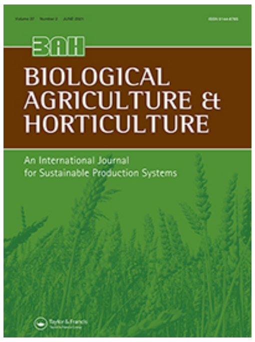

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Chemical and biological indicators of soil quality in organic and conventional Japanese plum orchards

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ABSTRACT

To evaluate the effect of the management system on soil quality, chemical and biological properties were measured in soils (0–20 cm depth) from Japanese plum orchards managed under organic and conventional conditions. The experiment was conducted between 2005 and 2014 in orchards established at the agricultural research station at Alcalá del Río, Seville, Spain. Overall, the soil chemical parameters in the organic orchard showed higher concentrations of soil organic matter, total N, available P and Mg. There was a trend of higher concentrations of K and Na, though for these elements the differences were significant only in two and one years, respectively. Similarly, concentrations of Mn and Zn tended to be higher in the organic plots, whereas contradictory trends were shown for Fe and Cu. No differences were found between the treatments for other physicochemical parameters such as pH and electrical conductivity. Regarding soil biological properties, the organic system was characterised with a significantly greater abundance of bacteria and fungi compared with the conventional system. The rhizobia population and the legume biomass were also higher in the organic orchard and the plants had more nodules. Likewise, the number of earthworms was higher in the organic system. The results showed that long-term organic management, including the use of cover crops and compost, resulted in a significant shift of the chemical and biological characteristics of the soil compared with the conventional management practice, thus improving soil quality and suggesting that organic farming may provide a potential solution to achieve sustainable agricultural systems.

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Organic agriculture; *Prunus salicina*; soil biological properties; soil fertility; sustainability

Introduction

Preserving and improving agroecosystem health should be considered a priority goal in agriculture management in Southern Europe. It is well known that the combination of traditional intensive orchard management and the Mediterranean-type climate in Andalusia can lead to unsustainable agroecosystems (Keesstra et al. 2018a). One way to address this problem is by implementing ecological principles to manage agroecosystems for environmental, economic and social benefits (Zhu et al. 2012; Cerdà et al. 2018). These concerns have led to changes in the agricultural policies of the European Union (EU), which have progressively incorporated environmental considerations and invested in development of nature-based solutions (NBS) under Horizon 2020, the EU research and innovation programme. Nature-based solutions (NBSs) have shown to have potential to provide cost-effective and long-term

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solutions for hydrological and land degradation risk situations. NBSs have been divided into two main groups: soil- and landscape-based solutions. Soil-based solutions aim to enhance the health and functions of the soil, through which ecosystem services can be maintained or restored. Agricultural soil strategies that focus on achieving improved soil health usually aim to improve the organic matter content and the structure of the soil to facilitate higher infiltration rates and lower runoff and erosion (Berge et al. 2017). Soil management strategies that improve and sustain the fertility and the biological activity of the soil are considered as appropriate measures to sustain long-term crop growth. Organic agricultural systems have a strong focus on this, and also on protecting the soil surface, with the use of techniques such as mulching, intercropping and the use of cover crops. Keesstra et al. (2018b) used an organic farming system in Spain as a key example to understand the superior effect of nature-based solutions to enhance the sustainability of cropping systems by promoting desirable soil and landscape functions. Other researchers have also suggested organic farming as a potential solution to achieve sustainable agricultural systems (Niggli 2015).

Organic farming is based on a given set of farm practices associated with soil health and which emphasise ecological sustainability. In organic systems, soil fertility is maintained through the use of organic amendments (e.g. compost and manure) and cover crops; practices known to increase in soil organic matter and to play a key role in terms of improving physical properties and nutrient cycling (Goss et al. 2013; Di Prima et al. 2018). The soil has a key role in organic farming and the quality of the soil has a direct effect on the quality and safety of the fruit grown in it (Cuevas et al. 2015).

Although some studies have shown that the concentrations of nutrients in organically managed soils have been higher than those in conventionally managed soils, overall, the results have been inconsistent. While Edmeades (2003) showed that soil supplied with organic amendments had significantly higher concentrations of soil macronutrients, other authors have shown lower macronutrient concentrations in organically managed soils (Gosling and Shepherd 2005) or no consistent differences in the status of the major nutrients (Domagała-Świątkiewicz and Gąstoł 2013). Several authors have demonstrated a long-term positive influence of organic farming on soil quality and microbial activity compared with conventional farming. This might be due to the use of crop rotations, the absence of synthetic nutrients and pesticides and/or due to an increase in the soil microbial biomass, aerobic bacteria and fungi after incorporation of organic substrates, such as straw and green manures (Hansen et al. 2001). The diversity of bacterial functional communities has been recorded to be greater in soils from organic farms, while species diversity was found to be similar (Liu et al. 2007). However, other authors found conflicting evidence in relation to the biological communities associated with different management practices, where the differences in soils were subtle with no consistent significant differences in the parameters studied (Shannon et al. 2002). Despite these inconsistencies, several studies have suggested that the use of organic amendments or cover crops resulted in changes in soil quality (Marinari et al. 2006; Goss et al. 2013). However, for fruit cropping systems, such as for plum orchards, there is still a lack of information on the long-term effects of organic management practices and on how they affect the chemical and biological parameters of the soil.

In Andalusia, a region in southern Spain, organic agriculture has experienced impressive growth over the past two decades, including for a wide range of crops. However, the production of organic stone fruit is still very limited. Measurement of different soil properties under different long-term management practices would provide a better understanding of the relationship between soil biological and chemical properties and provide a comprehensive assessment of soil quality. This study investigated the physicochemical soil characteristics and the abundance of soil organisms in two Japanese plum orchards, managed organically and conventionally.

