted phenomenon that addition of organic matter leads to the buildup of soil organic C [6, 30].

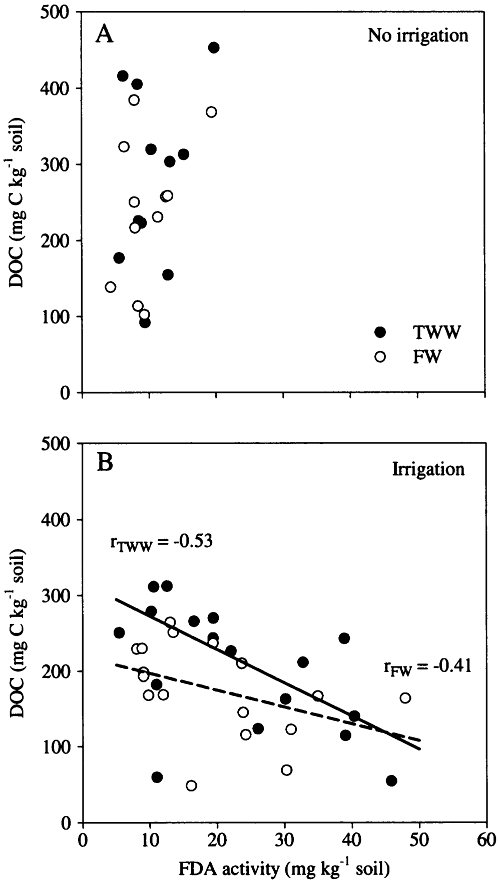


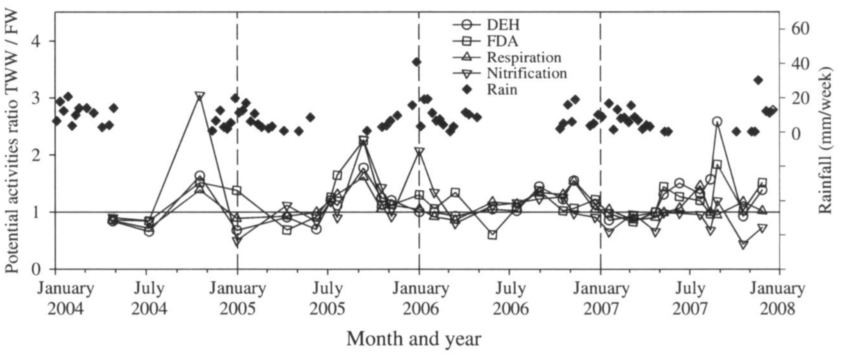
Figure 4 DOC concentration as a function of FDA activity during winter (**a**) and summer, and irrigation season (**b**) in soils irrigated with either TWW (*closed symbols*) or FW (*opened symbols*)

The addition of organic carbon from an external source (i.e., TWW) may influence the overall soil organic carbon quantity and quality and, as a result, its overall health. It was therefore important to evaluate whether this addition influences soil characteristics, especially microbial activity [11]. Soil microbial activity is considered an indicator of soil health, and therefore, when an application results in a decrease in soil activity, it suggests that its health is compromised [15]. Of all the parameters measured in the current study, hydrolysis activity was the only one correlated with DOC (Fig. 4). While there was very little FDA activity and no evidence of DOC disappearance in the soil sampled during the winter (Fig. 4a), there were high FDA levels and a negative correlation between FDA and DOC in the soils collected during the irrigation season (summer) (Fig. 4b), in both water qualities. This suggests that increased FDA activity, in part due to higher temperature during summer, resulted in decreased DOC in both soils. However, as this correlation was higher and significant in the TWW-irrigated soils, it is possible that the DOC added with the TWW is more available for microbial degradation. Similar increase in FDA hydrolysis was previously described as a response to irrigation of a citrus orchard with urban wastewater in Italy [6].

Similarly, microbial community respiration and overall oxidation were higher in the TWW-irrigated soil in the majority of the samples taken over time with no correlation to DOC. Even though there were no apparent seasonal trends in these activities, the addition of DOC from the TWW may maintain higher respiration and dehydrogenase activities in the TWW-irrigated soil. The increased activities in TWW-irrigated soils, especially during the irrigation season, are clearly seen in Fig. 5. This is in accordance with other studies that measured respiration and dehydrogenase activity in croplands where compost or TWW were applied [15]; in hazelnut orchards that were flooded periodically with urban and industrial wastewater [14]; and in barley croplands to which pig slurry was applied for 5 years in Spain [31]. However, the long-term (80 years) irrigation with wastewater in Mexico affected respiration only slightly [9].

