

Nutrient dynamics, soil properties and microbiological aspects in an irrigated olive orchard managed with five different management systems involving soil tillage, cover crops and compost

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ABSTRACT

The aim of the present study was to determine the short-term effects (three years) of four sustainable management systems including different carbon inputs (compost, pruning residues and cover crops, applied solely or combined) on the mineral content of soil, olive trees and weeds, on some specific groups of soil microorganisms, and on weed mycorrhizal colonization in an olive orchard compared to a conventional system involving soil tillage and only mineral forms of fertilizers. The study was performed between 2013 and 2015 in a 40-year-old olive plantation. The results showed that soil organic matter, as well as main macro- and micronutrients, were markedly improved following three years of increased biomass inputs. Data related to the mycorrhizal colonization of spontaneous weed flora and to actinobacteria, *Azotobacter* and proteolytic bacteria suggest favourable effects on soil biology and agro-ecosystem complexity. Sowing a mixture of winter cover crops for three successive years also contributed to soil enrichment in biological as well as mineral nutrient aspects. Adoption of the sustainable management here applied practices is in complete agreement with the European policy on the transition from a linear to a circular economy and would provide significant benefits for rural stakeholders and ecosystems in the long term.

Key words | carbon inputs, mycorrhiza, *Olea europaea*, soil microbiota, soil nutrients, sustainable agriculture

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INTRODUCTION

In the Mediterranean area, 16% of the total cultivable land is used for fruit orchards (Palese *et al.* 2013). Olives (*Olea europaea* L.) have been cultivated in the Mediterranean for centuries, covering over 10 Mha (Rallo *et al.* 2018 with data from IOC, www.internationaloliveoil.org) and they are an integral part of the Mediterranean landscape (Pergola *et al.* 2013). Climate change has significant impacts on olive vegetative and reproductive functions (Koubouris *et al.* 2015a, 2015b). In the semi-arid areas, where olive is cultivated, a new approach in orchard management is imposed by environmental emergencies, such as soil degradation,

decline in soil fertility, pollution of the environment and water shortage (Gómez *et al.* 2009a, 2009b; Ramos *et al.* 2010; Kourgialas *et al.* 2016, 2017a). In this context, the knowledge of the chemical and microbiological properties of soils of olive orchards is nowadays necessary.

In olive orchards, sustainable management practices with low environmental impact, such as minimum tillage or no-tillage, recycling of locally derived organic matter and adequate irrigation, rational fertilization and pruning are required to improve soil physicochemical characteristics, water retention, crop productivity and profitability,

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and, in addition, to reduce erosion, environmental pollution, pauperization in soil organic matter and groundwater contamination (Hernández *et al.* 2005; Al-Absi *et al.* 2009; Gómez *et al.* 2009a, 2009b; Palese *et al.* 2014). These sustainable practices can also have positive effects on soil microbial community, increasing its microbial biomass, activity and complexity (Moreno *et al.* 2009; Pascazio *et al.* 2009; Sofo *et al.* 2010, 2014). In olive groves, a positive influence of sustainable orchard management systems on soil biochemical characteristics and soil microbial genetic diversity has been observed, and important physiological groups of microorganisms related to soil fertility were identified (Hernández *et al.* 2005; Moreno *et al.* 2009; Sofo *et al.* 2010), including important mycorrhizal species (Calvente *et al.* 2004; Porrás-Soriano *et al.* 2009; Tataranni *et al.* 2012).

Whereas microbial biomass and metabolism of soil microbial communities change significantly in response to both long-term and short-term soil management, improvement of soil chemical properties and soil nutritional status is usually evident in long-term (>10 years) adequate soil treatments (Marschner *et al.* 2003; Persiani *et al.* 2008; Sofo *et al.* 2010, 2014). It is known that soil and plant chemistry at the elemental level plays a key role in soil fertility. Olives, like other higher plants, on top of the C, H and O found in natural sources, require macro- (N, S, P, K, Mg, Ca) and micronutrients (Fe, Zn, Cu, Mn, B, Cl) in appropriate amounts for continuing growth and yield (Celano *et al.* 1999; Connor & Fereres 2010). Mineral content in olive organs – particularly leaves – and mineral partitioning in the whole orchard is a reliable method for diagnosing tree nutritional status and represents an important tool for determining the efficacy of an agronomic treatment (Fernández-Escobar *et al.* 1999, 2004). The loss of chemical soil quality and the decrease in soil minerals, essential for plant growth (in particular N and P), is a process that mostly affects areas where intensive agriculture and an indiscriminate use of external energetic inputs (fertilizers, pesticides, water) are adopted (Moreno *et al.* 2009; Ramos *et al.* 2010). Soil microbiota itself influences soil fertility and plant growth by regulating nutrient availability and increasing their turnover (Wu *et al.* 2016; Zheng *et al.* 2016). In this view, sustainable plant and soil management systems in olive growing could determine an optimal plant nutritional equilibrium.

The aim of the present study was to determine the short-term effects (three years) of five management systems involving different carbon inputs (compost, pruning residues and cover crops) in an irrigated olive orchard on the mineral content of soil, olive plants and weeds, on the abundance of some specific groups of soil microorganisms, and on weed mycorrhizal colonization. The hypothesis is that the optimization and innovation of agricultural practices with a low negative environmental impact could allow the normal levels of both chemical, mineral and microbial soil quality and fertility to be maintained or recovered.

