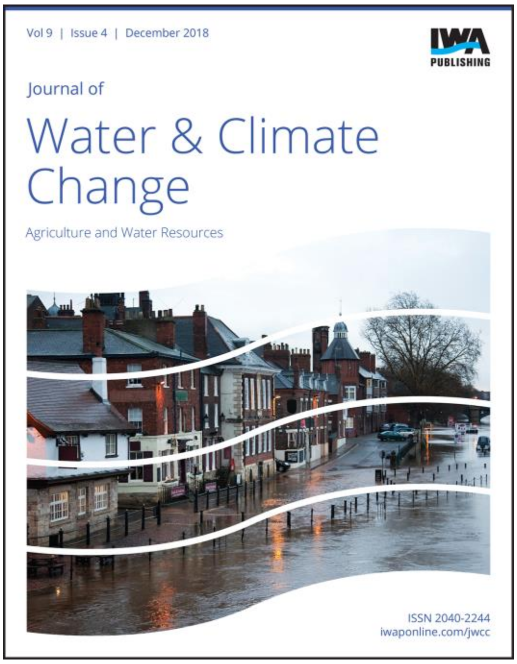

Nutrient dynamics, soil properties and microbiological aspects in an irrigated olive orchard managed with five different management systems involving soil tillage, cover crops and compost

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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; Porras-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°28′36.76″ N, 24°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 mg kg^{-1} NO_3- *N*, 8.53 mg kg^{-1} $^{\text{Available}}$P and 72 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 (NO_3^-) 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 aboveground 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 HNO_3 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 $CaCl_2$ L^{-1}, 0.05 g $NaHCO_3$ 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 NO_3^--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 NO_3^--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 ($n = 3$)

	Size distribution (%)		
Treatment	**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

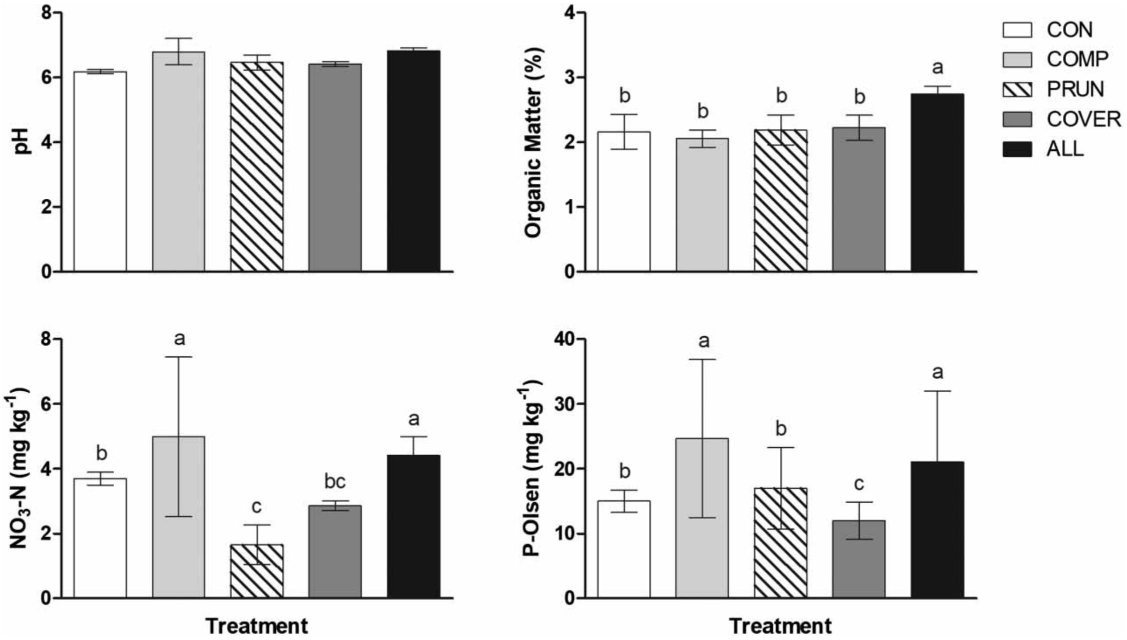

Figure 1 | Selected chemical properties of the 0–30 cm soil depth under the five different treatments. Average values ± SE are shown ($n = 3$). Different letters denote significance at $P < 0.05$.