Materials and methods

Description of the experimental plots

The study was conducted from 2005 to 2014 in two experimental orchards (5500 m^2 each) located at the IFAPA Centro 'Las Torres-Tomejil' in the province of Seville in the Guadalquivir River Valley, south west Spain (37° 30′ 48" N; 5° 57′ 46"W). The orchards, which were 150 m apart to avoid interference between the different management systems, were located at an altitude of 11 m a.s.l. on a loam soil classified as a Xerofluvent (Soil Survey Staff 1999). The two areas for the orchards had been selected on the basis of having similar physical and chemical characteristics of the soil (Table 1). The experimental orchards were subjected to different management regimes, organic versus conventional management. The organic orchard had been certified for organic production, in accordance with the EU Regulations on organic farming (OJEU 2007, 2008) since 2005. The region has a Type C Mediterranean climate, according to the Köppen classification (Peel et al. 2007). In both orchards, 14 Japanese plum (*Prunus salicina* Lindl) cultivars had been planted in January 2005. The details of the cultivars and their characteristics have previously been described by García-Galavís et al. (2009). The experimental set up in each orchard was a randomised block design with three replicates, each containing six trees of each cultivar.

Soil fertility regimes and other management practices of the orchards

In the organic orchard, the fertility regime included annual application of composted animal manure at a rate of 3–4 kg m^{-2} and sowing different leguminous green cover crops, such as beans (*Vicia faba* L). and a mixture of vetch (*Vicia spp.*) and oats (*Avena sativa* L.). Occasionally, other cover crops, such as rapeseed (*Brassica napus* L.) + vetch (*Vicia* spp.) mixtures or spontaneous weeds were also used. In the summer of 2004, before planting the trees, a cover crop of soybean (*Glycine max* (L.) Merr.) was grown. The cover crops were sown in November and incorporated into the superficial soil layer (15 cm depth) in the following February or March by means of mechanical methods that included rolling, and partial rototilling. Early termination of the cover crops was carried out to avoid competition for the trees for nutrients and, particularly, for water during spring and summer (a relevant factor in these pedoclimatic conditions). The input of macronutrients supplied through the incorporation of the cover crops in the successive years is shown in Table 2 and the nutrient concentration of the animal manure compost is shown in Table 3.

Table 1. Physicochemical analysis of the soil in the organic (O) and conventionally (C) orchards at the time of planting the plum trees (2005).

	C	O
OM (%)	1.3	1.1
pH	8.6	8.6
EC (dS m^{-1})	0.28	0.27
N total (mg kg^{-1})	507	482
P_{Olsen} (mg kg^{-1})	5.5	9.3
K (meq 100 g^{-1})	0.72	0.68
Ca (meq 100 g^{-1})	9.39	10.44
Mg (meq 100 g^{-1})	1.97	1.84
Na (meq 100 g^{-1})	0.66	0.71
B (mg kg^{-1})	0.96	1.06
Fe (mg kg^{-1})	6.64	7.28
Zn (mg kg^{-1})	2.42	2.54
Mn (mg kg^{-1})	7.96	10.04

OM: organic matter. EC: electric conductivity. K, Ca, Mg, Na: extractable ACNH$_4$. Fe, Mn, Zn, B: extractable Mehlich-3). $n = 24$.

Table 2. Gross contribution of above-ground biomass (dry matter) and macronutrients by the different cover crops used in the organic orchard (n = 24).

Cover	Sown	Incorporated	Dry biomass (kg ha^{-1})	N kg ha^{-1}	P kg ha^{-1}	K kg ha^{-1}
Soybean	May 2004	August 2004	10,600	320	34	206
Beans	October 2005	March 2006	6320	180	14	185
Rape + Vetch	December 2006	April 2007	2500	70	8	75
Spontaneous vegetation	-	March 2008	2913	56	9	75
Vetch + Oats	October 2008	March 2009	8320	180	19	220
Beans	October 2009	March 2010	10,353	225	24	274
Vetch + Oats	October 2010	March 2011	4100	140	12	120
Beans	November 2011	February 2012	nd	nd	nd	nd
Vetch + Oats	October 2012	March 2013	2850	83	12	108
Beans	October 2013	January 2014	6164	232	17	293

nd: not determined

Table 3. Average concentrations and annual applications of nutrient elements in the animal manure compost applied during the period 2006–2013 in the organic orchard (n = 8).

Element	mg kg^{-1}	kg ha^{-1}
N	5474	136
P	1626	41
K	10,262	256
Ca	23,927	598
Mg	2149	54
Na	2712	68
B	9.10	0.23
Fe	2126	53
Cu	13.59	0.34
Zn	34.78	0.87
Mn	92.56	2.31

Calculation based on the application of animal manure compost at 25,000 kg ha^{-1} year^{-1}.

In the conventional orchard, the same conventional fertilisation programme was followed each year of the study and this was based on the application of an NPK complex fertiliser (11–11-11, 800 kg ha^{-1}) in February, ammonium nitrate (400 kg ha^{-1} NH_4NO_3, 33.5% N) in April and potassium sulphate (450 kg ha^{-1} SO_4K_2, 50% K) at the end of May or June, to provide 150 units of N, 55 of P and 150 of K. No cover crops were used in the conventional orchard.

The management of pests and diseases in the conventional and organic plots were adjusted in accordance with the regulations for integrated production (BOE 2002; BOJA 2003) and for organic farming (OJEU 2007; 2008), respectively, with the specific products and treatments for both orchards previously described by García-Galavís et al. (2009). During the dry season, both orchards were irrigated by gravity along two rows parallel to the line of trees with identical volumes and frequency. In both orchards, the land management consisted of a reduced tillage regime similar in both areas, consisting of one annual surface pass with disc harrow (at 200 mm top layer). In both areas, weeds were controlled by means of superficial tillage with a disc harrow and the grass between the tree lines was controlled using a manual brush cutter in the organic orchard and by the application of herbicides in the conventional orchard. When necessary, grass was removed in the line of the trees by a pass with hydraulic rotating heads attached to an orchard tractor.

