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Microbial-based soil quality indicators in irrigated and rainfed soil portions of Mediterranean olive and peach orchards under sustainable management

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ABSTRACT

The main objective of this study was to apply microbial indicators of soil quality in drip-irrigated olive and peach orchards managed with sustainable agricultural practices. Soil characterization was carried out in different areas of the orchards along the row, under the drippers (R_{dr}), and along the inter-row, rainfed (IR_{rf}), to evaluate the effects of irrigation. Two parameters were followed during one year: a) a biochemical soil indicator (N_c/N_k ratio) based on soil N/C turnover and soil enzyme activities, and b) the abundance of three important N-cycling genes (*nifH*, *amoA* and *nosZ*). Localized irrigation caused higher values of water content in the R_{dr} areas, compared to IR_{rf}. The N_c/N_k ratio exhibited all the attributes of a reliable soil fertility indicator, being generally higher in irrigated R_{dr} areas. The abundance of *nifH* and *amoA* in the soil showed a trend similar to N_c/N_k, being affected by higher soil water content, while *nosZ* abundance was generally insensitive to irrigation. Both N_c/N_k and gene abundances, much more than the measured chemical, biochemical and molecular soil parameters considered alone, can give a precise idea on N and C soil dynamics, that in turn, affect soil quality and fertility.

1. Introduction

Soil quality plays a double role in the agro-ecosystems, as it is essential for high production as well as from an environmental point of view (Ding et al., 2013; Jónsson et al., 2016). Soil quality can be defined as the ability of the soil to decompose organic matter and release nutrients from it (Karlen et al., 1997).

Among all the agronomic practices adopted in an orchard and able to affect soil quality and fertility, a key role is played by irrigation (Sofo et al., 2014; Dal Ferro et al., 2016). From one side, it is important for fruit production and the maintenance of soil fauna and microbiota (Miller et al., 2005; Montanaro et al., 2012). On the other side, if not well planned, irrigation can lead to increases of soil mineralization and respiration, that in turn cause decreases of soil organic carbon and nutrients, and repercussions on the environment due to CO_2 emission and nitrate leaching (Mikha et al., 2005; Miller et al., 2005). Irrigation is able to influence the dynamics of soil microorganisms, in terms of mobility, growth, nutrient absorption and respiration, can strongly affect the rates of N and C mineralization, and consequently soil quality (Kruse, 1986; Graf et al., 2014; Sofo et al., 2014).

A high number of physico-chemical, microbiological and biochemical parameters are responsible for the fertility of a soil. However, due to the impossibility of considering all of them, it is inevitable to select the most informative and reliable ones (Gil-Sotres et al., 2005). Generally, the physico-chemical parameters are of scarce utility as indicators, as they are altered often when soils are subjected to drastic disturbances (Filip, 2002). On the other side, some soil biochemical properties are sensitive to smaller changes occurring in a soil (Yakovchenko et al., 1996; Wallenstein and Vilgalys, 2005; Muscolo et al., 2015). On this basis, the selection of biochemical indicators closely related to soil microbial dynamics could be essential for the quantification of soil quality and its resilience to stresses, two basic requisites of soil fertility. These

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indicators should be a measure that provides reliable and easy to interpret information and they should not be affected by the fluctuations related to the season and the positional effect, because this could prevent the identification of changes due to perturbations, damages or environmental stresses (Arshad and Martin, 2002).

On this basis, the main objective of this study was to analyze some microbial indicators in soils from drip-irrigated olive and peach orchards, two of the most representative fruit crops of the Mediterranean basin, managed with sustainable agricultural practices. This characterization was carried out in different areas of the orchards (along the row, under the drippers; and along the inter-row, rainfed), in order to understand the effects of irrigation. In order to characterize the two agroecosystems and better understand if soil moisture can affect soil quality in the different parts of a drip-irrigated orchard, two indicators were considered: a) a biochemical soil indicator that can be used quantitatively as a measure of the degree of soil quality or degradation (Trasar-Cepeda et al., 2000) (N_c/N_k ratio) based on soil N and C turnover; and b) the abundance of three major N-cycling functional bacterial genes (nitrogenase reductase, *nifH*; ammonia mono-oxygenase, *amoA*; and nitrous oxide reductase, *nosZ*).

2. Materials and methods

2.1. Experimental site, orchard management and soil sampling

The first trial was carried out in a 2-ha mature olive grove (*Olea europaea* L., cv. Maiatica di Ferrandina; plants with an age of approximately 60 years, trained to vase at a distance of 8 × 8 m) located in Ferrandina (Southern Italy, Basilicata region; N 40°30′, E 16°27′) and managed using organic agricultural practices since 2000. The area has a semi-arid climate, annual precipitation of 561 mm (mean 1976–2015), falling mostly in the winter, and mean annual temperature ranging from 15 to 17 °C. The soil is a sandy loam, a Haplic Calcisol (FAO, 2016) with a mean bulk density of 1.49 g cm^{-3}. The top 30 cm of the soil had the following characteristics: pH 8.0; electric conductivity = 0.159 mS cm^{-1}; organic carbon content = 13.9 g kg^{-1}; extractable phosphorus (Olsen method) and potassium = 8 and 180 mg kg^{-1}, respectively; cation exchange capacity = 11.70 meq 100 g^{-1}; base saturation = 100%. Olive plants were drip irrigated from March to October (2800 m^3 ha^{-1} $year^{-1}$) with urban wastewater (chemical parameters in Supplementary Table 1). Six drip emitters discharging 8 L h^{-1} over a 1-m radius were placed for each plant. Plants were lightly pruned every year in winter. The soil was permanently covered by spontaneous self-seeding weeds (mainly Fabaceae and Poaceae), mowed twice a year for avoiding competition for water and nutrients. Cover crop residues and prunings were shredded and left along the row as mulch. No mineral nitrogen addition was needed.