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,

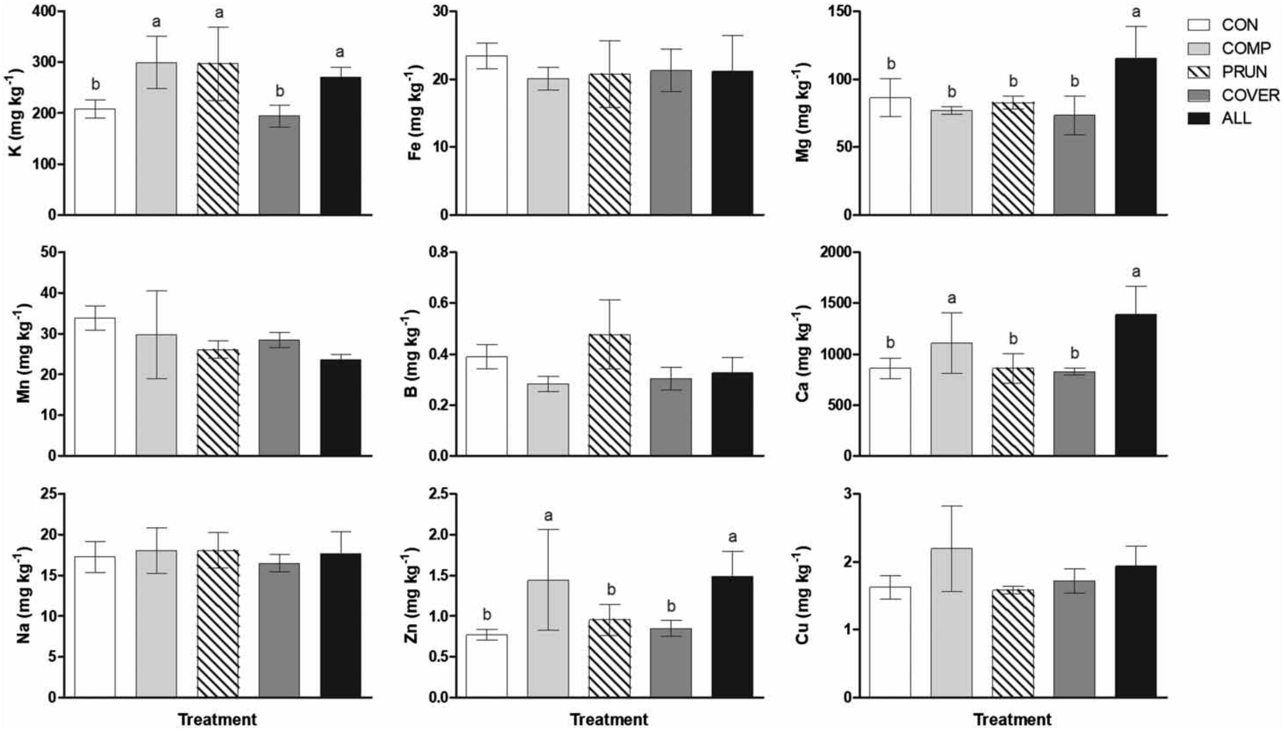

Figure 2 | Soil nutrient status of the 0–30 cm soil depth under the five different treatments. Average values ± SE are shown ($n = 3$). Different letters denote significance at $P < 0.05$.

