

# How soil microbial biodiversity is modified by soil chemical parameters in differently managed olive orchards

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## Abstract

**Soil restoration is an important challenge of the 21<sup>st</sup> century, facing the increasing soil degradation, characterized by decline in quality and decrease in ecosystem goods and services. Several studies confirmed that sustainable orchard management practices might sequester atmospheric CO<sub>2</sub> into soil, tree biomass and litter, enhancing soil organic carbon (SOC) stock and biodiversity. Higher biodiversity in ecosystems leads to greater stability and multifunctionality. In bacteria-plant interactions, both the bacteria and the plant profit from each other. These interactions play an important role in agriculture, positively affecting plant status and improving product quality. This study aimed at evaluating soil N/C parameters and microbial communities in soil, leaf (aerial part) and xylem sap between olive trees managed under sustainable practices for 17 years (i.e., no-tillage, drip irrigation with urban wastewater and recycling of polygenic carbon sources, like cover crops and pruning material) and trees managed under conventional ones (i.e., soil tillage, burning of pruning residues, mineral fertilization, rainfed), in a mature olive grove located in Southern Italy. In March 2017, samples of soil, leaf and xylem sap were collected in both treatments for DNA extraction and metagenomic analysis of the microbial communities. Soil samples were also collected for chemical and metabolic analyses. Results revealed that the long-term adoption of sustainable agricultural practices increased SOC, organic-N, and microbial biodiversity, with positive effects on plant growth protection and crop quality of olive plants.**

**Keywords:** metagenomic analysis, soil organic carbon, resilience of ecosystem, circular economy

## INTRODUCTION

Soil degradation provoked physical loss and reduction in quality of topsoil, the decrease in ecosystem goods and services, by affecting agricultural production (Lal, 2009, 2015a; Scherr, 2001).

Soil degradation trends can be reversed by conversion to a restorative land use and adoption of sustainable orchard management practices. The advantages of a change to sustainable soil management including no-tillage, recycling of polygenic carbon sources like cover crops and pruning material, use of organic manure or compost, have been extensively studied in olive groves. Such benefits include a high level of soil microbial genetic/functional diversity and complexity in soil (Sofa et al., 2014; Pascazio et al., 2015), a faster C and N turnover (Pascazio et al., 2018), higher soil organic carbon (SOC) (Montanaro et al., 2012) and soil water content (Celano et al., 2011), and better soil physical (Palese et al., 2014) and chemical characteristics (Sofa et al., 2010).

The olive tree (*Olea europaea* L. subsp. *europaea*) is one of the Mediterranean basin's main crops with great cultural and economic importance. From a commercial perspective, many olive tree varieties grow in the Mediterranean basin and 99% of the world's olive oil is produced (Muzzalupo and Perri, 2008). Among Mediterranean countries, Italy is the main exporter of olive oil in the world (Muzzalupo and Perri, 2008) and about 2 million ha of olive



groves are situated mainly in the southern Italian regions.

In olive groves, the sustainable soil management also gives considerable economic advantages to the farmers (Pergola et al., 2013) and provides efficient ecosystemic and sociocultural services (Montanaro et al., 2017). In addition, the sustainable soil management applied to olive orchards can reduce the negative repercussions on the environment linked to nutrient leaching/runoff, particularly weighty for nitrates (Palese et al., 2015).

Soil microorganisms, major responsible of soil fertility and quality (Bünemann et al., 2018), are strongly affected both by the type of soil management and irrigation (Enwall et al., 2007; Jeanbille et al., 2016). The functionality and metabolism of soil microorganisms are related to soil quality and fertility, as they influence the soil C and N contents, being bacteria an essential part of C and, even more, of N cycling processes (de Vries and Shade, 2013; Mooshammer et al., 2014; de Vries and Wallenstein, 2017; Zhang et al., 2014; Li et al., 2018).

Recent studies on olive groves have shown that sustainable soil management practices have positive effects on microbiological soil fertility and influence the microbes living on olive fruit and leaves (Ferreira et al., 2013; Sofu et al., 2014; Pascazio et al., 2015; Sastre et al., 2016). In the semi-arid Mediterranean agricultural lands, a new approach in fruit orchard management has become evident in order to improve or maintain soil quality, health and fertility (Hochstrat et al., 2006; Govaerts et al., 2008).

