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

Silvia Pascazio[a], Carmine Crecchio[a], Marina Scagliola[a], Alba N. Mininni[b], Bartolomeo Dichio[b], Cristos Xiloyannis[b], Adriano Sofo[c,*]

[a] Department of Soil, Plant and Food Sciences (DiSSPA), Università degli Studi di Bari "Aldo Moro", Via Amendola, 165 – 70126 Bari, Italy
[b] Department of European and Mediterranean Cultures: Architecture, Environment and Cultural Heritage (DiCEM), Università degli Studi della Basilicata, Via San Rocco, 3 – 75100, Matera, Italy
[c] School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), Università degli Studi della Basilicata, Viale dell'Ateneo Lucano, 10–85100 Potenza, Italy

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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

* Corresponding author.
E-mail address: adriano.sofo@unibas.it (A. Sofo).

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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.