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

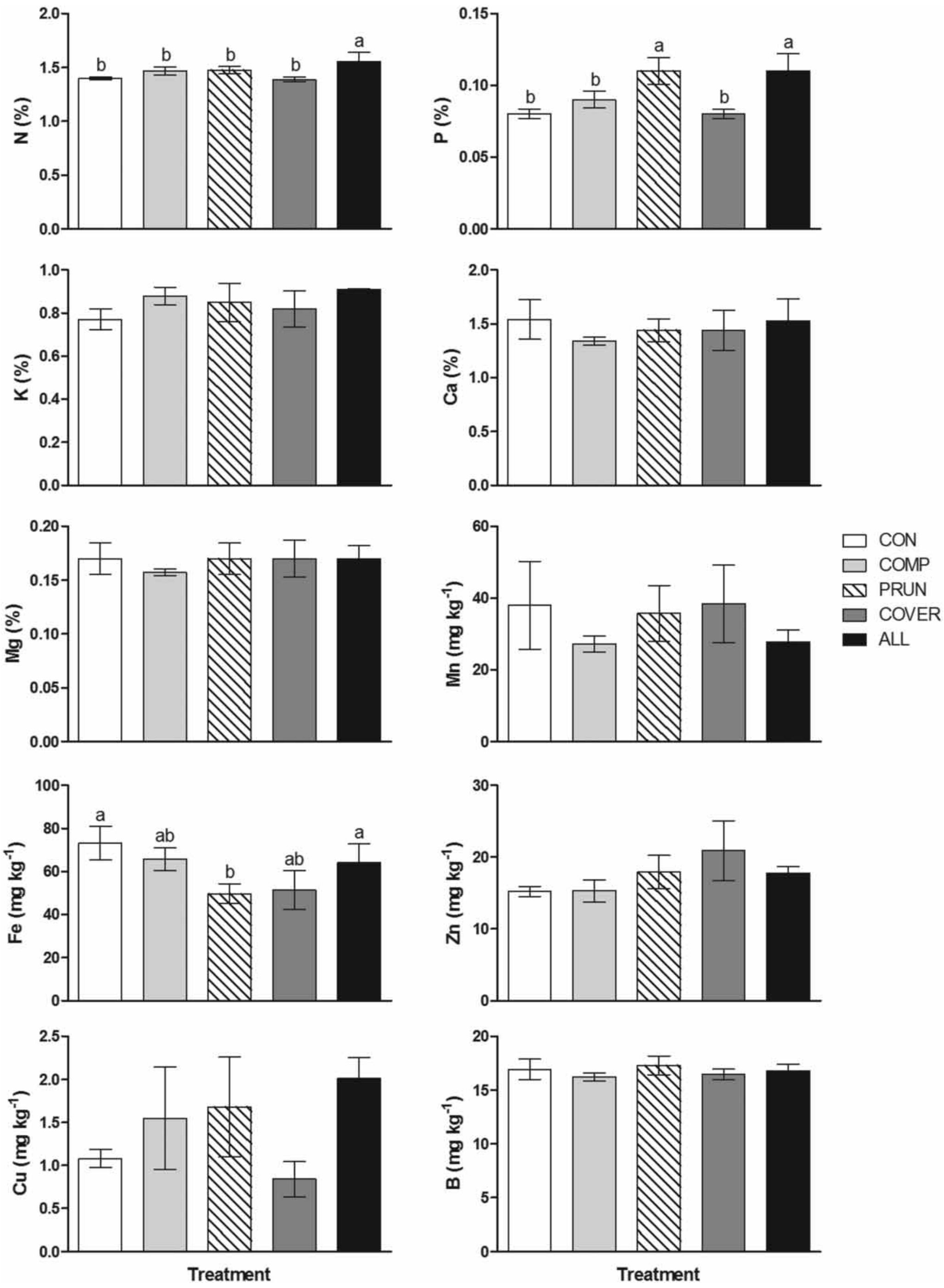

Figure 3 | Concentrations of inorganic elements in olive leaves. Average values ± SE are shown ($n = 3$). Different letters denote significance at $P < 0.05$.