Because of the complexity and site-specificity of soils, defining soil quality is not an easy task (Bünemann et al., 2018). The study of the soil N-cycling bacteria and of the N dynamics could help to understand how soil management can affect soil status (Pascazio et al., 2018; Li et al., 2018). On this basis, the main objective of this study was to evaluate microbial diversity related to soil chemical parameters between olive trees managed under sustainable practices for 17 years and trees managed under conventional one in a mature olive grove located in southern Italy.

## **MATERIALS AND METHODS**

### **Experimental site, orchard management and soil, leaf and xylem sap sampling**

The trial was done in a 2-ha olive orchard ('Maiatica', 80-year-old plants, 8×8 m planting density) located in Ferrandina (southern Italy, Basilicata region) divided into two plots managed according to sustainable agricultural practices (S) and conventional ones (C) for 17 years.

Trees of the S plot were drip-irrigated by treated urban wastewater and pruned every year and residues shredded and left on the ground as mulch, soil was not tilled and covered by spontaneous self-seeding weeds mowed twice a year. The treated irrigation water was supplied from May to October by drip irrigation. The C plot was grown under rain-fed conditions and managed according conventional practices: soil tillage usually performed 2-3 times a year by plowing up to a depth of 25 cm, empirical soil fertilization, heavy pruning every two years and burning of pruning residues.

In March 2017, composite soil samples ( $n=3$ ) were collected from 0 up to 90 cm depth for performing chemical analyses. Soil, leaf and xylem sap samples were collected from three trees per treatment for 16S-based metagenomics analysis. Each composite soil sample was formed from ten subsamples pooled on site. About 10 shoots were cut off each tree and were collected. Three replicates ( $n=3$ ) of xylem sap, each from one single plant, were collected using a Scholander pressure chamber (Model 600, PMS Instruments, Corvallis, OR) pressurized with N<sub>2</sub> by applying high pressure (5-7 MPa).

### **Soil chemical analysis**

FAO guidelines (2006) were used for soil profile description of both S and C plots. On soil composite samples, total organic carbon (TOC), total N (TN) and pH were determined. All the soil samples were air-dried at approximately 25°C and then sieved through a 2-mm stainless steel sieve. Soil pH and total nitrogen (TN) were measured by Pansu and Gautheyrou method (2006). Total organic carbon (TOC) was determined by Walkley and Black method (1934). The statistical analysis of the soil chemical data was performed using Sigmastat 3.1

SPSS Inc. software (SPSS Inc., Quarry Bay, Hong Kong). The means of all the measured parameters were treated by one-way analysis of variance (ANOVA) with the orchard management type (S and C) and soil depth as factors.

### **16S-based metagenomics analysis**

DNA was extracted from an amount of 0.5 g of soil and phyllosphere using the method described by Pascazio et al. (2018). The sap collection was used for bacterial DNA extraction according to the protocol of the DNeasy Blood Tissue kit (Qiagen). For each sample of soil, leaf and xylem sap the taxonomic characterization up to the species level of bacterial communities were carried out through a 16S-based metagenomics approach. 16S library preparation and sequencing were performed by IGA Technology Services S.r.l. (Udine, Italy). The resulting libraries were pooled and sequenced from both ends on an Illumina MiSeq and more than 100,000 reads of about 300 bp were generated. The most abundant and long reads in each OTU (Operational Taxonomic Unit) were used for the taxonomy assignments using the GreenGene database, version 2013\_8 (<http://greengenes.secondgenome.com>) as a reference database. The Kruskal-Wallis test ( $P < 0.05$ ) was used to find differences in relative abundances of bacterial genera in soil and leaves samples under different management (<http://emperor.colorado.edu>).

## **RESULTS AND DISCUSSION**

### **Soil chemical parameters**

TOC and TN levels in the topsoil (0-5 cm) were significantly higher ( $p \leq 0.05$ ) in the S, compared to the C. Due to its high content of organic matter deriving from crop and pruning residues, litter provided S topsoil (0-5 cm) with higher TOC and TN contents, compared to the C (Figure 1). The C/N ratios in the S were significantly lower at 5, 10, 20, 30 and 60 cm. In the litter of the S, the values of TOC, TN and C/N were  $443.90 \pm 17.93$  (SD)  $\text{g kg}^{-1}$ ,  $5.60 \pm 0.58$   $\text{g kg}^{-1}$ , and  $79.27 \pm 8.91$ , respectively. In both the soil management systems, the trends of TOC and TN generally decreased with increasing soil depth (Figure 1).