Soil, manure and cover crop analyses

Soil samples from the upper layer (0–20 cm deep) were taken twice in each year, once prior to sowing the cover crops (October) and then approximately 45 days after the incorporation of the cover crops (April). On each occasion, four samples were taken from each block. For soil chemical analysis, the samples taken prior to sowing the cover crops were used. The samples were air-dried, sieved to 2 mm, and stored in plastic containers before analysis. To determine the biomass of the cover crops, above-ground biomass was harvested from four squares (each with an area of 0.5 m^2) placed randomly within each block. After drying the biomass for a week, first air-dried and then dried in the oven at 40 ºC, the biomass was weighed and then milled for analysis. The nutrient composition of the organic amendments (manure compost and cover crops) was determined by the MAPA method (1994). Soil pH and electrical conductivity were determined using a 1:2.5 soil:water extract. Total N concentration was determined by Kjeldahl digestion and organic carbon (OC) by potassium dichromate oxidation using the Walkley and Black method, as modified by Jackson (1958). Available P was measured using the Olsen method (Olsen et al. 1954), available K, Mg, and Na were measured using extraction with ammonium acetate (MAPA 1994) and extractable elements Cu, Zn, Fe and Mn were assessed using the Mehlich-3 method (Mehlich 1984). Element concentration was measured using an atomic absorption spectrophotometer. Extractable B was also assessed using extraction with Mehlich-3 and the concentration was estimated using a spectrophotometric method (Wolf 1974)

Determination of microorganisms

The quantification of the cultivable microbial population in the top 20 cm of the soil was carried out using serial dilutions and plating on specific media (Mukerji and Mandeep 1998; Ellis et al. 2003). Four soil samples in each block were taken. The low nutrient medium used for bacteria isolation consisted of a nutrient broth diluted at 2 g L^{-1} (Nutrient Broth acc., Scharlau) solidified by adding 2% agar and the pH was adjusted to 7.3. After sterilisation by autoclaving for 20 min at 120 ºC, cycloheximide (50 mg kg^{-1}) was added. For fungal isolation, potato dextrose agar (PDA) medium (Sigma Chemical Co.) supplemented with 50 mg kg^{-1} of chloramphenicol and kanamycin was used.

Determination of Rhizobium in soils and nodules on the legumes

The *Rhizobium* population in the soil was estimated using the most-probable-number (MPN) technique (Vincent 1970). To compare the *Rhizobium* nodulation efficiency in the different orchards, samples of the cover crops were also sown in the conventional orchard in two of the years (2010 and 2011). For evaluation of nodulation on the roots of *Vicia faba* L. and *Vicia sativa* L., 12 plants of each species were collected at random from each orchard. Roots were separated from shoots and the nodules were carefully removed from the roots and counted. Both the nodules and the shoots were dried at 65°C until constant weight and were then weighed.

Determination of abundance of mycorrhizal fungi

Mycorrhizae were studied in the roots of the herbaceous species *Conyza bonariensis* (L) Cronq. and *Vicia faba* L. (five plants per block) present in both plots. In addition, in 2012 and 2013, the degree of mycorrhizal colonisation in roots of two of the plum cultivars (Souvenir and Showtime) was analysed. Root samples were taken from three trees per block and treatment (nine samples for treatment) and were then cut, bleached and dyed with trypan blue (0.05% in lactic acid) (Phillips and Hayman 1970). The estimate of the percentage of mycorrhizal colonisation was performed by observation of stained roots under magnification and by counting the number of pieces showing vesicular-arbuscular mycorrhizal (VAM) per 100 pieces (Trouvelot et al. 1986).

Earthworm abundance

Earthworms, *Lumbricus terrestris* L., were assessed at different times between 2007 and 2014, by hand-sorting soil samples collected from 50 × 50 cm square grids, 0–40 cm depths on an area of 0.5 ha. Five samples were collected from each of the organic and conventional orchards. The population density and the biomass of the earthworms were determined. Characterisation of the earthworms at the species level was not undertaken.

Statistical analysis

Statistical analyses were performed using Statistix software (version 9.0, NH Analytical Software, USA). All data were subjected to analysis of variance using one-way ANOVA. For the different soil parameters investigated, the significance of the differences between conventional and organic treatments were calculated by using the unpaired Student t-test.

Results and discussion

Cover crop biomass

Different cover crops were used in the organic orchard in this study. Because nitrogen in the organic management system can often be a limiting factor for the development of the trees, legume cover crops were incorporated as green manures to the soil in the organic orchard to provide nitrogen. Prior to planting the trees, soybean was grown as a green manure; a crop that produced abundant biomass to help with the conversion of the soil. After the first year, a legume cover crop (*Vicia faba* beans) or a legume-grain mixture of vetch (*Vicia* spp) and oats (*Avena sativa* L) were generally alternated. The vetch/oats mixture was used to also increase the soil organic matter; this mixture combines the benefits of the nitrogen fixing legume (vetch) and a cereal (oats) that provides more organic matter, helps the vetches to climb, and as is cold-tolerant the mixture is suitable for sowing in early autumn. Furthermore, autumn sown cover crops are known to produce other benefits such as improved use of nutrients, reduced nitrate leaching, increased biodiversity of the system and abatement of soil erosion (Dorais and Alsanius 2015). Different results for the aboveground biomass were obtained due to the different climatic conditions across the study. However, under these conditions, the soybean and the bean crops clearly produced more biomass and higher inputs of nutrients from the biomass than the vetch/oats mixture or the spontaneous vegetation. (Table 2). The total nitrogen input from the cover crops was generally high, though the percentage of N derived from biological nitrogen fixation was not determined. The addition of K by the cover crops was also very high, whilst that for P it was lower. Moreover, the animal manure compost used contained particularly high concentrations of Ca, K and N, and various microelements, especially Fe and Mn (Table 3).