The second trial was conducted in a peach orchard located in Policoro (Southern Italy, Basilicata region; N 40°19′, E 16°66′). Plants were trained to vase at a distance of 5 × 4 m. Since 2007, the peach orchard has been managed organically (law Reg. CEE 834/07), with no-tillage, no use of synthetic fertilizers, and recycling of pruning residues. Compost (12 t ha^{-1} $year^{-1}$) was applied once a year along the row (chemical parameters in Supplementary Table 2). Plants were drip irrigated from March to October with freshwater (3300 m^3 ha^{-1} $year^{-1}$) by two drip emitters per plant discharging 16 L h^{-1}, placed at a distance of 4 m each other. In cases of days particularly hot in July-August, a micro-jet irrigation system is also present in the orchard. The soil is a sandy clay loam, a Haplic Calcisol (FAO, 2016) with a mean bulk density of 1.48 g cm^{-3}. The top 30 cm of the soil had the following characteristics: pH 7.7; electric conductivity = 0.624 mS cm^{-1}; organic carbon content = 31.8 g kg^{-1}; extractable phosphorus (Olsen method) and potassium = 23 and 821 mg kg^{-1}, respectively; cation exchange capacity = 18.82 meq 100 g^{-1}; base saturation = 100%. The soil was permanently covered by spontaneous self-seeding weeds (mainly Fabaceae and Poaceae), mowed four times a year (February, May, July, September) for avoiding competition for water and nutrients. Cover crop residues and pruning material were shredded and left along the row as mulch.

In March, June and October 2015, bulk soil of both the orchards were sampled. For each treatment, three composite samples of bulk soil were randomly collected from the topsoil layer (0–20 cm). Each composite sample was formed from ten subsamples: cores of 7-cm-diameter sampled within a 0.5 m radius to minimize spatial variability and pooled on site (Tian et al., 2004). For both the orchards, two sampling areas were identified: along the row, under the drip emitters (R_{dr}) and along the inter-row, rainfed (IR_{rf}). After removal of crop residues, the soil samples were stored immediately at 4 °C in sterilized plastic pots and analyzed after 24 h.

Soil water content (SWC) was determined from the weight differences of soil samples before and after drying at 105 °C for 24 h and expressed as percentages of water on dry weight.

2.2. Soil biochemical parameters and N_c/N_k ratio

From each of the three composite soil samples, microbial biomass carbon (MBC) was determined by the fumigation-extraction method (Brookes 1995). The microbial biomass carbon was calculated by the equation of Vance et al. (1987): $MBC = E_c \times 2.64$, where E_c is the difference of biomass carbon between fumigated soil and non-fumigated soil, expressed as μg C kg^{-1} dry soil (DS). Mineralizable N (N_m) was evaluated as the difference of inorganic N at the beginning and at the end of a 10-day incubation period (Trasar-Cepeda et al., 1998). Inorganic N was determined by distillation after its extraction in 2 M KCl, as reported by Bremner and Keeney (1966), and expressed in mg kg^{-1} DS. Urease activity was measured according to Tabatabai and Bremner (1972) and expressed as μg NH_4-N g^{-1} DS h^{-1}. Phosphomonoesterase (PME) activity was measured by the method of Eivazi and Tabatabai (1977), and expressed as μmol *p*-nitrophenol g^{-1} DS h^{-1}. The activity of *β*-glucosidase (*β*-glu) was determined by the method of Eivazi and Tabatabai (1988), and expressed as μmol *p*-nitrophenol g^{-1} DS h^{-1}. All analyses were performed in triplicate.

The degree of soil quality was expressed by the N_c/N_k ratio, where N_k is total soil N, determined by Kjeldahl, and N_c is a linear function of microbial biomass carbon and N mineralization capacity, combined with three enzyme activities, calculated by the following equation (Trasar-Cepeda et al., 2000):

$$N_c = (0.38 \cdot 10^{-3})MBC + (1.4 \cdot 10^{-3})N_m + (13.6 \cdot 10^{-3})\,PMEactivity + (8.9 \cdot 10^{-3})\beta\text{-gluactivity} + (1.6 \cdot 10^{-3})\,ureaseactivity$$

2.3. Nucleic acids extraction

Total microbial genomic DNA from each of the three composite soil samples was extracted from 0.5 g of soil using the FastDNA® SPIN Kit for soil combined with the Thermo Savant FastPrep® System homogenizer (MP Biomedicals LLC, Cleveland, OH, USA). Total genomic DNA of pure bacterial cultures was extracted and purified using the DNeasy Blond & Tissue kit (Qiagen, GmbH, Valencia, CA, USA). DNA quality was checked by TEAE agarose gel electrophoresis (0.7% w/v) while DNA quantity was determined spectrophotometrically using a NanoDrop® ND-1000 UV–vis spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Extracted DNA was stored at −20 °C.

Table 1
Primers selected for the amplification of nitrogen functional bacterial genes and qPCR conditions.