METHODS

Field site and treatments

The study was performed between 2013 and 2015 in a 40-year-old olive plantation (*Olea europaea* L., cv. Kalamata; 1.1 ha, distances between trees 7×7 m) located in the experimental station of the Institute of Olive Tree, Subtropical Crops and Viticulture (Nerokourou, Crete island, Southern Greece ($35^{\circ}28'36.76''$ N, $24^{\circ}02'36.44''$ E; 51 m a.s.l.). According to the meteorological station placed in the Institute, the annual average air temperature was 18°C , the relative humidity (RH) was 64% and the annual rainfall was 700 mm. The soil was sandy loam, pH 7.2, with very low contents in main macro-elements ($7.24 \text{ mg kg}^{-1} \text{ NO}_3\text{-N}$, $8.53 \text{ mg kg}^{-1} \text{ Available P}$ and $72 \text{ mg kg}^{-1} \text{ ex-K}$ at 0–40 cm depth) (detailed soil properties in Kourgialas *et al.* 2017b) and irrigation was implemented weekly according to evapotranspiration through drippers (five per tree), each with a discharge rate of 4 L h^{-1} and wetting a ~ 1.0 m wide strip along the in-row. Five treatments, performed according to a completely randomized design, with three replicates per treatment ($n = 3$, for a total of 15 plots; each plot included four olive trees, covering about 200 m^2 of soil), were considered:

- (a) 'CON': Control consisted of no addition of organic materials and maintaining soil free of weeds through tillage and herbicides.
- (b) 'COMP': The compost used for the experiment was a commercial product consisting of recycled olive mill by-products (olive leaves, fruit pulps and stones and

liquid waste) and was added to the soil without tillage at rates of 6 t ha⁻¹ in February 2013, 9 t ha⁻¹ in March 2014 and 9 t ha⁻¹ in March 2015. The compost had the following characteristics: C/N=18, pH 7.8; 49.76% (w/w, on dry matter basis) total C, 2.77% (w/w) total N, 2.26% (w/w) total K, and 0.18% (w/w) total P.

- (c) 'PRUN': Soil was mulched with chopped pruning residues, deriving from the olive trees of each plot, without tillage at rates of 20 t ha⁻¹ in April 2013, 20 t ha⁻¹ in May 2014 and 20 t ha⁻¹ in July 2015. Pruning residues had 51–55% (w/w; dry matter basis) total C, 0.6–1.8% (w/w) total N, 0.4–1.2% (w/w) total K and 0.4–1.2% (w/w) total P.
- (d) 'COVER': A 150-kg ha⁻¹ seed mix of leguminous crops (*Vicia sativa*, *Pisum sativum* subsp. *arvense*, *Trifolium alexandrinum*, *Vicia faba* var. *minor* and *Medicago sativa*) and 30 kg ha⁻¹ of seeds of *Avena sativa* were seeded in December of 2013, 2014 and 2015. In each subsequent spring, plants were mowed without being incorporated into the soil.
- (e) 'ALL': This treatment included the application of the above treatments (b, c and d) simultaneously.

Soil physicochemical properties

From each plot, three composite soil samples were collected in January 2016, at the depth of 0–20 cm ($n = 3$). Each composite sample was formed from ten 7 cm-diameter cores sampled within a 0.50 m radius to minimize spatial variability and pooled on site. Soil samples were air-dried at room temperature, disaggregated in a ceramic pestle and mortar and sieved through a 2 mm sieve. The <2 mm fraction of the soil, representing the percentage of clay, silt and sand, was used for all soil analyses. Electrical conductivity and soil pH were measured in a 1:2.5 soil/distilled water (w/v) suspension. The exchangeable cations (Ca, K and Mg) were determined by extraction of soil samples in 1 N ammonium acetate. Nitrate (NO₃⁻) was extracted with 1 M KCl for 1 h and measured spectrophotometrically by the Cd reduction method (Keeney & Nelson 1982). Soil organic matter was determined by the Walkley-Black procedure (Nelson & Sommers 1982). Soil available P was determined

according to Olsen *et al.* (1954). The bioavailable fraction of micronutrients and heavy metals (B, Cu, Fe, Mn, Zn, Ni, Cr, Cd and Pb) was determined after extraction with 0.005 M diethylene triamine pentaacetic acid (DTPA) (pH 7.3) using the method of Lindsay & Norvell (1978). The concentrations of exchangeable cations and bioavailable metals were measured by inductively coupled plasma (ICP-OES, Optima 8300, Perkin Elmer).

Plant tissue minerals

Plant tissue nutrient analyses were carried out both in olive leaves and in spontaneous *Oxalis pes-caprae* L. (the most abundant spontaneous weed species in the experimental field) for the determination of the effects of the different treatments in soil fertility and plant nutrition. For olive leaf analysis, three samples of 200 leaves each were collected from each plot and treatment ($n = 3$). In addition, three samples of 200 leaves each were collected from the above-ground part of *Oxalis* from each plot and treatment ($n = 3$).