Soil organic matter

For the soil chemical parameters, the results from the fourth year (2009) onwards of the study have been presented here, as significant differences between the systems were observed from this point. During the first 3 years of the study, the organic system was in a conversion period (i.e. not yet fully organic) and it is known that the chemical characteristics of the soil change relatively slowly. From 2009, the content of soil organic matter (OM) was found to be higher in the organic plots compared with that in the conventional plots (Figure 1a) and these differences were statistically significant. For the organic treatment, the OM values ranged between 1.65% and 2.89%, while for the soil in the conventional orchard the values ranged between 1.08% and 1.65%. These results agreed with those published by Romanyà et al. (2012), reporting these values as representative of the wet

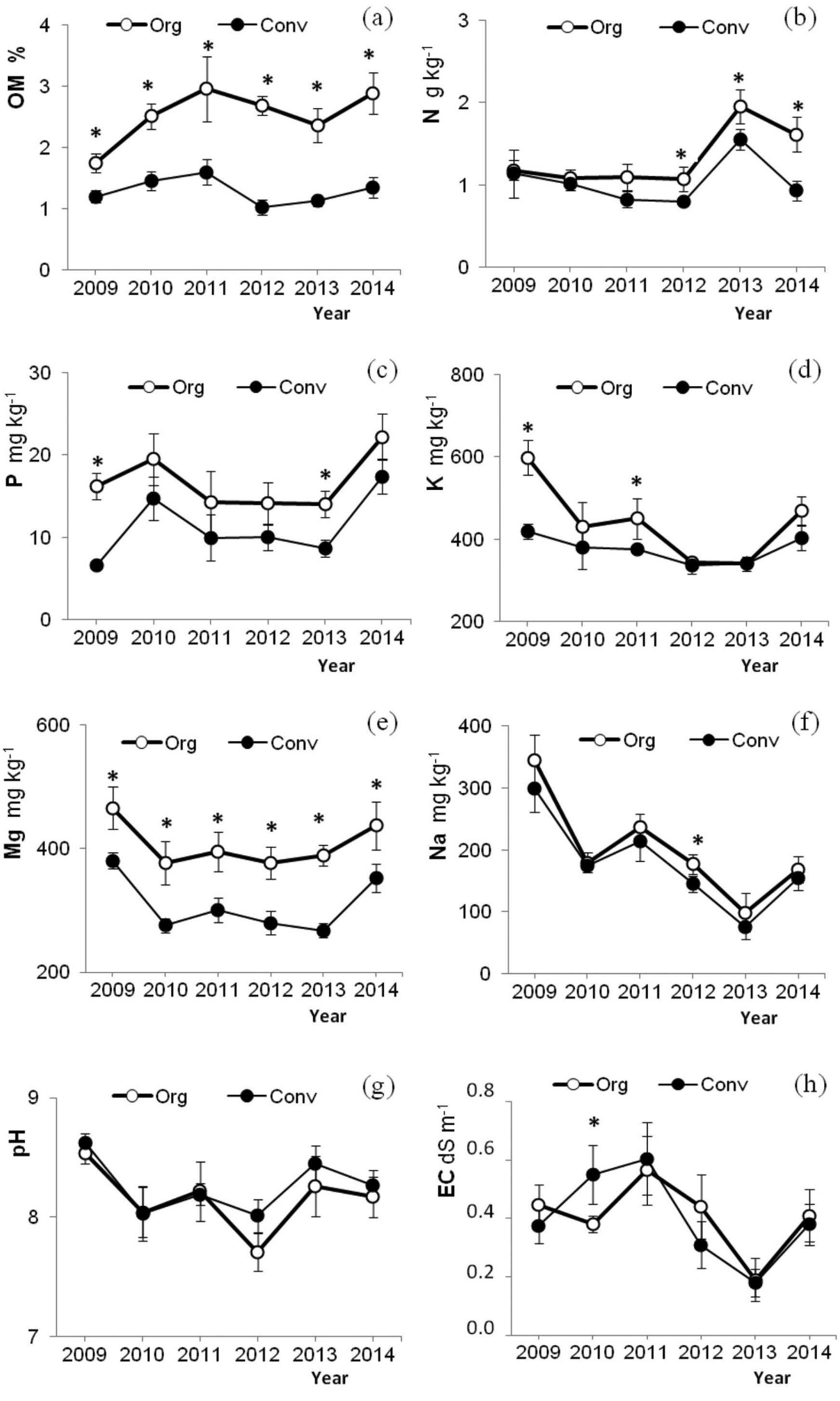

Figure 1. Concentrations of organic matter (OM), Kjeldhal nitrogen (N), available phosphorus (P), available potassium (K), available magnesium (Mg), available sodium (Na), pH and electrical conductivity (EC) during the period 2009–2014 in soil subjected to different management strategies. Treatments: Org (– ○ –), organic management; Conv (– ● –), conventional management. Error bars indicate standard deviations of means (*n* = 12).

Mediterranean area of Spain and that organic soils had higher soil organic carbon (SOC) than conventional soils.

The application of the organic amendments (animal manure compost and cover crop residues) clearly increased soil OM, as has also been reported in other studies (Stockdale et al. 2001; Melero et al. 2006). Scow et al. (1994) reported that OM increased after alternate applications of compost and cover crops over 4 years. However, Gosling and Shepherd (2005) found no differences in soil OM with different soil fertility management regimes; possibly not too surprising as this can be due to different soil characteristics, the climate and to the rates and characteristics of the organic amendments. It was interesting that in this study reported here, the difference in OM was not significant until in the fourth year of the study (2009). This was in agreement with what has been shown in previous studies (Marinari et al. 2006), where the authors indicated that the delay in the increase in OM may be a consequence of the organic management regime; although considerable additions of labile carbon were added (green manures) to the soil in the organic system, at the start this was partially countered by increased decomposition of the OM by the soil micro-organisms that were favoured by the frequent tillage of the soil for mechanical weed control. Marinari et al. (2006) predicted an increase in the soil OM after a period longer than 7 years of organic management. Recently, Novara et al. (2019) demonstrated that an organic management regime combining the use of compost and cover crops was an effective strategy to restore or increase SOC concentrations in Mediterranean orchard systems.