Target gene	Primers (forward/reverse)	Thermal profile	Number of cycles	Amplicon length	References
NifH	AAAGGYGGWATCGGYAARTCCACCAC TTGTTSGCSGCRTACATSGCCATCAT	95 °C × 5 min 95 °C × 45 s 55 °C × 45 s 72 °C × 45 s	1 40	458	Rösch et al., 2002; Fiorentino et al., 2016
AmoA	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	95 °C × 5 min 95 °C × 10 s 55 °C × 26 s 72 °C × 1 min	1 40	491	Rotthauwe et al., 1997; Zhang et al., 2013
NosZ	CGYTGTTCMTCGACAGCCAG CGSACCTTSTTGCCSTYGCG	95 °C × 5 min 95 °C × 10 s 60 °C × 1 min	1 40	454	Throbäck et al., 2004; Zhang et al., 2013

2.4. Quantitative PCR

Quantitative PCR (qPCR) with non-specific dye SYBR green was performed to assess the abundance of some bacterial functional genes of soil N cycling. Primer sequences for amplifying nitrogenase reductase (*nifH)*, ammonia mono-oxygenase (*amoA)* and nitrous oxide reductase (*nosZ*), and qPCR conditions are listed in Table 1. Pure bacterial cultures of strains containing the genes of interest were used as standards. The *nifH* containing strain was *Azospirillum brasiliense*, the *amoA* strain was *Nitrosomonas europea* and the *nosZ* strain was *Pseudomonas fluorescens Migular* (Supplementary Table 3). All qPCR reactions were conducted on a 7300 Real-Time PCR System (Applied Biosystem, Forter City, CA USA) and all samples, standards and negative controls were assayed in triplicate. Each amplification was performed in a 20 μL reaction mix containing 10 μL 2X iTaq™ Universal SyBR®Green Supermix (Bio-Rad, Hercules, CA, USA), 0.2 μL of each primers (10 μM) and 1 μL of DNA (3.5 ng), under conditions reported in Table 1.

At the end of each qPCR, melting curve analysis was performed (95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s) to ensure that a single product was generated. An 1.5% (w/v) TEAE agarose gel with the qPCR products was run to confirm the correct sized amplicons, too.

The intensity of SYBR fluorescent signals was analyzed by the SDS software (version 1.4.0), and the gene copy number was calculated using the regression equation that related the cycle threshold (Ct) value to the number of copies in the standard curve, obtained by serial dilutions of reference strain DNA. Assuming one gene copy per reference strain genome (Wallenstein and Vilgalys 2005), the copy numbers were calculated using the following reference strain genome size (in megabases): 3.1 for *Azospirillum brasiliense* (*nifH*) (Rivera et al., 2014), 2.8 for *Nitrosomonas europea* (*amoA*) (Chain et al., 2003), 6.0 for *Pseudomonas fluorescens* (*nosZ*) (Hernández-Salmerón et al., 2016) (Supplementary Table 3) and according to the equation:

$$\text{Copy number} = (\text{DNA amount} \times \text{Avogadro's number})/$$
$$(\text{length of reference genome} \times 1{\cdot}10^{9} \times \text{weight of a base pair})$$

The results were expressed as gene copy number per gram of dry soil.

2.5. Statistical analysis

Statistical analysis was performed using Sigmastat 3.1 SPSS Inc. software (SPSS Inc., Quarry Bay, Hong Kong). The means of all the measured parameters (three analytical replicates for each treatment from the three independent composite soil samples; $n = 3$) were treated by two-way analysis of variance (ANOVA) with orchard management and sampling time as factors.

Table 2
Soil water content in the olive and peach orchards.

Crop	Sampling time	Position	Water content (% DS)
Olive	March	R_{dr}	17.5 a
		IR_{rf}	15.0 b
	June	R_{dr}	15.8 b
		IR_{rf}	14.7 bc
	October	R_{dr}	17.8 a
		IR_{rf}	14.0 c
Peach	March	R_{dr}	23.0 a
		IR_{rf}	21.2 b
	June	R_{dr}	17.4 c
		IR_{rf}	15.5 d
	October	R_{dr}	17.5 c
		IR_{rf}	16.2 d

The soil samples were collected from topsoil layer (0–20 cm). Statistical differences at $P < 0.05$ are indicated with different letters. Data are reported as means of three independent composite replicates ($n = 3$). DS: dry soil; IR_{rf}: areas along the inter-row; R_{rd}: areas under the drip emitters.

Means were separated according to Fisher's LSD test at $P \leq 0.05$. Correlation analysis was performed to determine the relationship between the measured parameters, computing Pearson correlation coefficients as parametric measure of the linear relationship between the variables. For the correlation analysis, significant differences among means were determined according to Fisher's LSD test at $P \leq 0.05$ and $P \leq 0.01$.

3. Results

3.1. Soil water content and biochemical parameters

In both olive and peach orchards, the localized irrigation caused higher values of water content in the R_{dr} areas, compared to IR_{rf} (Table 2).

In the olive orchard, total nitrogen (N_k), mineralizable nitrogen (N_m) and microbial biomass carbon (MBC) were generally statistically higher in the R_{dr} treatment (Table 3). Some exceptions were observed for N_k in March (not statistically different between the two treatments), for N_m in June (below the limit of detection), and for MBC in October (statistically higher in the IR_{rf} treatment) (Table 3). The enzyme activities for olive were generally higher in the R_{dr} treatment or not statistically different between the two-water management systems (Table 3).