Another major TWW component that may affect soil quality and activity is organic N and NH_4^+. Nitrification potential may indicate how the microbial community is coping with the addition of NH_4^+ via TWW (mostly as ammonium but also as a result of mineralization of organic nitrogen). While there was no change in soil NH_4^+ concentrations over time, the NO_3^- concentrations were higher at the end of the irrigation seasons of each year compared to their level before the irrigation season. The

Figure 5 The ratio between the activities in TWW- vs. FW-irrigated soils and the average weekly average rainfall



high NO_3^- concentrations corresponded to the high nitrification potential during the irrigation season and to absorption of NH_4^+ by plants [32, 33]. Both NO_3^- concentration and the nitrification potential did not differ between the water qualities. This may suggest that the irrigation with TWW may not affect this activity in the soil. However, as the NH_4^+ were equalized in both treatments, the nitrification potential for soil irrigated with unamended FW was not possible. It is highly likely that if ammonium was not added to the FW, the nitrification potential would have been higher in the TWW-irrigated soils. For example, nitrification was the highest in corn fields amended with dairy compost compared to $(NH_4)_2SO_4$ [34, 35]. Nitrification was also higher in soils irrigated with TWW compared to freshwater in a laboratory experiment [36].

One aspect that should be considered is the potential activity of exogenous bacteria arriving with the TWW to irrigated soils. The survival of WW bacteria in soil was investigated worldwide and under different field conditions and found to vary between days to 2–3 months [37, 38]. BOD values (Table 1) suggested that there was an active community present in the TWW. While it is clear that some exogenous bacteria arrive with the TWW to the fields, the function of this bacteria in the soil is largely unknown, and it is only possible to assume that this exogenous population may have some role in the total community activity.

In conclusion, the microbial community activity responded to the application of TWW in the soil. It also seems that there was a re-bounce of the community activity to a baseline after each irrigation season and during the winter time. Thus, the microbial effects of TWW irrigation, on the annual and longer scale, were relatively minor. Similarly, long-term irrigation with TWW did not affect soil enzyme activities in California [15]. In Germany, the microbial activity in soil irrigated with primary effluent for 100 years was significantly higher than in soil that was never irrigated [18]. However, when some plots were left un-irrigated for 20 years, the microbial activity decreased and approached the soil activity levels measured in non-irrigated plots. This, combined with the results observed in the present study, suggests that soil has the capability to recover even from long-term irrigation with wastewater, and it is possible that it may not have harmful long-term effects on soil microbiological quality. However, in this case study, irrigation was used only seasonally, and it would be beneficial to evaluate the effects of constant irrigation on soil and microbial community properties.

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References

1. Filip Z, Kanazawa S, Berthelin J (2000) Distribution of microorganisms, biomass, ATP, and enzyme activities in organic and mineral particles of a long-term wastewater irrigated soil. J Plant Nutr Soil Sci 163:143–150
2. Petersen SO, Henriksen K, Mortensen GK, Krogh PH, Brandt KK, Sørensen J, Madsen T, Petersen J, Grøn C (2003) Recycling of sewage sludge and household compost to arable land: fate and effects of organic contaminants, and impact on soil fertility. Soil Tillage Res 72:139–152
3. Alpert P, Osetinsky I, Ziv B, Shafir H (2004) Semi-objective classification for daily synoptic systems: application to the eastern Mediterranean climate change. Int J Climatol 24:1001–1011
4. Jueschke E, Marschner B, Tarchitzky J, Chen Y (2008) Effects of treated wastewater irrigation on the dissolved and soil organic carbon in Israeli soils. Water Sci Technol 57:727–733
5. Whetton PH, Fowler AM, Haylock MR, Pittock AB (1993) Implications of climate change due to the enhanced greenhouse effect on floods and droughts in Australia. Climate Change 24:289–317
6. Meli S, Porto M, Belligno A, Bufo SA, Mazzatura A, Scopa A (2002) Influence of irrigation with lagooned urban wastewater on chemical and microbiological soil parameters in a citrus orchard under Mediterranean condition. Sci Total Environ 285:69–77
7. Speir TW (2002) Soil biochemical properties as indices of performance and sustainability of effluent irrigation systems in New Zealand - a review. J Roy Soc New Zeal 32:535–553
8. Bouwer H (2000) Groundwater problems caused by irrigation with sewage effluent. J Environ Health 63:17–20
9. Friedel JK, Langer T, Siebe C, Stahr K (2000) Effects of long-term waste water irrigation on soil organic matter, soil microbial biomass and its activities in central Mexico. Biol Fertil Soils 31:414–421
10. Ramirez-Fuentes E, Lucho-Constantino C, Escamilla-Silva E, Dendooven L (2002) Characteristics, and carbon and nitrogen dynamics in soil irrigated with wastewater for different lengths of time. Bioresour Technol 85:179–187
11. Harrison EZ, Oakes SR, Hysell M, Hay A (2006) Organic chemicals in sewage sludges. Sci Total Environ 367:481–497
12. Oppel J, Broll G, Loffler D, Meller M, Rombke J, Ternes T (2004) Leaching behavior of pharmaceuticals in soil-testing-systems: a part of an environmental risk assessment for groundwater protection. Sci Total Environ 328:265–273
13. Siebe C, Fischer WR (1996) Adsorption of Pb, Cd, Cu and Zn by two soils of volcanic origin under long-term irrigation with untreated sewage effluent in Central Mexico. J Plant Nutr Soil Sci 159:357–364
14. Gianfreda L, Rao MA, Piotrowska A, Palumbo G, Colombo C (2005) Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. Sci Total Environ 341:265–279
15. Chen W, Wu L, Frankenberger JWT, Chang AC (2008) Soil enzyme activities of long-term reclaimed wastewater-irrigated soils. J Environ Qual 37:S36–S42
16. Brzezinska M, Tiwari SC, Stepniewska Z, Nosalewicz M, Bennicelli RP, Samborska A (2006) Variation of enzyme activities, CO_2 evolution and redox potential in an Eutric Histosol irrigated with wastewater and tap water. Biol Fertil Soils 43:131–135
17. Garcia-Gil JC, Plaza C, Soler-Rovira P, Polo A (2000) Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biol Biochem 32:1907–1913