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

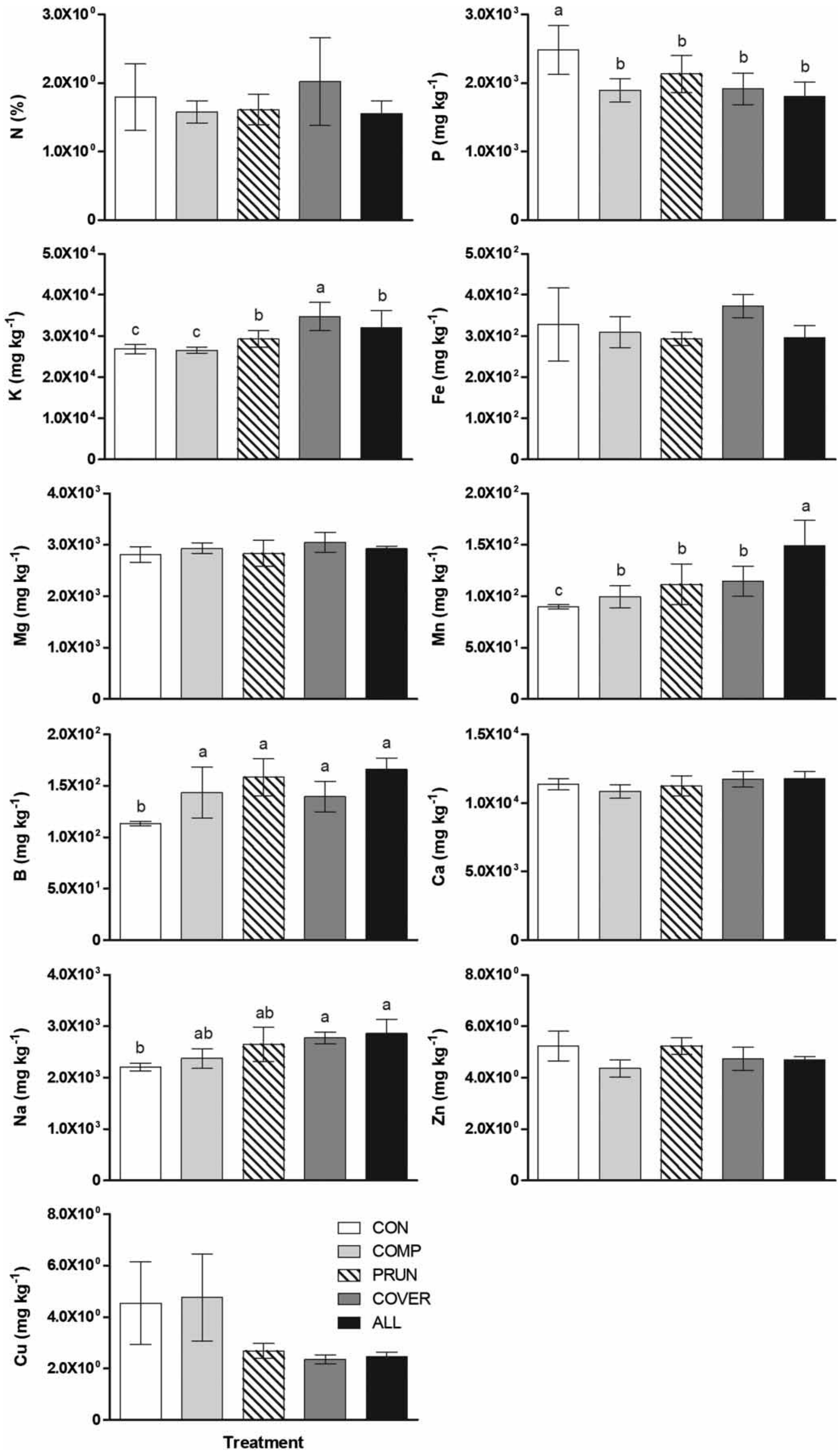

Figure 4 | Concentrations of inorganic elements in *Oxalis* leaves. Average values ± SE are shown ($n = 3$). Different letters denote significance at $P < 0.05$.