The values of N_k, N_m and MBC in the peach orchard were generally higher in the IR_{rf} treatment for all the three sampling dates (Table 3). The exceptions were N_m values below limit of detection (LOD) in March and October and not different in June and MBC higher in R_{dr} in June. In the peach orchard, the three enzyme activities were generally higher in the IR_{rf} treatment or not significantly different between the two soil managements, excepting PME activity in June (Table 3).

Table 3
Biochemical parameters in soils from the olive and peach orchards.

Crop	Sampling time	Position	N_k (mg N g^{-1} DS)	N_m (mg NH_4-N g^{-1} DS)	MBC (μg C g^{-1} DS)	β-glu (μmol p-nitrophenol g^{-1} DS h^{-1})	PME (μmol p-nitrophenol g^{-1} DS h^{-1})	Urease (NH_4-N g^{-1} DS h^{-1})
Olive	March	R_{dr}	1.01 ± 0.04 c	2.97 ± 4.05 b	381.48 ± 69.07 c	17.92 ± 2.2 c	12.76 ± 2.48 a	1.52 ± 0.47 a
		IR_{rf}	1.02 ± 0.03 c	1.51 ± 1.99 c	253.44 ± 3.73 d	8.90 ± 1.45 d	6.67 ± 1.52 b	1.06 ± 0.03 b
	June	R_{dr}	1.64 ± 0.03 a	<LOD	459.36 ± 67.20 ab	37.17 ± 6.25 ab	9.63 ± 2.89 b	0.65 ± 0.23 c
		IR_{rf}	1.26 ± 0.06 b	<LOD	380.16 ± 22.40 c	17.69 ± 2.30 c	7.11 ± 0.73 b	0.60 ± 0.02 c
	October	R_{dr}	1.48 ± 0.13 a	4.38 ± 0.30 a	245.52 ± 33.60 d	7.42 ± 0.90 d	13.11 ± 2.48 a	1.06 ± 0.12 b
		IR_{rf}	1.27 ± 0.03 b	2.24 ± 3.02 b	290.4 ± 37.34 c	6.28 ± 0.65 d	10.74 ± 0.76 a	0.84 ± 0.17 c
Peach	March	R_{dr}	1.68 ± 0.20 c	<LOD	484.44 ± 9.33 b	33.05 ± 0.99 b	25.64 ± 2.03 a	1.30 ± 0.14 c
		IR_{rf}	1.85 ± 0.12 b	<LOD	560.34 ± 8.40 a	37.55 ± 4.81 ab	23.09 ± 3.43 a	2.00 ± 0.19 b
	June	R_{dr}	1.90 ± 0.05 b	4.38 ± 1.00 a	538.56 ± 22.40 a	33.27 ± 6.33 b	24.10 ± 3.41 a	0.91 ± 0.13 de
		IR_{rf}	2.12 ± 0.11 a	4.38 ± 18.56 a	409.86 ± 19.60 c	43.74 ± 5.21 a	19.11 ± 2.54 b	2.08 ± 0.13 b
	October	R_{dr}	1.74 ± 0.21 c	<LOD	368.28 ± 102.67 d	12.81 ± 1.18 c	19.94 ± 4.50 b	1.54 ± 0.28 c
		IR_{rf}	2.14 ± 0.21 a	<LOD	513.48 ± 84.00 a	14.69 ± 2.08 c	20.81 ± 2.08 b	2.72 ± 0.28 a

Statistical differences at $P < 0.05$ are indicated with different letters within columns. The data are reported as means of three independent composite replicates ± standard deviation ($n = 3$). N_k: total soil nitrogen; N_m: mineralizable soil nitrogen; MBC: microbial biomass carbon; β-glu: β-glucosidase activity; PME: phosphomonoesterase activity; urease: urease activity; DS: dry soil; LOD: limit of detection; IR_{rf}: areas along the inter-row; R_{rd}: areas under the drip emitters.

Table 4
The ratio N_c/N_k in soils from the olive and peach orchards.

Crop	Sampling time	Position	N_c/N_k (%)
Olive	March	R_{dr}	47.77 a
		IR_{rf}	26.47 d
	June	R_{dr}	38.86 b
		IR_{rf}	31.71 c
	October	R_{dr}	23.34 d
		IR_{rf}	27.48 d
Peach	March	R_{dr}	49.35 a
		IR_{rf}	46.72 b
	June	R_{dr}	44.01 b
		IR_{rf}	38.41 c
	October	R_{dr}	30.32 d
		IR_{rf}	28.66 d

Statistical differences at $P < 0.05$ are indicated with different letters within column. The data are reported as means of three independent composite replicates ($n = 3$). IR_{rf}: areas along the inter-row; R_{rd}: areas under the drip emitters.

3.2. N_c/N_k ratios

The ratios N_c/N_k, calculated from these values and reported in Table 4, were significantly higher in R_{dr} (almost doubled in March) compared to IR_{rf}, and with no significant differences in October (Table 4). As observed for olive, N_c/N_k ratios in the peach orchard presented significantly higher values in R_{dr}, compared to IR_{rf}, with no significant differences in October (Table 4).

3.3. N-cycling genes

Th gene *nifH* was generally more abundant in R_{dr} compared to IR_{rf} for olive and only in June for peach (Figs. 1 and 2A, respectively). The abundance of *amoA* was always significantly higher in R_{dr} than in IR_{rf}, both in olive and peach orchards (Figs. 1 and 2B, respectively). The abundance of *nosZ* was statistically different between R_{dr} and IR_{rf}, in particular in October for the olive orchard (Fig. 1C), while it was almost comparable between the two treatments in the peach orchard (C).