Soil nitrogen

In the last three years of the study, significantly higher concentrations of total N were observed in the soil from the organically managed plots compared with that from the conventional plots (Figure 1b). In the organic orchard, soil N values ranged between 1.05 and 1.96 g kg^{-1}, while in the conventional orchard the values ranged between 0.90 and 1.55 g kg^{-1}. Soil N values were low in both plots until 2009 (data not shown), probably as a result of the previous cultivation regime, but from 2012 significantly higher levels were observed in the organically managed soil, although the N values in the conventional soil were also within the adequate range. In previous studies, higher levels of N were also observed in soils managed with organic regimes as compared with the use of mineral fertilisers (Melero et al. 2006; Herencia et al. 2008a), and other studies have also highlighted the importance of the use of cover crops, particularly legumes (Perdigão et al. 2010). The use of cover crops has also been shown to significantly reduce the concentrations of inorganic N in the soil in the autumn and thus reduce the risk of nitrate leaching and thereby making more N available for crops (Stockdale et al. 2001). The N applied in the form of an organic amendment is only available to the plants after a period of mineralisation (Sims 1995), so changes in availability can only be detected after a period of time.

Soil phosphorus

The results indicated that the available P concentration in the soil in the organic orchard was higher compared with that in the conventional orchard (Figure 1c). However, the concentrations of P fluctuated, and the differences between the two treatments were not significant at all dates. In both of the orchards, but particularly in the conventional one, P values were, at some dates, below the levels considered adequate (16–25 mg kg^{-1}). In the organic treatment the available P values ranged between 14 and 23 mg kg^{-1}, whilst the values in the conventional treatment ranged between 6.7 and 17.4 mg kg^{-1} measured at the beginning and at the end of the study, respectively. There is considerable evidence in the literature to suggest that the application of organic material to soil may increase the solubility of P (Andrews et al. 2002; Edmeades 2003) and various mechanisms have been proposed to explain this increase: the higher P concentration of the organic amendments, the increase of organic acids, and the increase of microbial biomass in organic plots. In addition, in the calcareous soils used in this study, the organic anions from organic amendments may have decreased fixation of P by calcium increasing the available P concentration (Herencia et al. 2008a). In addition, the application of green manures to soils has been found to increase soil P supply and plant-available P (Arcand et al. 2010). Arcand et al. (2010) reported that although the low concentrations of P in the organic amendments were not usually sufficient to satisfy crop demand, the incorporation of the amendment provided a carbon substrate that supported increased microbial activity in the soil and as a result the liberation of P from adsorption sites and chemical precipitation, making it available to plants.

Available potassium

Although the concentrations of available K tended to be higher in the organically managed plots compared with the conventional plots (Figure 1d), the differences were only significant in two of the 6 years (2009 and 2011). In the organic treatment the available K values ranged between 344 and 598 mg kg^{-1}, while in the conventional plots the values ranged between 336 and 418 mg kg^{-1}. In both systems, the values were higher than the levels considered adequate (156–295 mg kg^{-1}). One explanation for the similar K availability in the two treatments, may be that illite-type clay minerals, dominant in the soil, fix K strongly. The data also revealed great fluctuations between the cultivation cycles, as previously shown by Liu et al. (2007) in a 3-year study. In fact, previously published results have shown contradictory results. While some studies have shown lower K concentration in organically managed soils (Gosling and Shepherd 2005), other studies have reported an increase in available K after application of organic amendments (Edmeades 2003). In a 3-year study, Andrews et al. (2002) found higher K values in the organic system, which was attributed to the high K concentration of the compost applied and the increase of exchange sites due to the added organic matter, which does not fix K strongly.

Available magnesium

The available Mg concentration in the organically managed plots was significantly higher compared with that in the conventional plots (Figure 1e), ranging from 376 to 466 and 267 to 380 mg kg^{-1} for organic and conventional plots, respectively. The organic matter application can build up the humus and the clay-organic complexes in the soil, which increases the soil's nutrient-exchange capacity and the available cations, such as Mg. Similar results were also shown in short- or long-term studies in organic systems using a combination of compost, cover crops and incorporation of crop residues into the soil (Schjonning and Christensen 1994; Liu et al. 2007). In many studies, the concentrations of Mg (and Na) have not been assessed and it is well known that the uptake of these elements, as well as of Ca and K, is strictly dependent on the relationship between the concentrations of these elements in soil. Mg cannot be dealt with separately from other nutrients, such as K and Ca, with which it has antagonistic relationships in terms of uptake (Tůma et al. 2004). High ratios of Ca:Mg and K:Mg can be a limiting factor for the uptake of available Mg. Nevertheless, Senbayram et al. (2015) indicated that high concentrations of Ca and K in the soil solution interfered with Mg uptake by plants, while higher Mg concentration in the soil solution did not generally disturb K uptake. Although in this study reported here, the average K:Mg ratio was lower in the organic treatment (1.07) than in the conventional treatment (1.24), so the risk for Mg deficiency was lower in the organic plot.

Available sodium

Although Na concentration tended to be slightly higher in the organic plots, there were no significant differences between the treatments, except in 1 year, 2012 (Figure 1f). In addition, a gradual decrease in the concentration of Na was observed in both soils over the course of the study, though the differences between the years were not statistically verified. Similar to Mg, the increase observed in the organic plots may have been due to the Na concentration in the organic amendment and the increase in the soil's nutrient-exchange capacity due to the addition of OM. The gradual decrease could be due to the retention capacity of cations on negatively charged exchange sites, where possibly other cations, Ca^{2+}, Mg^{2+}, K^{+}, displaced the Na of the exchangeable sites and were lost by leaching. Although sodium does not seem to be essential for plants, sometimes it is used, particularly when K is low (Maathuis 2014). Other studies of Na levels are scarce, though Liu et al. (2007) showed, in a 3-year study, higher Na concentration in the organic system and Reganold et al. (2010), who compared 13 pairs of commercial organic and conventional agroecosystems, showed that Na levels were significantly higher in the organically farmed soils while, other authors found no differences in a long-term study (Schjonning and Christensen 1994).

pH and electrical conductivity

The pH values showed no overall significant differences between the organic and the conventional plots, though there was a tendency of a lower pH in the organic plots during the last years of the study (Figure 1g). In this system, the decrease in pH was small probably due to the buffering characteristics of the OM in the soil and the high carbonate content; the soil was calcareous and in these types of soil the pH correlate with the partial pressure of CO_2 that in alkaline medium produces bicarbonate by consuming OH^- avoiding pH increases. In addition, respiring plant roots and microorganisms produce CO_2 and this may be another reason for a lower pH related to the higher biological parameters in the organic plots. Another aspect to highlight was that the mean pH of the compost used was lower than that of the calcareous soil in the plots due to basic cations content in the compost.