For the determination of mineral content, the plant tissues were dried at 70 °C to a constant weight and then finely ground with a stainless steel beater analytical mill. Total N concentration was determined colorimetrically after wet digestion (Kjeldhal method) of a subsample of 0.05 g of dry tissue (Bilbao *et al.* 1999). Another subsample of 1 g was ashed in a muffle furnace at 550 °C for 6 h and then digested with 5 mL of concentrated HNO₃ 69% (Jones & Case 1990). The concentrations of nutrients (Ca, Mg, K, Cu and Mn) were determined by ICP-OES, phosphorus by the vanado-molybdate method (Jones & Case 1990) and boron was measured colorimetrically according to the method of Gaines & Mitchell (1979).

Mycorrhizal colonization

Three composite samples of *Oxalis* roots from each treatment ($n = 3$) were collected at 0–20 cm soil depth in February 2016 for the determination of mycorrhizal colonization. Once the roots were collected, they were washed under tap water to free them from the soil and to distinguish the healthy roots from the damaged ones. The mycorrhizal colonization was determined by staining according to the method of Phillips & Hayman (1970) with the modifications

of Koske & Gemma (1989) and Merryweather & Fitter (1991). The washed roots were immersed in 2% KOH solution for 24 h, then the roots were rinsed thoroughly with deionized water and immersed in 2% HCl for 20 min. Afterwards, the roots were rinsed thoroughly with deionized water and placed in 0.05% Trypan blue dye solution for 24 h. After the staining, the roots were stored in glycerol solution until the measurement of root colonization in the microscope, according to the method of McGonigle et al. (1990).

Soil microbial analysis

From each plot, one composite soil sample, consisting of three sub-samples, was collected at the depth of 0–20 cm. Two replicates of 5 g fresh soil of each soil sample were suspended in 45 mL sterile 0.1% sodium phosphate one quarter-strength Ringer solution (2.25 g NaCl L⁻¹, 0.105 g KCl L⁻¹, 0.045 g CaCl₂ L⁻¹, 0.05 g NaHCO₃ L⁻¹ and 0.034 g citric acid L⁻¹) and sonicated for 2 min to disperse microbial cells. Ten-fold serial dilutions of the supernatants were made in sterile ultrapure water. Aliquots were spread plated in triplicate on 1:10 strength tryptic soy agar medium amended with 0.1 mg mL⁻¹ cycloheximide (Sigma Aldrich, New York, USA) for bacterial counting.

Actinobacteria were isolated by using modified casein starch agar supplemented with 0.12 mg cycloheximide mL⁻¹. *Azotobacter* spp. were isolated by modified Brown substrate, whereas proteolytic bacteria were quantified by the MPN method in a culture medium containing gelatin (Oxoid Ltd, Hampshire, UK) (Sofo et al. 2010). Counting took place after suitable incubation at 28 °C for 3 days for total bacteria and 15 days for the specific bacterial groups (actinobacteria, *Azobacter* and proteolytic bacteria).

Statistical analysis

Data were analysed using the Sigmastat 3.1 SPSS Inc. software (SPSS Inc., Chicago, IL, USA) and were subjected to one-way analysis of variance (ANOVA). Significantly different means between control and treatments were statistically analysed by Fisher's LSD test at $P \leq 0.05$. The number of replicates (n) for each measured parameter is specified in the figure captions.

RESULTS AND DISCUSSION

Soil physicochemical properties

No significant differences were found for sand, silt and clay soil contents (w/w) (Table 1). Significant increases in organic matter content occurred only in ALL but not in the other three treatments with organic matter inputs (Figure 1). Significant higher levels of NO₃-N and available P were found in COMP and ALL, compared to the other treatments (Figure 1). The soil pH did not significantly change among the five treatments (Figure 1).

The ex. K content was significantly higher in COMP, PRUN and ALL compared to the other two treatments (Figure 2). The levels of B, Cu, Fe, Mn and Na were not significantly different among the treatments (Figure 2), whereas those of Zn and Ca were significantly higher in COMP and ALL, and that of Mg only in ALL (Figure 2).

The absence of differences in soil texture and pH (Table 1) demonstrates that the observed differences in the rest of the soil parameters examined were not due to soil texture.

Similar values in soil pH among the five treatments (Figure 1) imply that the adoption of sustainable practices did not cause any soil acidification, at least for the examined short period. This result can be related to the soil minerals available for plants, as the soil pH is a simple but reliable diagnostic test that can predict availability of some nutrients, such as Mn and Fe (Connor & Fereres 2010). The observed increases in soil organic matter (Figure 1), and consequently of NO₃-N and available P (Figure 1), in ALL treatment (where all the sustainable practices are combined) are in

Table 1 | Particle size distribution at soil depth 0–30 cm under the five different land-use regimes. Data represent average values ± standard error ($n = 3$)

Treatment	Size distribution (%)		
	Sand (20–200 μm)	Silt (2–20 μm)	Clay (<2 μm)
CON	56.0 ± 1.1	25.3 ± 1.8	18.7 ± 2.4
COMP	59.3 ± 1.3	23.3 ± 0.7	17.3 ± 1.8
PRUN	58.7 ± 1.3	25.3 ± 0.7	16.0 ± 1.1
COVER	56.0 ± 1.1	26.7 ± 0.7	17.3 ± 1.8
ALL	54.7 ± 2.4	26.7 ± 0.7	18.7 ± 2.9