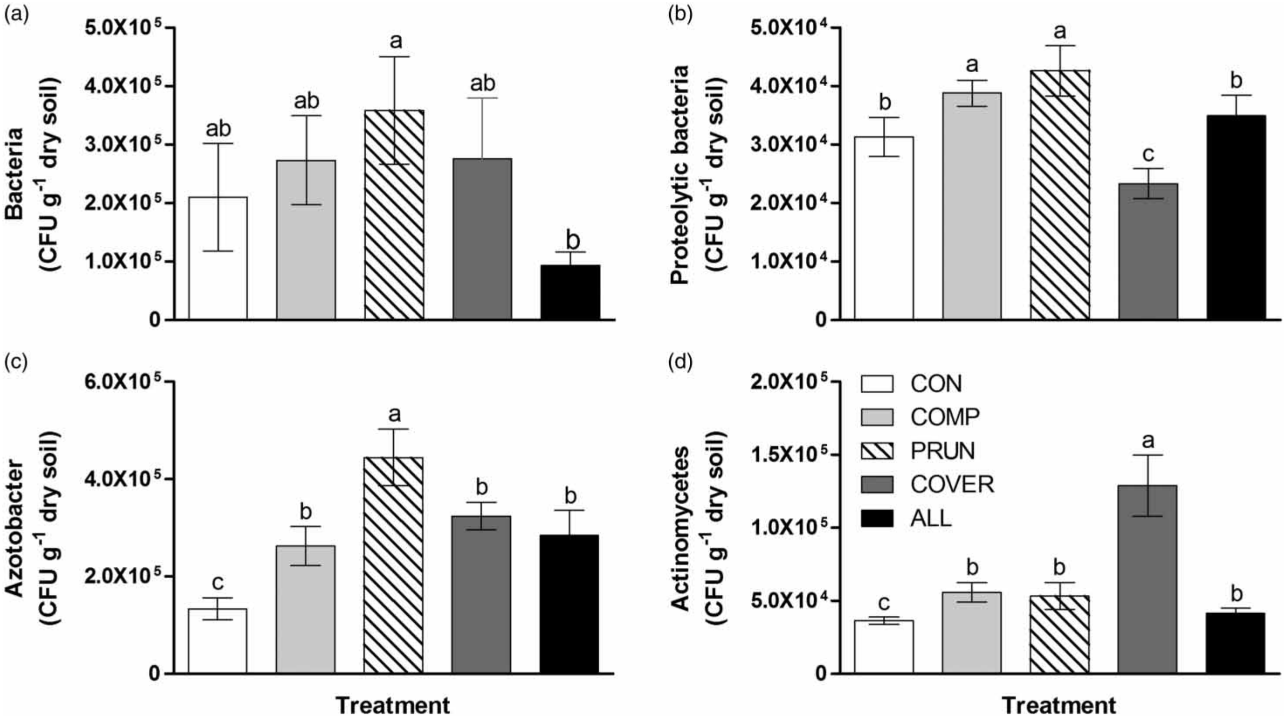

Figure 5 | Total cultural bacterial (a), proteolytic bacterial (b), azotobacterial (c) and actinomycetes (d) counts in the five treatments. Average values ± SE are shown ($n = 3$). Different letters denote significance at $P < 0.05$.

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 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

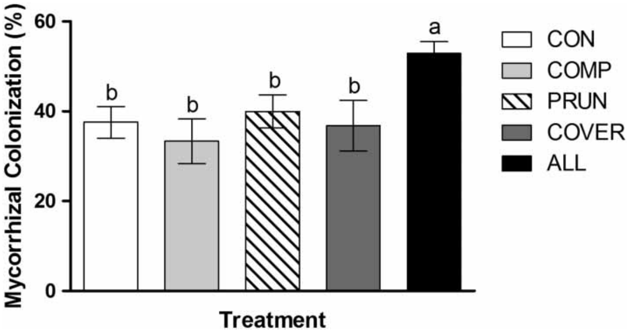

Figure 6 | Mycorrhizal colonization in *Oxalis* roots under the five different land-use regimes. Average values ± SE are shown ($n = 3$). Different letters denote significance at $P < 0.05$.

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; Porras-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; Porras-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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REFERENCES

Al-Absi, K. M., Al-Nasir, F. M. & Mahadeen, A. Y. 2009 Mineral content of three olive cultivars irrigated with treated industrial wastewater. *Agricultural Water Management* **96**, 616–626.

Bilbao, B., Giraldo, D. & Hevia, P. 1999 Quantitative determination of nitrogen content in plant tissue by a colorimetric method. *Communications in Soil Science and Plant Analysis* **30**, 1997–2005.