In terms of electrical conductivity (EC), the results obtained in this study showed that there were no significant differences between organic and conventional plots (Figure 1h) apart from in 1 year (2010) and neither the conventional or the organic regimes appeared to cause soil salinisation.

Micronutrients

The results for the concentrations of micronutrients were highly variable. Although in some cases, there were no significant differences between the treatments, the available Fe and Cu tended to be higher in the conventionally managed soil whereas Mn and Zn tended to be higher in organic plots. No differences between treatments were found for B. The concentrations of Fe extracted from soil samples from organic and conventional plots were similar, ranging from 72.6 to 118.6, and 78.2 to 121.4 mg kg^{-1}, respectively (Figure 2a). The concentrations of Cu ranged from 7.10 to 9.93 mg kg^{-1} and 6.09–8.50 mg kg^{-1} for conventional and organic plots, respectively (Figure 2b). In the organic plots the concentration of extracted Cu was significantly lower than that in the conventional soil in 2010 and 2012. The Mn extracted in the soil from the organic plots tended to be higher than that in conventional plots, ranging from 65.60 to 108.15 mg kg^{-1} and 62.90–87.19 mg kg^{-1}, respectively (Figure 2c), but this difference was statistically significant only in 1 year (2011). The concentration of Zn ranged from 2.69 to 3.37 mg kg^{-1} and 2.45–3.25 mg kg^{-1} for organic and conventional plots, respectively, (Figure 2d) and was significantly higher in the organically managed plots in 2010, 2011 and 2013. Some authors indicated that OM exerted a relevant and direct effect on micronutrient availability (Rodríguez-Rubio et al. 2003) and it is known that the concentrations of metals are negatively associated with concentration of calcium carbonate that can bind the metals (Bashir et al. 2019). Kabata-Pendias (2001) indicated that in soils with high pH such as in calcareous soils, as in the present study, complexation promotes maintenance of micronutrients dissolved, increasing their availability in the soil. The addition of exogenous OM containing functional groups with the ability to form complexes promotes the availability of Zn in soils with high pH. In this study, values