In the S, the values of soil pH measured both in water and  $\text{CaCl}_2$  increased with rising soil depth from 0 to 10 cm, then remained relatively stable, and increased again at 90 cm, whereas soil pH in the C increased constantly from 0 to 90 cm in water (excepting for the value at 60 cm), in  $\text{CaCl}_2$  the trend was similar but with a slight decrease at 90 cm.

The higher content of organic matter, likely transported in the deeper soil layers in a dissolved form by soil solution, caused lower soil pH in the S system, whose values ranged from 7.68 to 8.16 (in water) due to the carbonatic nature of the soil. The average difference of topsoil (0-5 cm) pH measured in water and  $\text{CaCl}_2$  was higher in S (0.98) than in C (0.71), so demonstrating that the soil exchangeable  $\text{H}^+$  in S mainly derived from organic matter functional groups (Figure 1) (Sofo et al., 2019).

### **Microbial communities in soil, leaf and xylem sap**

Soil samples had the highest OTUs number followed by leaves and xylem sap, while the total reads detected in the phyllosphere were greater than in soil and xylem sap (Figure 2), too. By comparing the three different compartments, in soil and in the phyllosphere the relative abundance was higher compared to xylem sap at phylum level, indicated by number of taxa detected in each compartments, as specified below. At the phylum level, 17 bacterial taxa were present in soil (Figure 2a), 19 taxa predominated in the phyllosphere (Figure 2b) and 9 in the xylem sap (Figure 2c) (Fausto et al., 2019).

### **Identification of N-cycling bacteria**

In S and C soils, 18 bacterial genera differed in relative abundance at a degree higher than five-folds. The abundance of the bacterial taxa involved in N transformations were statistically different ( $p \leq 0.05$ ) between S and C.

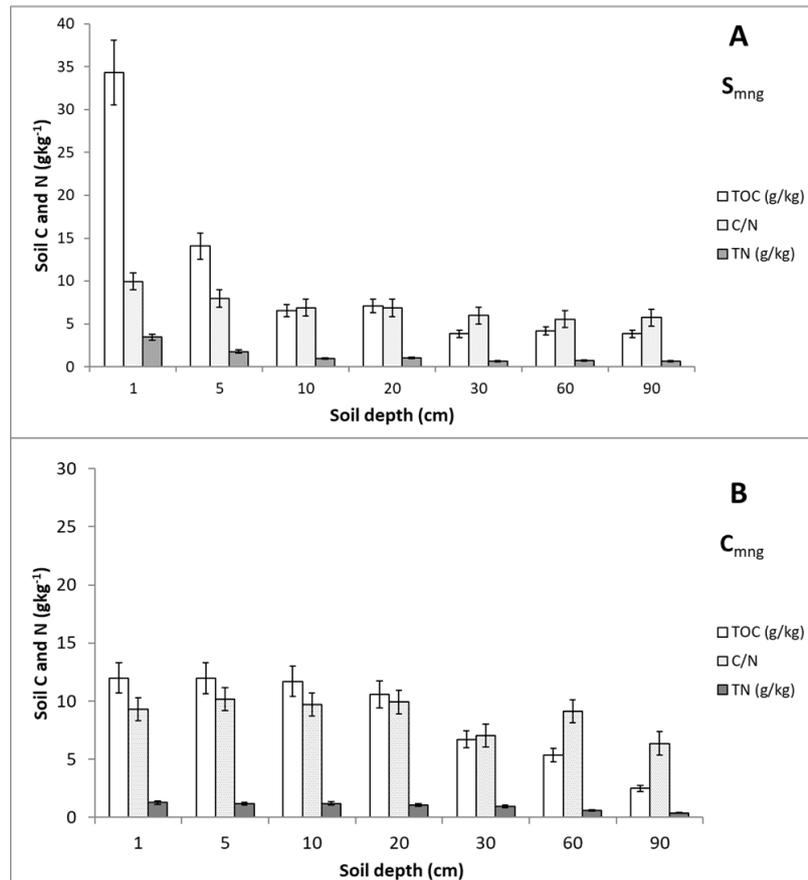


Figure 1. Soil total organic carbon (TOC), total nitrogen (TN) and C/N ratio of soils from (A) the sustainable (S) and (B) the conventional (C) systems measured at different soil depths. Each value represents the mean ( $\pm$ SD) from composite soil samples ( $n=3$ ) (redrawn from Sofo et al., 2019).