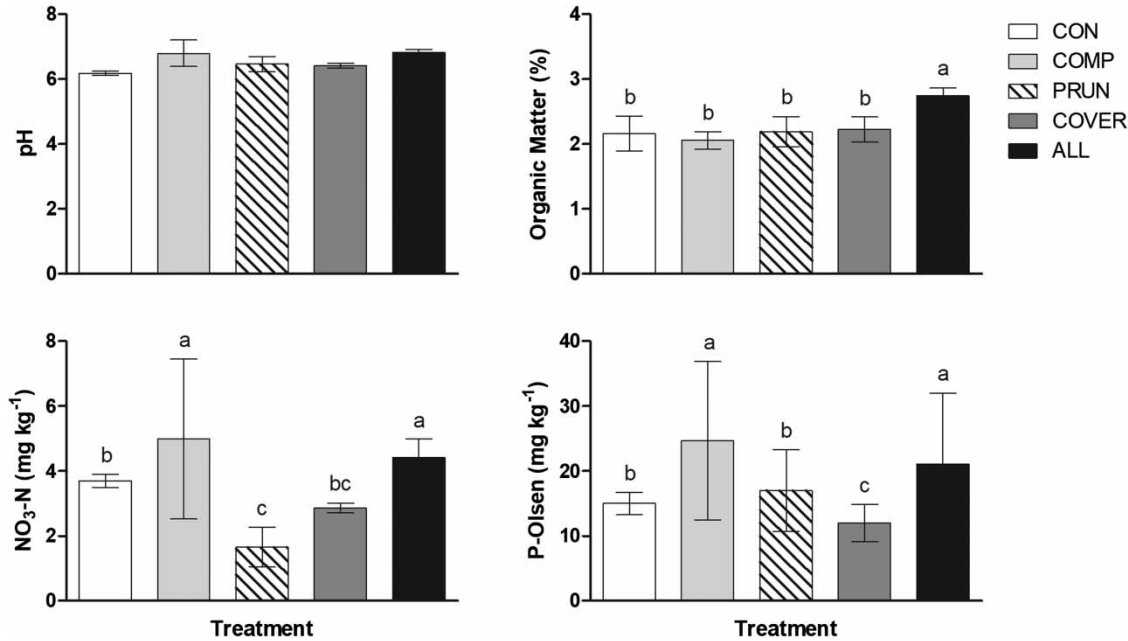


Figure 1 | Selected chemical properties of the 0–30 cm soil depth under the five different treatments. Average values \pm SE are shown ($n = 3$). Different letters denote significance at $P < 0.05$.

line with previous short- and medium-term experiments in olive orchards managed with a set of conservative agricultural practices (Castro *et al.* 2008; Sofo *et al.* 2010; Palese

et al. 2014). Particularly, in the study of Palese *et al.* (2014), total soil organic carbon increased by 0.317% (w/w) in the 0–10 cm soil layer after two years of different management,

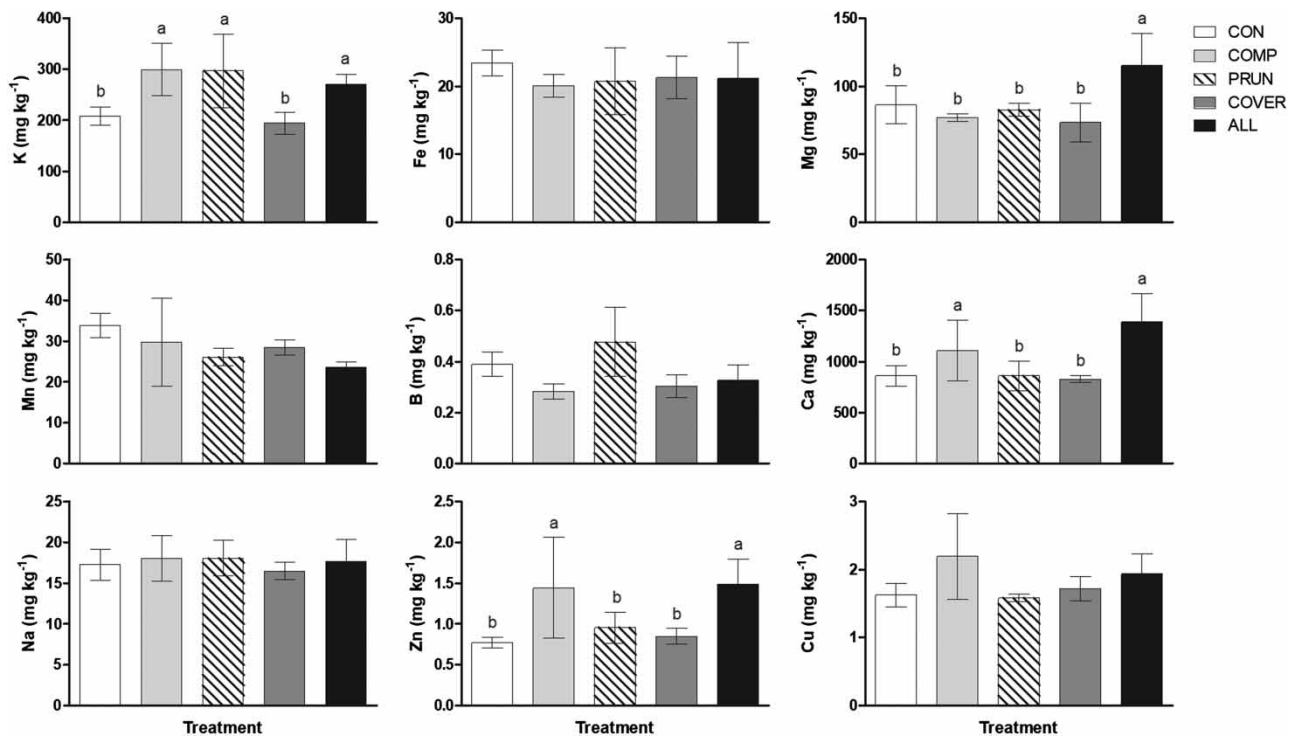


Figure 2 | Soil nutrient status of the 0–30 cm soil depth under the five different treatments. Average values \pm SE are shown ($n = 3$). Different letters denote significance at $P < 0.05$.