4. Discussion

In the calculation of N_c/N_k, the values of MBC and N_m assume a key importance. Indeed, despite the soil microbial biomass is only a small and labile reserve of the main organic compounds, it is a powerful catalyst in nutrient transformations, assuming a vital role in biogeochemical cycles and ecosystem functioning. In a previous research from our group in the same olive orchard here studied

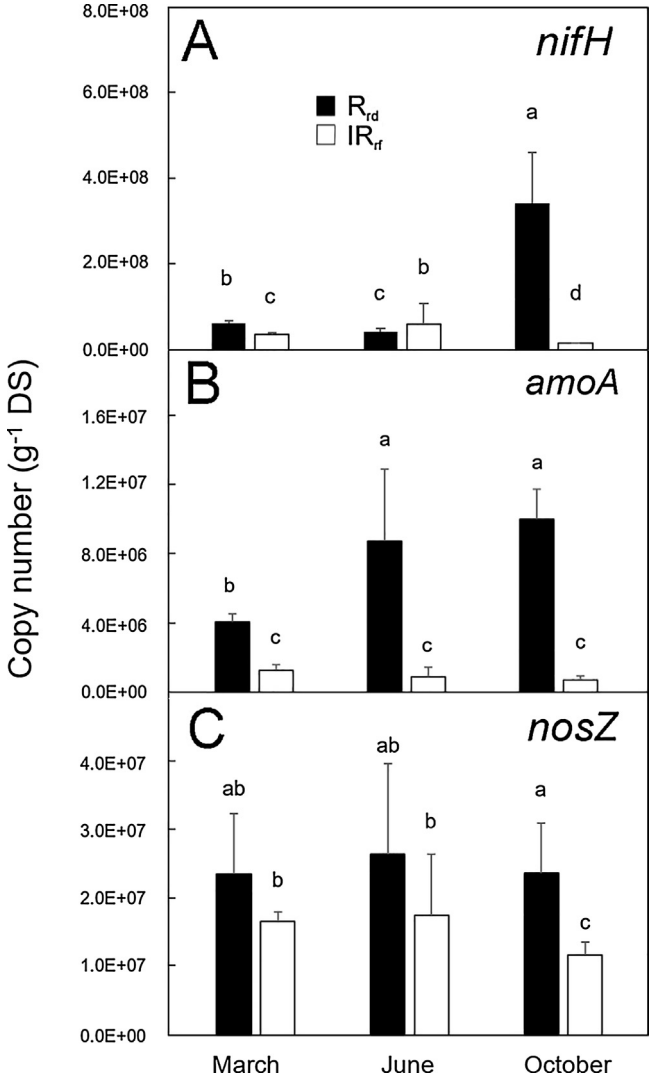

Fig. 1. Abundance of the N-cycling genes *nifH*, *amoA* and *nosZ* measured by qPCR in soils from the olive orchard.
Statistical differences at $P < 0.05$ are indicated with different letters. The values (±standard deviation) are means of three independent replicates ($n = 3$). DS: dry soil; IR_{rf}: areas along the inter-row (white columns); R_{rd}: areas under the drip emitters (black columns).

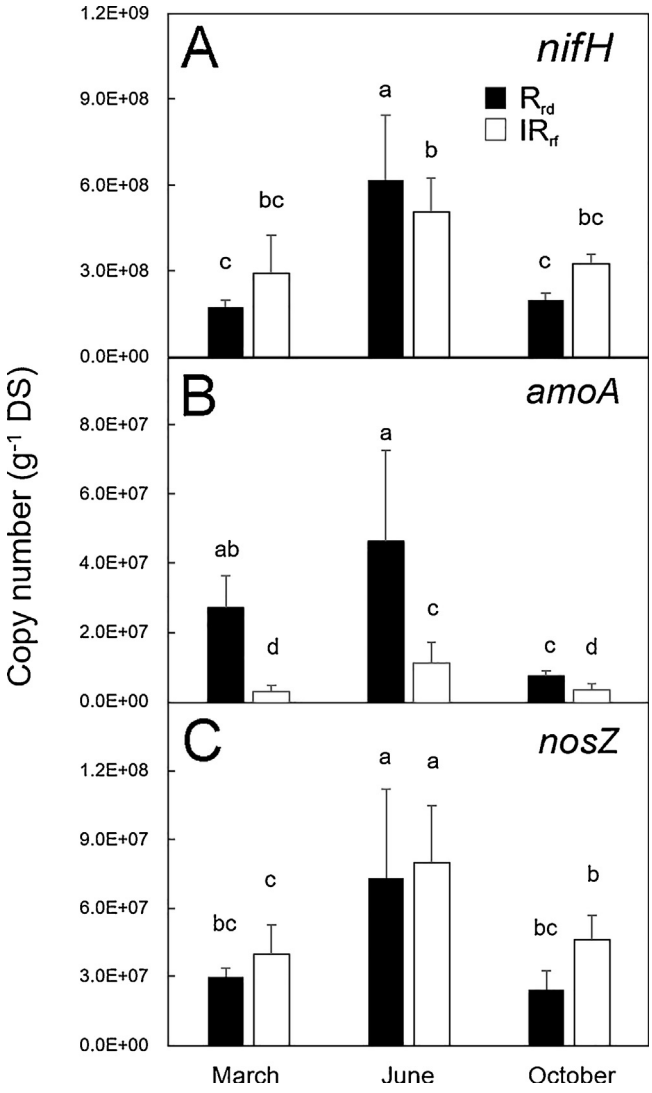

Fig. 2. Abundance of the N-cycling genes *nifH*, *amoA* and *nosZ* measured by qPCR in soils from the peach orchard.
Statistical differences at $P < 0.05$ are indicated with different letters. The values (±standard deviation) are means of three independent replicates ($n = 3$). DS: dry soil; IR_{rf}: areas along the inter-row (white columns); R_{rd}: areas under the drip emitters (black columns).