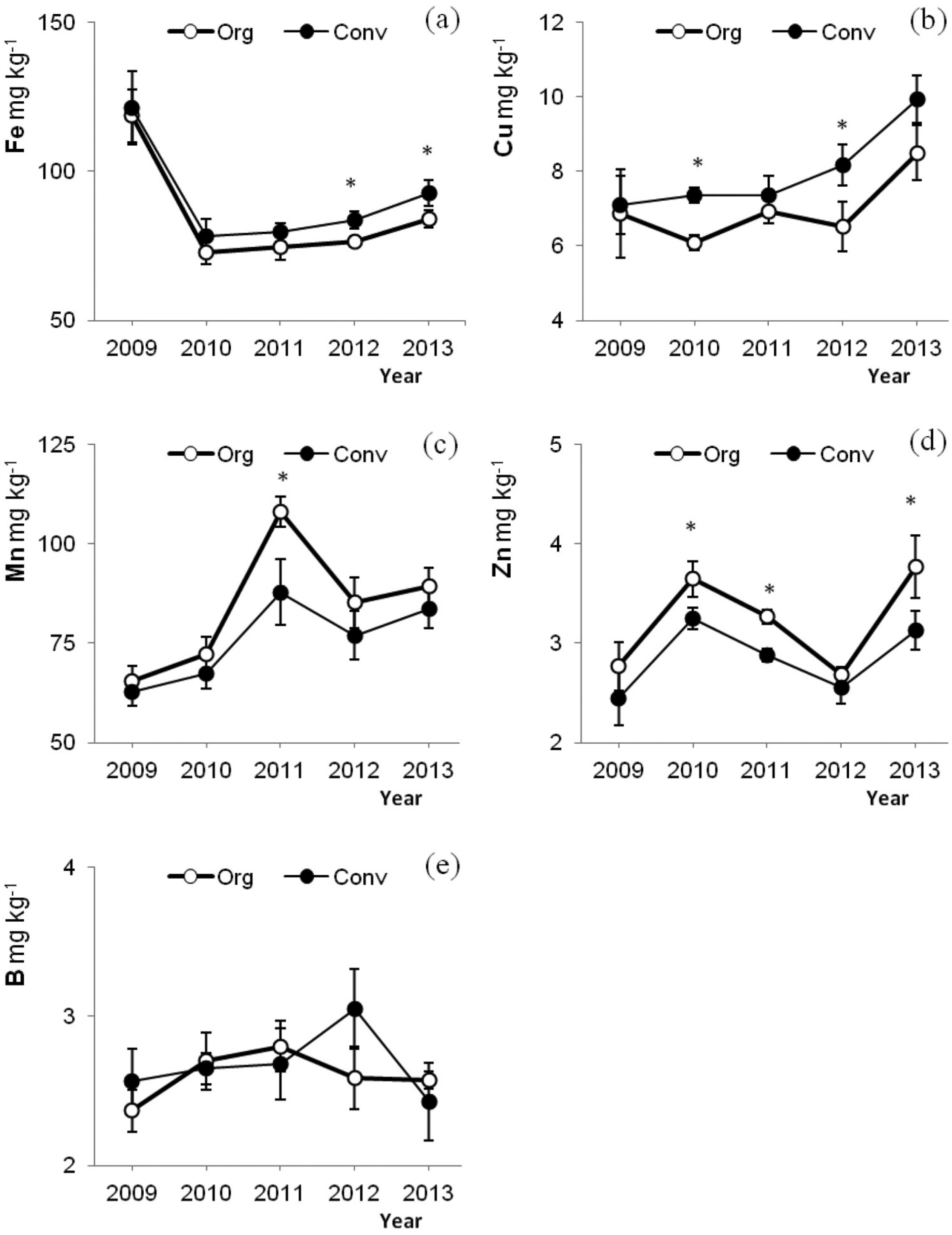

Figure 2. Concentrations of available iron (Fe), copper (Cu), manganese (Mn), zinc (Zn) and boron (B) during the period 2009–2014, in soil subjected to different management regimes. Treatments: Org (– ○ –), organic management; Conv (– ● –), conventional management. Error bars indicate standard deviations of means (*n* = 12).

of Zn were higher in the organically managed plots than in the conventional plots. However, the fact that Cu did not have the same pattern may be due to that Cu may associate in stable complexes with humic acids immobilising the metal. In this sense, Rodríguez-Rubio et al. (2003) concluded that carbonate is the main component responsible for Cu retention in calcareous soils. In addition, calcium carbonate has also been reported to increase Fe absorption and to induce lime chlorosis in plants grown in calcareous soils (Mengel 1994). In this study, higher concentrations of Mn were recorded in the organic plots which, as previously suggested, may have affected the solubility and availability of Fe in the soil, decreasing the extractable Fe concentration (Kabata-Pendias 2001). The role of OM in complexing Mn is important because it may affect the redox status of soils. Microbial soil activity is known to be responsible for the oxidation and reduction of Mn compounds. The microbial decomposition of added OM creates reducing conditions that favour Mn solubilisation (Kabata-Pendias 2001). In the present study, the microbial populations were higher in the organic plots than in the conventional plots. In a previous study undertaken at the same location, increases in available Fe, Cu, Mn, and Zn with the application of OM were found over a 6-year period (Herencia et al. 2008b), similarly to results published previously for Mn, Zn and Cu (Liu et al. 2007). Reganold et al. (2010) found that levels of extractable micronutrients were similar in both systems, except for zinc, boron and iron that were significantly higher in the organically farmed soils. One possible explanation is that the different extraction methods are known to have different capacity for extracting soil micronutrients (Kabata-Pendias 2001).

With regard to the concentration of B, no differences were found between the treatments and a high variability was found for this element (Figure 2e). Several soil factors such as pH, texture, moisture, temperature, OM, and clay mineralogy are known to affect the availability of B to plants (Goldberg 1997). Most of the available B is found in soil organic matter (Yermiyahu et al. 1995), as has also been reported by Reganold et al. (2010). When the organic matter is decomposed, the B is released, and a portion is absorbed by plants. However, under alkaline soil conditions, it can be tied up and made unavailable to plants. One reason of the low B plant availability in soils with clay may be due to the strength by which B is held on the clay surfaces. In addition, B is precipitated due to calcium carbonate and it becomes quite unavailable for plant growth (Goldberg 1997). The results in this study suggested that the supply of the organic matter may have increased the concentration of B, but other aspects such as the soil conditions, the antagonistic relationship between the micronutrients or the analytical methods used may have influenced the results.

Microbial counts

The use of plate counting methods to estimate the population/activity of micro-organisms are in general not considered as very robust as they underestimate the presence of microorganisms and only count the viable and active cells of the population. However, although these methods may estimate only about 10% of the total soil microflora, the cultivable microorganisms have been suggested as those that are most functionally active in the soil (Ellis et al. 2003). It was therefore assumed that this portion of the micro-organisms were the most relevant in this comparative study, as in the organic system the viable and active microorganisms are the principal component in the mineralisation of the organic matter. The numbers of culturable microorganisms recorded for the different sampling dates and for the two management systems are shown in Table 4. The results suggested that the organic management practice stimulated the soil microflora, as the estimates of colony-forming units (CFU) of bacteria and fungi were generally higher in the organic treatment, with significant differences at most sampling dates. The number of colony-forming units of bacteria at the different dates ranged from 1.29×10^7 to 15.01×10^7 CFU g^{-1} (7.11–8.18 log CFU g^{-1}) of soil dry weight and 0.61×10^7 to 12.71×10^7 CFU g^{-1} (6.79–8.10 log CFU g^{-1}) of soil dry weight for organic and conventional plots, respectively. In addition, the total population of fungi ranged from 0.87×10^5 g^{-1} (4.94–5.72 log CFU g^{-1}) to 5.22×10^5 and 0.36×10^5 to 4.32×10^5 g^{-1} (4.56–5.63 log CFU g^{-1}) for organic and conventional plots, respectively.

In general, bacteria utilise the readily available substrates, such as simple sugars and amino acids, while many fungi can utilise more complex plant constituents such as cellulose, hemicellulose, and lignin. Although the ratios of bacteria to fungi have been reported to be lower in the plots with organic treatments than in the conventional treatments (Bossio et al. 1998), other studies have reported the opposite (Marschner et al. 2003). In this study reported here, the organic management regime resulted in higher numbers of CFUs for bacteria and fungi and in a lower ratio of bacteria:

Table 4. Number of colony-forming units (CFU) of bacteria and fungi g^{-1} dry soil in the organic (O) or conventionally (C) managed plum orchards in the years of the study (n = 12).