a value very close to that of this experiment (+0.588% in ALL compared to CON, after three years). In an earlier soil analysis, after two years of increased carbon inputs, soil organic matter increased at 0–10 cm but not in 10–20 cm (Koubouris *et al.* 2017), indicating that long-term sustainable soil management is needed to achieve remarkable improvement in soil fertility indicators. Several external factors may also affect changes in soil quality in olive orchards due to carbon inputs such as irrigation conditions, air temperature and precipitation (Kavvadias *et al.* 2017). Generally, the combined sustainable treatments of ALL provoked an increase of some important plant micro- and macronutrients, and particularly that of K, that often is a limiting soil nutrient for olive trees (Celano *et al.* 1999; Connor & Fereres 2010).

Plant minerals

The levels of the main macronutrients (N and P) in olive tree leaves were both significantly higher in ALL (P was also higher in PRUN) compared to the control (Figure 3). The mineral level did not show any differences for K, Ca, Mg, Mn, Zn, Cu and B (Figure 3). Compared to the other treatments, Fe was significantly lower in PRUN (Figure 3).

In *Oxalis* weed leaves, significant increase was observed in K content in PRUN, ALL and especially COVER compared to CON (Figure 4). All sustainable treatments were equally efficient in increasing B content compared to CON (Figure 4). *Oxalis* weed leaf Mn content was significantly higher in all sustainable treatments and especially in ALL. The content of Na was significantly higher in COVER and ALL compared to CON (Figure 4). No statistical differences were observed for N, Fe, Mg, Ca, Zn and Cu while P content was higher in CON compared to all other treatments (Figure 4).

Research on olive leaf mineral contents provide valuable information about tree nutritional status (Celano *et al.* 1999; Fernández-Escobar *et al.* 2004; Al-Absi *et al.* 2009; Connor & Fereres 2010). Good nutritional status and active growth of olive trees were indicated by the levels of the main macronutrients (N and P), both significantly higher in ALL (P is also higher in PRUN) (Figure 3). The synergic action of the three treatments (COMP, PRUN and

COVER) was confirmed also in this case. Olive has the capacity to mobilize N and P to meet its needs for several years before leaf deficiency symptoms are visible and many years can pass before the detection of a recovery response to the addition of soil N (Connor & Fereres 2010). Accordingly, the observed increases in both soil and olive leaf N and P in ALL (Figures 1 and 3) demonstrated the beneficial effects of a combined sustainable orchard management.

Oxalis is the most dominant spontaneous weed in Greece and in this experimental field. It was chosen in this experiment instead of olive roots because of its shallow root system, close to the soil surface, where the organic inputs were spread, and because it is likely more responsive to the sustainable practices adopted here. On the other hand, it is known that olive roots grow deeper and further from the soil surface. The higher content of B and Mn in *Oxalis* in groves subjected to sustainable managements alone or in combination is of primary importance, as soil B and Mn are needed at low concentrations in olive trees and their excess can cause phytotoxic effects in this species (Fernández-Escobar *et al.* 2004; Al-Absi *et al.* 2009); however, quite often, B deficiencies are evident in olive culture. The significantly higher level of K in COVER, compared to the COMP and CON treatments (Figure 4), could be attributed to the interactions between cover crops and wild vegetation in this treatment. The content of Na, significantly higher in ALL compared to all other treatments (Figure 4), indicated that *Oxalis* could mitigate phenomena of soil salinity and water deficit, when soil water potential decreases and olive trees cannot absorb enough water (Dichio *et al.* 2006).

Microbial analysis

Total bacteria were higher in PRUN compared to all other treatments but this difference was statistically significant only compared to ALL (Figure 5). Proteolytic bacteria were significantly higher in COMP and PRUN, compared to the other treatments while the lowest levels were observed in COVER (Figure 5). A significant increase in *Azotobacter* counts compared to CON was observed in all sustainable treatments and especially in PRUN (Figure 5). Actinomycetes counts revealed a significantly higher