(Sofo et al., 2010), we observed a higher bacterial metabolic activity and diversity in the soils wetted by drippers compared to the inter-row rainfed areas, that is closely related to microbial biomass. Considering the different conditions in the parts of the orchard along the row (R_{dr}), where cover crop and pruning residues were shredded, compost (in the case of the peach orchard) was applied, and soil moisture was higher due to irrigation, we expected differences in MBC, compared to the inter-row (IR_{rf}) areas. In the case of olive, MBC was higher in March and June in R_{dr} (Table 3), so reflecting the importance of the irrigation more than that of soil carbon endogenous inputs in increasing microbial biomass. The trend of MBC appears to be less clear in the peach orchard, where the values in March and October are higher in IR_{rf}, underlining the predominant effect of added compost, and lower in June, when irrigation plays a key role in determining increases in microbial carbon (Table 3).

The other key factor, N_m, is a measure of the potential conversion of organic N into the respective N mineral forms (mainly NO_3^- and NH_4^+) through biochemical processes mediated by soil microorganisms, affected by temperature, humidity and pH. In the olive orchard, the highest N_m values were found in the irrigated areas of R_{dr}, where N mineralization and turnover is faster, as already pointed out by Sofo et al. (2010). Also in this case, the trend of N_m in the peach orchard is less defined, being detectable only in June (Table 3).

Soil microbial activity correlates well with the activities of many key soil enzymes (Xu et al., 2015). The extracellular enzymatic activity in soils is due to the enzymes synthesized and secreted by microbial cells during their life cycle, or that, following the cell lysis, are found in the extracellular space or associated with cell fragments.

Particularly, the activity of urease, that catalyzes the hydrolysis of urea to CO_2 and NH_3, is a good soil quality index, as it tends to increase in relation to rises of soil organic matter (Tabatabai, 1982). Indeed, urease activity was generally higher in the peach orchard, where measured organic matter is higher, compared to the olive one (Table 3). The results also show that the effect of irrigation on urease activity was clear in the olive, where it was higher in R_{dr}, respect to rainfed IR_{rf} areas (Table 3).

Phosphomonoesterases (PMEs) are present in soil, where they catalyze the hydrolysis of the organic phosphorus, with the production of inorganic phosphorus that can be absorbed by plants (Shi, 2011). In our experiment, PMEs activity in both the orchards was significantly affected by SWC, as assessed by the higher values in R_{dr} areas (Table 3).

Finally, the activity of ß-glucosidase represents an index of the evolution of soil organic matter and soil carbon cycle, catalyzing the last reaction of cellulose degradation by hydrolysing cellobiose into glucose (Geisseler et al., 2009). The trends of this enzyme activity in the two orchards were similar to those of urease (Table 3).

Trasar-Cepeda et al. (1998) pointed out that the biochemical balance, which is characteristic of a mature stable soil, can be expressed using mathematical models based on a combination of several microbiological and biochemical soil properties. The same authors found that the N_c/N_k value corresponds to 100% for climax soils, as a kind of reference; the greater the deviation from this value, the higher is the loss of soil quality. Thus, the N_c/N_k values generally higher in both orchards, under the drippers, clearly indicate that a localized irrigation can contribute to improve soil quality. It is also remarkable that the index summarizes, better than single parameters, those effects. In fact, as reported in Tables 5 and 6, N_c/N_k were positively correlated to water content in both orchards, while microbial biomass values and enzymes activities were in general poorly correlated.

In order to compare and integrate information rising from a biochemical index to those from molecular approaches, we applied a reliable method to determine the abundance of three essential N-cycling bacterial genes (*nifH*, *amoA* and *nosZ*). This aspect is of key importance, as the quantification of N-cycling functional genes allows to evaluate the interactions between microbial communities and nitrogen dynamics in field. qPCR studies allow to examine the medium- and long-term effects of soil management on the abundance of microbial genes present in soil (Wallenstein and Vilgalys, 2005; Cederlund et al., 2014). Soil microorganisms are key players in N biogeochemical cycle, that is due to soil bacterial communities with the functional genes responsible for N transformations, including nitrogen fixation (*nifH*), ammonia oxidation (*amoA*), and nitrous oxide reduction (*nosZ*) (Wallenstein and Vilgalys 2005; Jones et al., 2014).

In this study, *nifH*, a key gene of N fixation, was generally more abundant in R_{dr} compared to IR_{rf} for olive and only in June for peach (Figs. 1 and 2A, respectively). From a previous research in the same olive grove, Sofo et al. (2010) demonstrated that wet soils under drip emitters contained a high number of free N-fixer bacteria (*Azotobacter*), as determined by plate counting, and thus a N enrichment due to higher N fixation rates in the wet soils, according

Table 5
Correlation coefficients among the measured parameters in the olive orchard.