Calvente, R., Cano, C., Ferrol, N., Azcón-Aguilar, C. & Barea, J. M. 2004 Analysing natural diversity of arbuscular mycorrhizal fungi in olive tree (*Olea europaea* L.) plantations and assessment of the effectiveness of native fungal isolates as inoculants for commercial cultivars of olive plantlets. *Applied Soil Ecology* **26**, 11–19.

Castro, J., Fernández-Ondoño, E., Rodríguez, C., Lallena, A. M., Sierra, M. & Aguilar, J. 2008 Effects of different olive-grove management systems on the organic carbon and nitrogen content of the soil in Jaén (Spain). *Soil and Tillage Research* **98**, 56–67.

Celano, G., Dichio, B., Montanaro, G., Nuzzo, V., Palese, A. M. & Xiloyannis, C. 1999 Distribution of dry matter and amount of mineral elements in irrigated and non-irrigated olive trees. *Acta Horticulturae* **474**, 381–384.

Connor, D. J. & Fereres, E. 2010 The physiology of adaptation and yield expression in olive. *Horticultural Reviews* **31**, 155–229.

Dag, A., Yermiyahu, U., Ben-Gal, A., Zipori, I. & Kapulnik, Y. 2009 Nursery and post-transplant field response of olive trees to arbuscular mycorrhizal fungi in an arid region. *Crop and Pasture Science* **60**, 427–433.

Dichio, B., Xiloyannis, C., Sofo, A. & Montanaro, G. 2006 Osmotic regulation in leaves and roots of olive tree (*Olea europaea* L.) during a water deficit and rewatering. *Tree Physiology* **26**, 179–185.

Fernández-Escobar, R., Moreno, R. & García-Creus, M. 1999 Seasonal changes of mineral nutrients in olive leaves during the alternate-bearing cycle. *Scientia Horticulturae* **82**, 25–45.

Fernández-Escobar, R., Benlloch, M., Herrera, E. & García-Novelo, J. M. 2004 Effect of traditional and slow-release N fertilizers on growth of olive nursery plants and N losses by leaching. *Scientia Horticulturae* **101**, 39–49.

Gaines, T. P. & Mitchell, G. A. 1979 Boron determination in plant tissue by the azomethine H method. *Communications in Soil Science and Plant Analysis* **10**, 1099–1108.

Gómez, J. A., Guzmán, M. G., Giráldez, J. V. & Fereres, E. 2009a The influence of cover crops and tillage on water and sediment yield, and on nutrient, and organic matter losses in an olive orchard on a sandy loam soil. *Soil and Tillage Research* **106**, 137–144.

Gómez, J. A., Sobrinho, T. A., Giráldez, J. V. & Fereres, E. 2009b Soil management effects on runoff, erosion and soil properties in an olive grove of Southern Spain. *Soil and Tillage Research* **102**, 5–13.

Govaerts, B., Mezzalama, M., Sayre, K. D., Crossa, J., Lichter, K., Troch, V., Vanherck, K., De Corte, P. & Deckers, J. 2008 Long-term consequences of tillage, residue management, and crop rotation on selected soil micro-flora groups in the subtropical highlands. *Applied Soil Ecology* **38**, 197–210.

Hernández, A. J., Lacasta, C. & Pastor, J. 2005 Effects of different management practices on soil conservation and soil water in a rainfed olive orchard. *Agricultural Water Management* **77**, 232–248.

Ianetta, P. P. M., Young, M., Bachinger, J., Bergkvist, G., Doltra, J., Lopez-Bellido, R. J., Monti, M., Pappa, V. A., Reckling, M., Topp, C. F. E., Walker, R. L., Rees, R. M., Watson, C. A., James, E. K., Squire, G. R. & Begg, G. S. 2016 A comparative nitrogen balance and productivity analysis of legume and non-legume supported cropping systems: the potential role of biological nitrogen fixation. *Frontiers in Plant Science* **7**, 1700.

Jones, J. J. B. & Case, V. W. 1990 Sampling, handling and analyzing plant tissue samples. In: *Soil Testing and Plant Analysis* (R. L. Westerman, ed.). Soil Science Society of America, Madison, WI, USA, pp. 389–427.