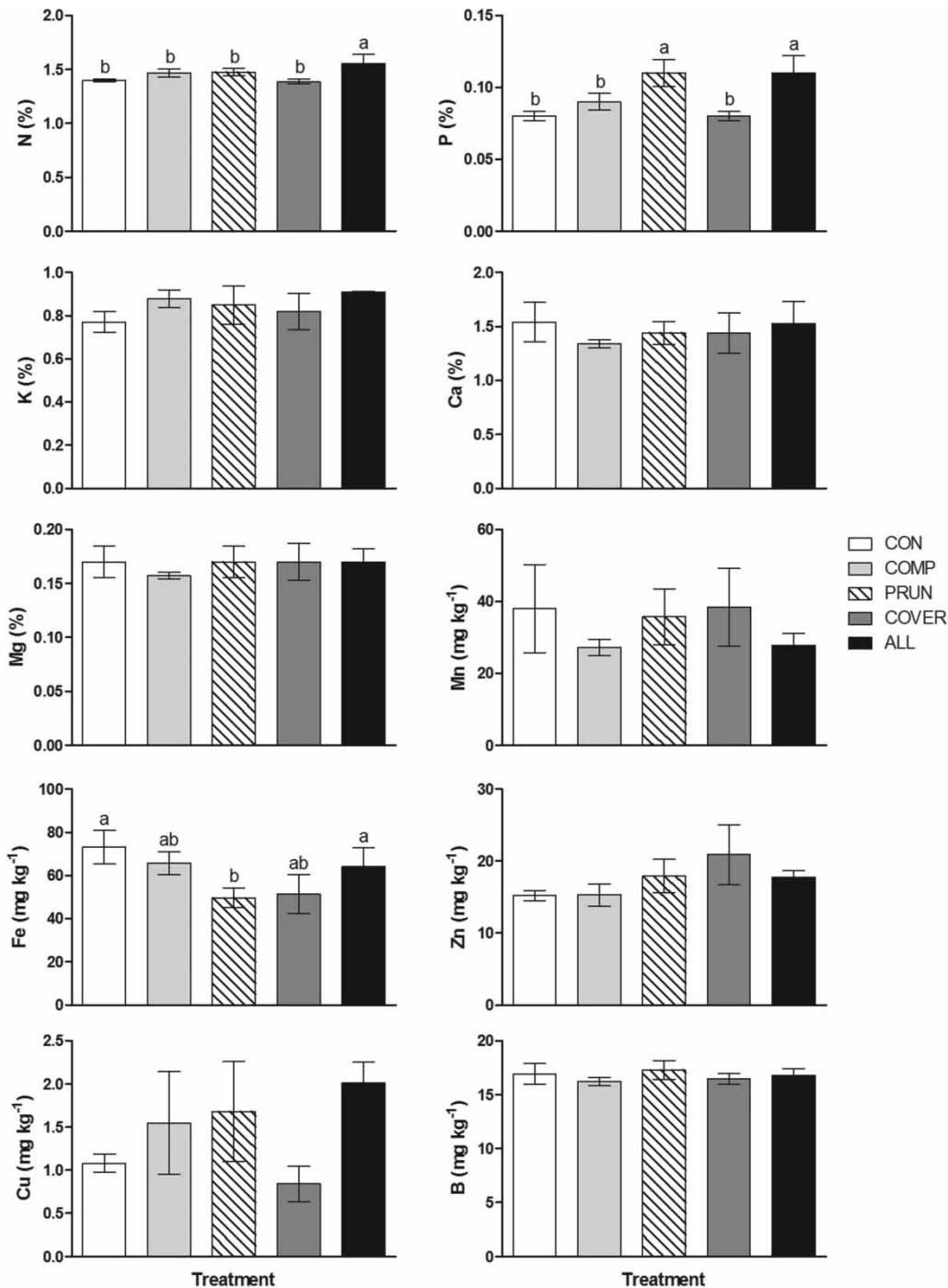


Figure 3 | Concentrations of inorganic elements in olive leaves. Average values \pm SE are shown ($n = 3$). Different letters denote significance at $P < 0.05$.

number in all four sustainable treatments, and particularly in COVER, compared to CON (Figure 5). Finally, mycorrhizal colonization of *Oxalis* roots was significantly higher in ALL, compared to the other four treatments (Figure 6).

It is known that the first soil layers are the habitat for a high number of microbial communities that play a key role in nutrients' availability and turnover. In a complex agroecosystem, as in the case of an olive orchard, particular