	R_{dr}								
	SWC	MBC	β-glu	PME	Urease	N_c/N_k	*nifH*	*amoA*	*nosZ*
SWC	1.00								
MBC	−0.38	1.00							
β-glu	−0.51	0.70**	1.00						
PME	0.62	−0.28	0.14	1.00					
Urease	0.37	−0.10	0.45	0.43	1.00				
N_c/N_k	0.88*	0.18	0.48	0.41	0.53	1.00			
nifH	0.70**	−0.21	−0.32	0.66**	−0.62	0.72**	1.00		
amoA	0.93*	0.30	0.13	0.36	0.49	0.89*	0.67**	1.00	
nosZ	0.31	0.57	0.63	−0.52	−0.50	0.22	−0.16	0.34	1.00
	IR_{rf}								
	SWC	MBC	β-glu	PME	Urease	N_c/N_k	*nifH*	*amoA*	*nosZ*
SWC	1.00								
MBC	−0.48	1.00							
β-glu	0.24	0.12	1.00						
PME	−0.60	0.25	−0.38	1.00					
Urease	0.75**	0.50	−0.42	−0.62	1.00				
N_c/N_k	0.67**	0.53	0.72**	0.47	0.49	1.00			
nifH	0.60	0.58	0.68**	0.50	0.52	0.74**	1.00		
amoA	0.65**	0.42	0.46	0.39	0.37	0.82*	0.71**	1.00	
nosZ	0.63	0.40	0.37	0.35	0.50	0.61	0.64	0.83*	1.00

*: significant difference at $P < 0.05$; **: significant difference at $P < 0.01$. MBC: microbial biomass carbon; β-glu: β-glucosidase activity, PME: phosphomonoesterase activity; urease: urease activity; N_c/N_k: N_c/N_k ratio; *nifH*: *nifH* copy number; *amoA*: *amoA* copy number; *nosZ*: *nosZ* copy number. IR_{rf}: areas along the inter-row; R_{rd}: areas under the drip emitters; SWC = soil water content.

Table 6
Correlation coefficients among the measured parameters in the peach orchard.

	R_{rd}								
	SWC	MBC	β-glu	PME	Urease	N_c/N_k	*nifH*	*amoA*	*nosZ*
SWC	1.00								
MBC	−0.45	1.00							
β-glu	−0.53	0.61	1.00						
PME	0.50	0.61	0.87*	1.00					
Urease	0.56	−0.58	0.36	−0.55	1.00				
N_c/N_k	0.75**	0.60	0.82*	0.63	0.34	1.00			
nifH	−0.33	0.70**	0.55	0.38	−0.67**	−0.23	1.00		
amoA	0.68**	0.65**	0.60	0.40	−0.65**	0.78**	0.65**	1.00	
nosZ	−0.34	0.67**	0.58	0.38	−0.70**	−0.13	0.69**	−0.10	1.00
	IR_{rf}								
	SWC	MBC	β-glu	PME	Urease	N_c/N_k	*nifH*	*amoA*	*nosZ*
SWC	1.00								
MBC	0.25	1.00							
β-glu	−0.34	−0.65**	1.00						
PME	0.65**	0.46	−0.54	1.00					
Urease	−0.58	−0.45	−0.60	0.15	1.00				
N_c/N_k	0.70**	0.39	0.62	0.33	0.27	1.00			
nifH	−0.37	−0.71**	0.64	−0.45	−0.55	0.43	1.00		
amoA	0.65**	−0.68**	0.65**	−0.42	−0.58	0.62	0.68**	1.00	
nosZ	−0.29	−0.71**	0.64**	−0.41	−0.46	0.44	0.72**	0.80*	1.00

*: significant difference at $P < 0.05$; **: significant difference at $P < 0.01$. MBC: microbial biomass carbon; β-glu: β-glucosidase activity, PME: phosphomonoesterase activity; urease: urease activity; N_c/N_k: N_c/N_k ratio; *nifH*: *nifH* copy number; *amoA*: *amoA* copy number; *nosZ*: *nosZ* copy number. IR_{rf}: areas along the inter-row; R_{rd}: areas under the drip emitters; SWC = soil water content.

to Hayden et al. (2010). The differences in *nifH* abundance between olive and peach orchards could be partly due to the "maturity" of the agroecosystem, as the olive orchard was managed with the same agricultural technique since 2000 (medium-term), while the peach orchard since 2007 (short-medium term), as pointed out by Wallenstein and Vilgalys (2005) and Hayden et al. (2010). Particularly, the high rates of nitrogen fixation in the irrigated soil of the olive orchard could partly explain the higher total soil nitrogen in R_{dr}, compared to IR_{rf} (Table 3). This hypothesis seems to be confirmed by the positive correlation existing between *nifH* copy number and N_c/N_k in both drip irrigated and rainfed areas, in olive orchard only (Table 5). Another explanation of the differences in *nifH* abundance between the two orchards could reside on the addition of organic compounds with the irrigation water in olive, as opposed to adding organic compounds as compost in peach.

The abundance of *amoA*, a key regulatory gene of N nitrification encoding for ammonia monooxygenase and positively related to ammonia oxidation rates (Petersen et al., 2012), was always significantly higher in R_{dr} than in IR_{rf}, both in olive and peach orchards (Figs. 1 and 2B, respectively). In particular, the copy number of *amoA* gene, that is the number of bacteria contributing to N nitrification in soil, is much higher in drip irrigated than rainfed areas in olive

orchard; although statistically relevant, in peach orchard seem less evident. Many variables influence *amoA* abundance (ammonium, nitrate, Olsen P and microbial biomass C) (Hayden et al., 2010), and these may account for differences in detection between R_{dr} than in IR_{rf}, as indicated by the correlation existing among copy number and water content, microbial biomass and enzymes activities (Tables 5 and 6).