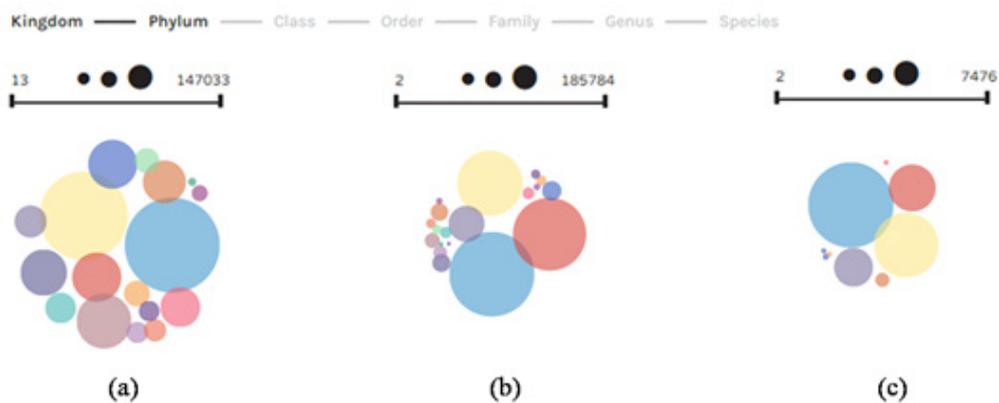


Figure 2. Bubble charts of relative abundance in (a) soil, (b) leaves and (c) xylem sap at the phylum level. Each bubble represents a taxon filled with a specific color, the size of which is proportional to the summary level of this taxon in the examined samples. The increasing scale of the total reads, represented by the smaller bubbles to the bigger ones, is displayed above each bubble chart (supplied by Phinch tool; Bik 2014) (redrawn from Fausto et al., 2019).

In the C system, the total abundance of free-living N-fixing Proteobacteria, Actinobacteria and Cyanobacteria was statistically higher than in the S system. The presence of Proteobacteria in the soil is an indicator of high soil nutrient-content, as most inhabit nutrient-rich soils (Castro et al., 2010; Gottel et al., 2011); owing to their ability to oxidise ammonia, they affect nitrogen availability for plants (Prosser 1990). Differently, N-fixing symbiont Proteobacteria belonging to the *Rhizobiales* order were more abundant in the S. Among them, the *Hyphomicrobiaceae* (genus *Rhodoplanes*) are mainly involved in C and N cycling and are also able to utilize different N substrates, such as N<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, or NH<sub>3</sub> (Kulichevskaya et al., 2006; Wang et al., 2016b). The genus *Rhodanobacter*, which includes many microorganisms involved in acidic denitrification in soils was found exclusively in S soil (van den Heuvel et al., 2010).

The abundance of two OTUs of denitrifying bacteria (*Denitrobacter* spp. and *Pseudomonas* spp.) was different between the two soil management systems, being higher in the S. The abundance of the three bacterial taxa involved in nitrogen oxidation from ammonia to nitrates (two Bacteria OTUs belonging to *Nitrospira* spp. and *Nitrosovibrio* spp., and the Archaea OTU *Nitrososphaera* spp.) was higher in the S, compared to C.

The difference between the C and S systems likely resides in the abundance of bacterial taxonomic groups having specific functions (qualitative differences) more than in parameters based on total bacterial metabolic activity (quantitative differences).

Our results depicted a scenario where N-cycling was generally upregulated in the S, compared to C. Interestingly, even if the total abundance of N-fixing bacteria was comparable in the two systems (610 in the S and 567 in the C), significant differences were found in the abundance of free N-fixing bacteria (higher in the C) and symbiont N-fixing bacteria (higher in the S) (Fausto et al., 2019; Sofo et al., 2019).

## CONCLUSIONS

From the soil chemical analysis, C and N stocks, and the genetic and functional diversity of soil bacteria, it emerged that a sustainable orchard management caused an improvement in soil organic matter, whose positive effects were reflected on the composition of microbial communities. In particular, soil N-cycling bacteria was revealed to be higher in sustainable management than conventional one, without affecting total bacterial metabolic activity and diversity. The potential benefits of the specific bacterial taxa detected under the S system could improve plant growth protection and provide a higher crop quality in olive plants.

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