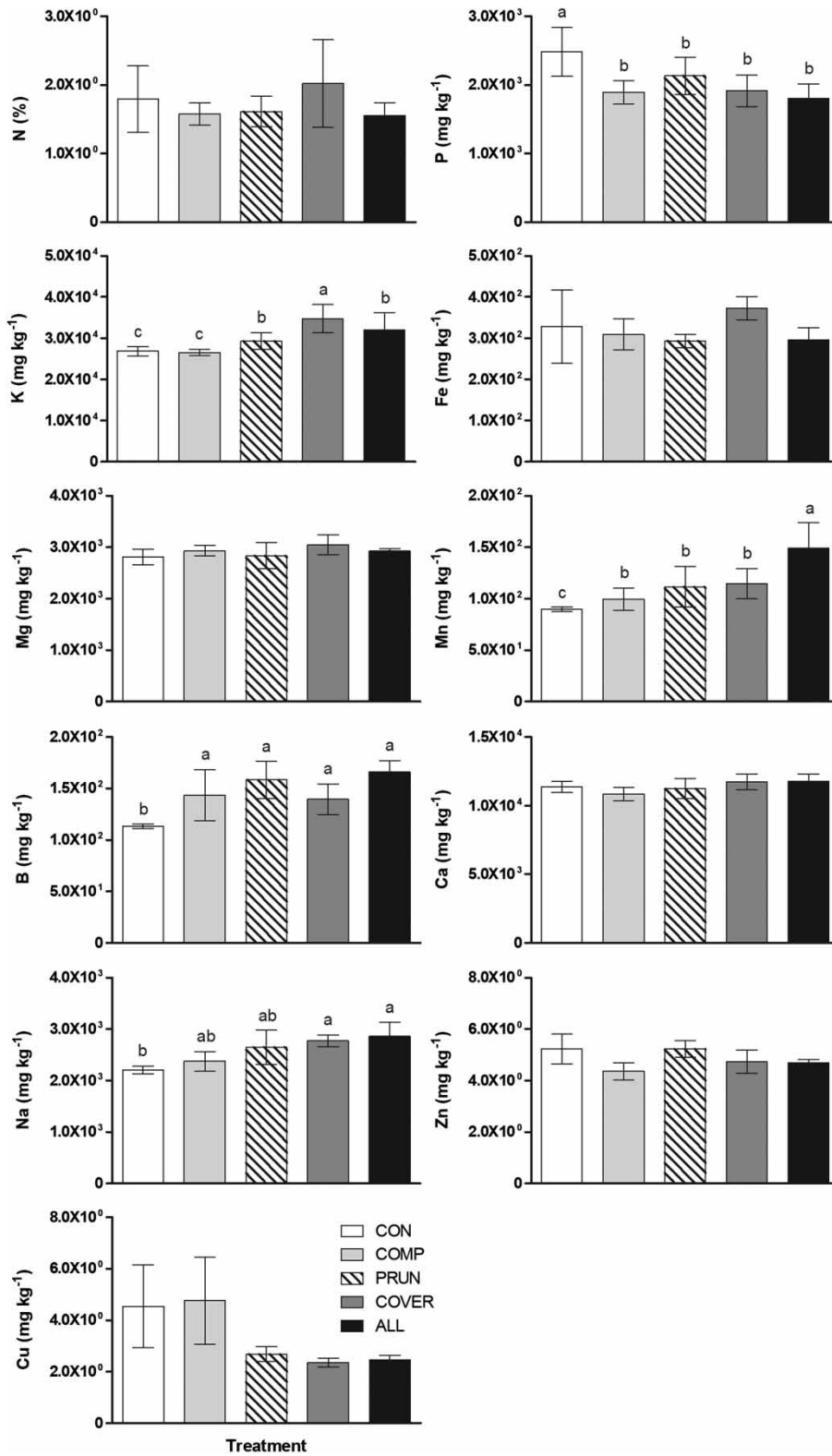


Figure 4 | Concentrations of inorganic elements in *Oxalis* leaves. Average values \pm SE are shown ($n = 3$). Different letters denote significance at $P < 0.05$.

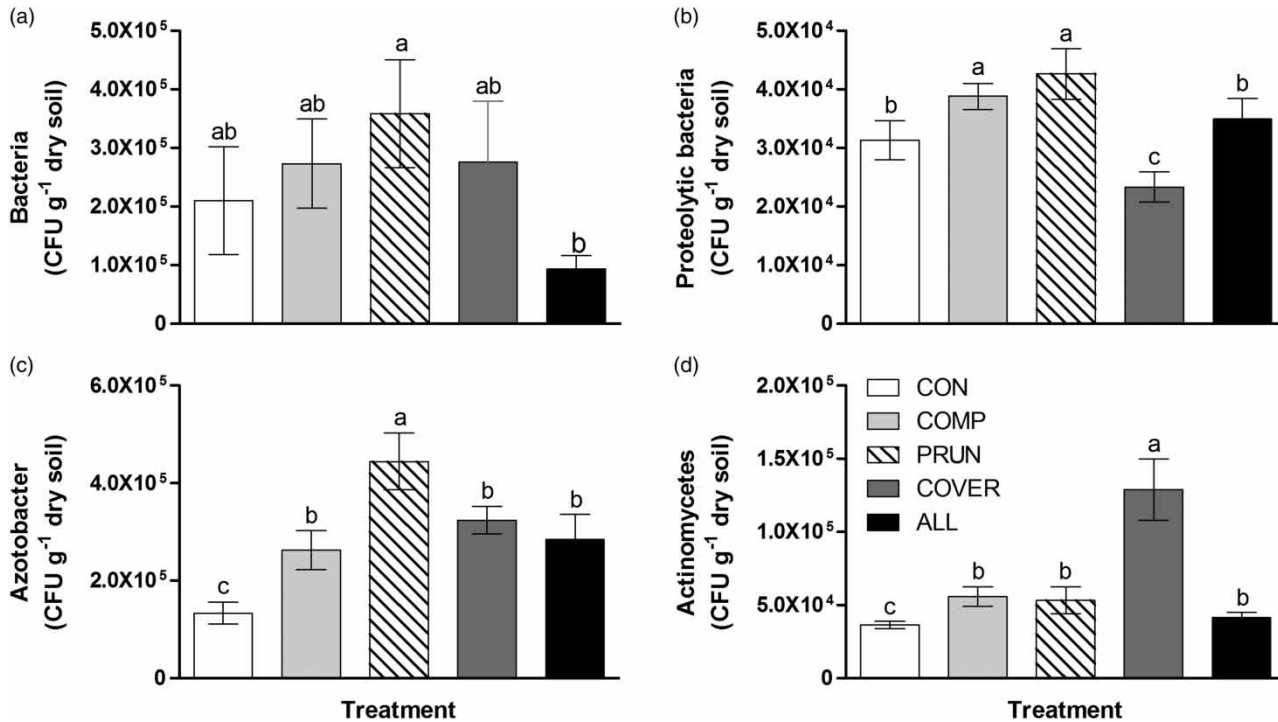


Figure 5 | Total cultural bacterial (a), proteolytic bacterial (b), azotobacterial (c) and actinomycetes (d) counts in the five treatments. Average values \pm SE are shown ($n = 3$). Different letters denote significance at $P < 0.05$.

emphasis could be given to the microorganisms involved in the nitrogen cycle, as nitrogen is the most important nutrient influencing vegetative growth, and yield quality and quantity of trees (Fernández-Escobar et al. 2004; Morales-Sillero et al. 2009; Wu et al. 2016).