Kapulnik, Y., Tsror, L., Zipori, I., Hazanovsky, M., Wininger, S. & Dag, A. 2010 Effect of AMF application on growth, productivity and susceptibility to *Verticillium* wilt of olives grown under desert conditions. *Symbiosis* **52**, 103–111.

Kavvadias, V., Papadopoulou, M., Vavoulidou, E., Theocharopoulos, S., Malliaraki, S., Agelaki, K., Koubouris, G. & Psarras, G. 2017 Effects of carbon inputs on chemical and microbial properties of soil in irrigated and rainfed olive groves. In: *Soil Management and Climate Change: Effects on Organic Carbon, Nitrogen Dynamics, and Greenhouse Gas Emissions* (M. A. Munoz & R. Zornoza, eds). Elsevier Inc., pp. 137–150. doi:10.1016/B978-0-12-812128-3.00010-0

Keeney, D. R. & Nelson, D. W. 1982 Nitrogen – inorganic forms. In: *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties*, 2nd edn (A. L. Page, R. H. Miller & D. R. Keeney, eds). American Society of Agronomy and Soil Science Society of America, Madison, WI, USA, pp. 643–698.

Koske, R. E. & Gemma, J. N. 1989 A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* **92**, 486–505.

Koubouris, G. C., Kavroulakis, N., Metzidakis, I. T., Vasilakakis, M. D. & Sofo, A. 2015a Ultraviolet-B radiation or heat cause changes in photosynthesis, antioxidant enzyme activities and pollen performance in olive tree. *Photosynthetica* **53** (2), 279–287.

Koubouris, G. C., Tzortzakis, N., Kourgialas, N. N., Darioti, M. & Metzidakis, I. 2015b Growth, photosynthesis and pollen performance in saline water treated olive plants under high temperature. *International Journal of Plant Biology* **6** (1), art. no. 6038.

Koubouris, G. C., Kourgialas, N. N., Kavvadias, V., Digalaki, N. & Psarras, G. 2017 Sustainable agricultural practices for improving soil carbon and nitrogen content in relation to water availability – an adapted approach to Mediterranean olive groves. *Communications in Soil Science and Plant Analysis* **48** (22), 2687–2700.

Kourgialas, N. N., Koubouris, G. C., Karatzas, G. P. & Metzidakis, I. 2016 Assessing water erosion in Mediterranean tree crops using GIS techniques and field measurements: the effect of climate change. *Natural Hazards* **83**, 65–81.

Kourgialas, N. N., Doupis, G., Psarras, G., Sergentani, C., Digalaki, N. & Koubouris, G. C. 2017a Soil management and compost effects on salinity and seasonal water storage in a Mediterranean drought-affected olive tree area. *Desalination and Water Treatment* **99**, 42–48.

Kourgialas, N. N., Karatzas, G. P. & Koubouris, G. C. 2017b A GIS policy approach for assessing the effect of fertilizers on the quality of drinking and irrigation water and wellhead protection zones (Crete, Greece). *Journal of Environmental Management* **189**, 150–159.

Lindsay, W. L. & Norvell, W. A. 1978 Development of a DTPA test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal* **42**, 421–428.

Marschner, P., Kandeler, E. & Marschner, B. 2003 Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biology and Biochemistry* **35**, 453–461.

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, D. L. & Swan, J. A. 1990 A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. *New Phytologist* **115**, 495–501.

Merryweather, J. W. & Fitter, A. H. 1991 A modified method for elucidating the structure of the fungal partner in a vesicular-arbuscular mycorrhiza. *Mycological Research* **95**, 1435–1437.

Morales-Sillero, A., Fernández, J. E., Ordovás, J., Pérez, J. A., Liñán, J., López, E. P., Girón, I. & Troncoso, A. 2009 Plant-soil interactions in a fertigated 'Manzanilla de Sevilla' olive orchard. *Plant and Soil* **319**, 147–162.

Moreno, B., Garcia-Rodriguez, S., Cañizares, R., Castro, J. & Benítez, E. 2009 Rainfed olive farming in south-eastern Spain: Long-term effect of soil management on biological indicators of soil quality. *Agriculture, Ecosystems and Environment* **131**, 333–339.