The gene *nosZ* codes for nitrous oxide reductase, which is active during the final step of denitrification and is positively correlated to denitrification rate, was used to quantify different groups of denitrifier microorganisms (Wallenstein and Vilgalys 2005). Its abundance was statistically different between R_{dr} and IR_{rf}, in particular in October for olive orchard (Fig. 1C), while it was almost comparable in peach orchard (Fig. 2C), comprising the hypothesis that a longer period of different water management and/or the different types of carbon inputs (wastewater or compost) could be responsible for these differences. It also seems the abundance of denitrifying bacteria is less correlated to the water content as well as to microbial biomass, enzymes activities, and in part to N_c/N_k.

Considering that, in both olive and peach orchards, drip-irrigation has already been started from the beginning of March and the soil sampling was carried out at the end of the same month, significant higher values of SWC in the R_{dr} areas were found in March (Table 2). This change in soil moisture was likely were the main responsible, together with wastewater (olive) and compost (peach), for the significant differences between R_{dr} and IR_{rf} in many cases observed in March for N_c/N_k (Table 4) and N-cycling genes abundances (2).

Summarizing, the results on gene abundance of *nifH*, *amoA* and *nosZ* taken together highlighted: 1) the positive relationship between SWC and N fixation (particularly in olive) and nitrification, and 2) that denitrification was not affected by water content. The differences in gene abundance observed between R_{dr} and IR_{rf} in the olive orchard reflect the higher values of microbial functional activity and diversity of irrigated areas, found in the same system by other authors (Sofo et al., 2010, 2014). Very likely, the less significant differences detected in the peach orchard may be due to the shorter time under which the orchard has been managed. The relationship between *nifH* and *amoA* with SWC was much stronger than observed for *nosZ*, suggesting that soil water can influence the biological potential for N fixation and nitrification (N inputs in the system) more than the biological potential for denitrification (N output).

5. Conclusions

In conclusion, N_c/N_k and gene abundance, more than the measured chemical, biochemical and molecular soil parameters considered alone, can give a precise idea on N and C soil dynamics, that in turn affect positively or negatively soil quality and fertility.

Results support the hypothesis that the adoption of localized irrigation, together with sustainable soil management practices, can significantly affect the soil quality of a drip-irrigated orchard. This study confirms that in an orchard management based on organic matter inputs associated with minimum tillage, irrigation plays a key role and an adequate irrigation management is fundamental for improving soil quality.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.agwat.2017.10.014.

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Supplementary material

Supplementary Table 1. Chemical parameters of treated municipal wastewater applied in the olive orchard.

Parameter	Unit of measure	Value
pH	-	7.6
Conductivity	($\mu S\ cm^{-1}$)	884
Organic C	($mg\ L^{-1}$)	20.4
N ($NO_3^- + NH_4^+$)	($mg\ L^{-1}$)	18.3
Na	($mg\ L^{-1}$)	121.3
Mg	($mg\ L^{-1}$)	13.8
Ca	($mg\ L^{-1}$)	67.8
B	($mg\ L^{-1}$)	1.0
K	($mg\ L^{-1}$)	17.0
P	($mg\ L^{-1}$)	1.0
COD	($mg\ L^{-1}$)	180

COD: chemical oxygen demand.

Supplementary Table 2. Chemical parameters of the compost applied in the peach orchard.

Parameter		Unit of measure	Value
Reaction (1:10)		pH	9.25
Electrical conductivity at 25°C (1:10)		mS cm^{-1}	0.970
Total nitrogen	(N)	% DS	2.10
Organic nitrogen	(N)	% DS	2.01
Organic nitrogen / Total nitrogen ratio		%	96
Dry matter	DM	%	46.4
Moisture		% FS	53.6
Organic matter		% DS	60.4
Organic carbon		% DS	35.1
C / N			17.6
Ashes		% DS	39.6
Calcium	(CaO)	% DS	14.7
Magnesium	(MgO)	% DS	1.16
Sodium	(Na)	% DS	0.60
Potassium	(K_2O)	% DS	1.93
Phosphorus	(P_2O_5)	% DS	1.05
Iron	(Fe)	mg kg^{-1} DS	2410
Lead	(Pb)	mg kg^{-1} DS	75
Manganese	(Mn)	mg kg^{-1} DS	370
Chrome	(Cr)	mg kg^{-1} DS	12
Copper	(Cu)	mg kg^{-1} DS	110
Cadmium	(Cd)	mg kg^{-1} DS	0,40
Zinc	(Zn)	mg kg^{-1} DS	170
Nickel	(Ni)	mg kg^{-1} DS	8
Boron	(B)	mg kg^{-1} DS	48
Arsenic	(As)	mg kg^{-1} DS	1.3
Mercury	(Hg)	mg kg^{-1} DS	< 0.1
Chromium VI	(Cr)	mg kg^{-1} DS	< 0.1

DS: dry soil; FS: fresh soil.

Supplementary Table 3. Strains used for the standard curves of qPCR.

Gene	Organism	Source	Genome size (Mb)	Reference
nifH	*Azospirillum brasiliense*	ATCC[a] 29145	3.1	Rivera et al., 2014
amoA	*Nitrosomonas europea*	ATCC[a] 19718	2.8	Chain et al., 2003
nosZ	*Pseudomonas fluorescens Migular*	DSMZ[b] 4358	6	Hernández-Salmerón et al., 2016

[a]ATCC American Type Culture Collection

[b]DSMZ German Collection of Microorganisms and Cell Cultures