The higher number of total bacteria in PRUN (Figure 5) has also been observed by Sofo et al. (2010, 2014) in an Italian olive orchard managed in a sustainable way with a

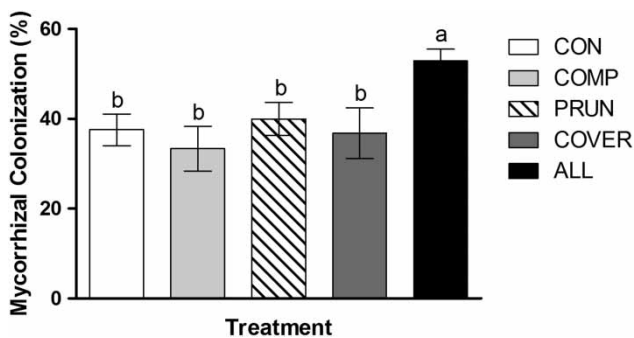


Figure 6 | Mycorrhizal colonization in *Oxalis* roots under the five different land-use regimes. Average values \pm SE are shown ($n = 3$). Different letters denote significance at $P < 0.05$.

wide range of organic carbon inputs. Actinobacteria are able to colonize rhizosphere and use root exudates as the carbon source, supply roots with easily assimilable nitrates and play a key role in the biological control of root pathogens and in the maintenance of soil health (Govaerts et al. 2008). On the other hand, *Azotobacter* are free bacteria able to reduce $N \equiv N$ to NH_3 for the biosynthesis of organic nitrogen compounds. Both bacteria groups play a key role in soil fertility processes (Sofo et al. 2010) and proved to be significantly higher in all the four sustainable treatments, compared to CON (Figure 5). Interestingly, the very high actinobacteria number in COVER (Figure 5) suggests that enrichment of weed flora biodiversity through leguminous crops has significant implications for soil microbiota. Indeed, prolonged monoculture was found to affect microbial diversity and activities and increased pathogenic microbes (Wu et al. 2016). For this reason, the inclusion of legumes in the agroecosystem, beyond N biological fixation, could affect soil biological processes, with positive influence on crop yields, as recently reviewed by Ianetta et al. (2016). Finally, proteolytic bacteria, responsible for soil protein

degradation in peptons, peptic acids and, finally, in amino acids, were significantly higher in COMP and PRUN (Figure 5), likely for the high inputs of organic N in these two treatments.

Mycorrhiza enhance health and vigour of the host plants (Calvente *et al.* 2004; Porrás-Soriano *et al.* 2009; Tataranni *et al.* 2012). In olive, arbuscular mycorrhizal (AM) symbioses, such as those of *Glomus* spp., are known to play a critical role in plant nutrition and mineral acquisition, growth, and tolerance to abiotic stresses (Calvente *et al.* 2004; Dag *et al.* 2009; Porrás-Soriano *et al.* 2009; Kapulnik *et al.* 2010; Tataranni *et al.* 2012). Interestingly, the high degree of mycorrhizal colonization of *Oxalis* roots, compared to the other four treatments (Figure 6), can be considered a positive parameter related to soil quality and fertility. Tataranni *et al.* (2012) found that mycorrhization of olive plants included positive effects on plant height, number of leaves, shoot dry weight, root dry weight and the root–shoot ratio. Moreover, Dag *et al.* (2009) reported that arbuscular mycorrhiza increased nutrient uptake by olive seedlings, including all the three macro-elements, N, P and K. Thus, the high amount of these beneficial fungi found in the ALL treatment (Figure 6) could increase the efficiency of plant roots to absorb water, macro- and micro-elements from the soil.

CONCLUSIONS

Crop management practices involving recycling agricultural waste, such as soil mulching with chopped pruning residue and composted by-products without soil mechanical disturbance, have great potential for enhancing soil biodiversity and fertility. In this study, soil organic matter, as well as the balance of macro- and micronutrients, was markedly improved following three years of biomass inputs. The results on biological indicators of soil quality, such as mycorrhizal colonization of spontaneous weed flora, actinobacteria, *Azotobacter* and proteolytic bacteria suggest favourable effects on soil biology and ecosystem complexity in the four studied sustainable treatments. Sowing a mixture of winter cover crops for three successive years contributed to soil biological enrichment, as well as mineral nutrient aspects.

A large part of cultivated soils in Southern Europe as well as in other continents is considered poor in nutrient and biological terms. The adoption of the sustainable management practices applied in this study is in complete agreement with the European policy on the transition from a linear to a circular economy and would provide significant benefits for rural stakeholders and ecosystems in the long term.

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