Nelson, D. W. & Sommers, L. E. 1982 Total carbon, organic carbon and organic matter. In: *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties*, 2nd edn (A. L. Page, R. H. Miller & D. R. Keeney, eds). American Society of Agronomy and Soil Science Society of America, Madison, WI, USA, pp. 539–579.

Olsen, S. R., Cole, C. V., Watanabe, F. S. & Dean, L. A. 1954 *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*. US Department of Agriculture Circular 939, US Government Printing Office, Washington, DC, USA.

Palese, A. M., Pergola, M., Favia, M., Xiloyannis, C. & Celano, G. 2013 A sustainable model for the management of olive orchards located in semi-arid marginal areas: some remarks and indications for policy makers. *Environmental Science and Pollution Research* **27**, 81–90.

Palese, A. M., Vignozzi, N., Celano, G., Agnelli, A. E., Pagliai, M. & Xiloyannis, C. 2014 Influence of soil management on soil physical characteristics and water storage in a mature rainfed olive orchard. *Soil and Tillage Research* **144**, 96–109.

Pascazio, S., Crecchio, C., Ricciuti, P., Palese, A. M., Xiloyannis, C. & Sofo, A. 2009 Phyllosphere and carposphere bacterial communities in olive plants managed with different cultivation practices. *International Journal of Plant Biology* **6**, 15–19.

Pergola, M., Favia, M., Palese, A. M., Perretti, B., Xiloyannis, C. & Celano, G. 2013 Alternative management for olive orchards grown in semi-arid environments: an energy, economic and environmental analysis. *Scientia Horticulturae* **162**, 380–386.

Persiani, A. M., Maggi, O., Montalvo, J., Casado, M. A. & Pineda, F. D. 2008 Mediterranean grassland soil fungi: patterns of biodiversity, functional redundancy and soil carbon storage. *Plant Biosystems* **142**, 111–119.

Phillips, J. M. & Hayman, D. S. 1970 Improved procedure for clearing roots and staining parasitic and vesicular–arbuscular fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**, 158–161.

Porras-Soriano, A., Soriano-Martín, M. L., Porras-Piedra, A. & Azcón, R. 2009 Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *Journal of Plant Physiology* **166**, 1350–1359.

Rallo, L., Díez, C. M., Morales-Sillero, A., Miho, H., Priego-Capote, F. & Rallo, P. 2018 Quality of olives: a focus on agricultural preharvest factors. *Scientia Horticulturae* **233**, 491–509.

Ramos, M. E., Benítez, E., García, P. A. & Robles, A. B. 2010 Cover crops under different managements vs. frequent tillage in almond orchards in semiarid conditions: effects on soil quality. *Applied Soil Ecology* **44**, 6–14.

Sofo, A., Palese, A. M., Casacchia, T., Celano, G., Ricciuti, P., Curci, M., Crecchio, C. & Xiloyannis, C. 2010 Genetic, functional, and metabolic responses of soil microbiota in a sustainable olive orchard. *Soil Science* **175**, 81–88.

Sofo, A., Ciarfaglia, A., Scopa, A., Camele, I., Curci, M., Crecchio, C., Xiloyannis, C. & Palese, A. M. 2014 Soil microbial diversity and activity in a Mediterranean olive orchard using sustainable agricultural practices. *Soil Use and Management* **30**, 160–167.

Tataranni, G., Dichio, B. & Xiloyannis, C. 2012 Soil fungi–plant interaction. In: *Advances in Selected Plant Physiology Aspects* (G. Montanaro, ed.). InTech, Rijeka, Croatia, pp. 161–188.

Wu, L., Wu, H., Chen, J., Wang, J. & Lin, W. 2016 Microbial community structure and its temporal changes in *Rehmannia glutinosa* rhizospheric soils monocultured for different years. *European Journal of Soil Biology* **72**, 1–5.

Zheng, S., Cao, H., Huang, Q., Liu, M., Lin, X. & Li, Z. 2016 Long-term fertilization of P coupled with N greatly improved microbial activities in a paddy soil ecosystem derived from infertile land. *European Journal of Soil Biology* **72**, 14–20.

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