18. Filip Z, Kanazawa S, Berthelin J (1999) Characterization of effects of a long-term wastewater irrigation on soil quality by microbiological and biochemical parameters. J Plant Nutr Soil Sci 162:409–413
19. Tarchitzky J, Hatcher PG, Chen Y (2000) Properties and distribution of humic substances and inorganic structure-stabilizing components in particle-size fractions of cultivated Mediterranean soils. Soil Sci 165:328–342
20. Swift RS (1996) Organic matter characterization. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (eds) Methods of soil analysis, part 3, chemical methods. SSSA Inc, Madison, pp 1011–1069
21. Hadas A, Kautsky L, Goek M, Kara EE (2004) Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. Soil Biol Biochem 36:255–266
22. Keeney DR, Nelson DW (1982) Nitrogen-inorganic forms. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, part 2 agronomy 9, 2nd edn. ASA, SSSA, Madison, pp 642–698
23. Schinner F, Oehlinger R, Kandeler E, Margesin R (1996) Methods in Soil Microbiology. Springer, Berlin Heidelberg
24. Schnürer J, Rosswall T (1982) Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl Environ Microbiol 43:1256–1261
25. Schmidt EL, Belser LW (1991) Autotrophic nitrifying bacteria. In: Mickelson SH, Bigham JM (eds) Methods of soil analysis, part 2, microbiological and biochemical properties. SSSA Inc, Madison
26. Angelakis AN, Marecos Do Monter MHF, Bontoux L, Asano T (1999) The status of wastewater reuse practice in the Mediterranean basin: need for guidelines. Water Res 33:2201–2217
27. Weber J, Karczewska A, Drozd J, Licznar M, Licznar S, Jamroz E, Kocowicz A (2007) Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts. Soil Biol Biochem 39:1294–1302
28. Haynes RJ, Tregurtha R (1999) Effects of increasing periods under intensive arable vegetable production on biological, chemical and physical indices of soil quality. Biol Fertil Soils 28:259–266
29. Kalbitz K, Schmerwitz J, Schwesig D, Matzner E (2003) Biodegradation of soil-derived dissolved organic matter as related to its properties. Geoderma 113:273–291
30. An PL, Hua JM, Franz M, Winter J, Gallert C (2004) Changes of chemical and biological parameters in soils caused by trickling sewage. Acta Hydroch Hydrob 32:286–303
31. Hernandez D, Fernandez JM, Plaza C, Polo A (2007) Water-soluble organic matter and biological activity of a degraded soil amended with pig slurry. Sci Total Environ 378:101–103
32. Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London
33. Mengel K, Kirkby EA (2001) Principles of plant nutrition, 5th edn. Kluwer academic publishers, The Netherlands
34. Habteselassie MY, Stark JM, Miller BE, Thacker SG, Norton JM (2006) Gross nitrogen transformations in an agricultural soil after repeated dairy-waste application. Soil Sci Soc Am J 70:1338–1348
35. Shi W, Miller BE, Stark JS, Norton JM (2004) Microbial nitrogen transformations in response to treated dairy waste in agricultural soils. Soil Sci Soc Am J 68:1867–1874
36. Master Y, Laughlin RJ, Stevens RJ, Shaviv A (2004) Nitrite formation and nitrous oxide emissions as affected by reclaimed effluent application. J Environ Qual 33:852–860
37. Gagliardi JV, Karns JS (2000) Leaching of *Escherichia coli* O157: H7 in diverse soils under various agricultural managements practices. Appl Environ Microbiol 66:877–883
38. Palese AM, Pasquale V, Celano G, Figliuolo G, Masi S, Xiloyannis C (2009) Irrigation of olive groves in Southern Italy with treated municipal wastewater: effects on microbiological quality of soil and fruits. Agric Ecosyst Environ 129:43–51