	Colony forming units $\log_{10}$ g^{-1} soil			
	Bacteria		Fungi	
Date	O	C	O	C
2006	7.85 a	7.61 a	5.28 a	5.20 a
April 2008	8.10 a	8.10 a	5.57 a	5.63 a
October 2009	7.96 a	7.83 b	5.45 a	5.16 b
February 2010	7.83 a	7.65 b	5.56 a	4.88 b
July 2010	7.76 a	7.70 a	5.57 a	5.34 b
March 2011	7.97 a	7.72 b	5.56 a	5.16 b
November 2011	7.11 a	6.79 b	5.26 a	4.56 b
February 2012	7.43 a	7.38 a	4.94 a	4.56 b
May 2012	7.98 a	7.96 a	5.44 a	4.94 b
June 2012	7.81 a	7.51 b	5.35 a	5.05 b
November 2012	8.17 a	7.96 b	5.72 a	5.42 b
February 2013	7.70 a	7.60 b	5.49 a	5.32 b
May 2013	7.66 a	7.59 a	5.48 a	5.23 b
July 2013	8.18 a	7.95 b	5.25 a	5.03 b
November 2013	7.51 a	7.64 b	5.14 a	5.16 a
February 2014	7.89 a	7.63 b	5.10 a	5.02 a
July 2014	7.64 a	7.44 b	5.17 a	5.06 a
October 2014	7.70 a	7.56 b	5.36 a	5.29 a

For each date, comparing estimates of bacteria and fungi separately, values followed by the same letter were not statistically different at $p < 0.05$.

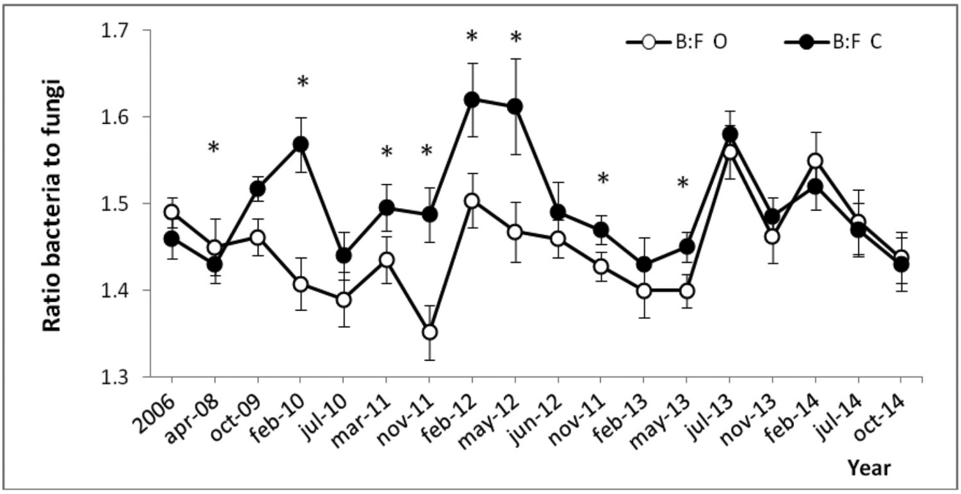

Figure 3. Ratios of bacteria to fungi (B:F) for the different treatments across the study period. O (– ○ –), organic treatment; C (– ● –), conventional treatment (n = 12).

fungi (indicating a higher proportion of fungi in the organic soil compared with that in the conventional soil) (Figure 3).

It is known that some of the regular management practices in organic systems, such as the addition of organic carbon substrates and the use of cover crops, exert substantial influence in the microbial community in the soil, though these changes are known to be the result of complex interactions with unique impacts on the microbial community structure (Bossio et al. 1998). The increase in microbial population may be due to the addition of labile organic matter (compost and plant residue) that is readily available for the microorganisms (Drenovsky et al. 2004). In this study, the cover crops used in the rotation included legumes and legume/cereal mixtures; the use legumes as cover crops is common practice in organic systems as the use of N_2-fixing species reduce the need for other inputs of N. Legumes generally have a low C:N ratio, which favours the bacterial activity and improves the decomposition of the soil organic carbon (SOC) (Six et al. 2006). Mbuthia et al. (2015) reported that a continuous period of growing a leguminous cover crop like vetch, enhanced the microbial activity and promoted the cycling of both C and N. Moreover, they concluded that the use of a nitrogen-fixing cover crop in combination with reduced tillage offered a more sustainable option than the use of inorganic N. On the other hand, the use of cover crops with a relatively high C:N ratio and lignin content, such as oats, produce fair amounts of residues that decomposed relatively slowly. It is known that higher concentrations of lignin in the residue and higher C:N ratios can impact positively on soil organic carbon accumulation (Paustian et al. 1992). Six et al. (2006) indicated that the quality of the substrate altered the fungal:bacterial ratios, with materials with high C:N favouring fungi and materials with low C:N favouring bacteria.

The results reported from this study, agreed with those reported by Six et al. (2006) concluding that the use of crop rotations, reduced or no-tillage practices, organic farming, and cover crops increased the total microbial biomass and shifted the community structure towards a more fungal-dominated community. Quantitative and qualitative improvements of SOM are generally observed in agroecosystems favouring a fungal-dominated community, but the mechanisms leading to this improvement are not completely understood (Six et al. 2006). Moreover, fungi are primarily responsible for the formation of soil macroaggregates that protect the OM and decompose more slowly. Thus, ecosystems with fungal-dominated soil communities may have higher C retention than those dominated by bacteria. It is possible that in long-term organic systems more available OM is continuously added and mineralised and the increase in soil OM could be due to more stable OM fractions favouring the fungal community. Microorganisms play a central role in maintaining the fertility of the soil and organic amendments were found to result changes in the bacterial and fungal communities (Yu et al. 2013). However, the magnitude and the specificity of these changes vary between sites, organic amendments, and microorganisms targeted, suggesting that the magnitude of the impact is dependent on the type of soil, organic input typology as well as on the particular ecology of microorganisms (Lejon et al. 2007).

Nodulation studies

To evaluate the performance of the vegetation, the efficiency of nodulation and to estimate the *Rhizobium* population in the soils, in 2010 and 2011 some of the green cover crops that were used in the organic orchard were also grown in the conventional orchard. The number and dry weight of the nodules formed on the two leguminous species were considerably greater in the organic plots compared with those in the conventional plots (Table 5); with the number of nodules on the organic beans being tenfold higher than that on the conventional beans and in the vetch the number of nodules was almost twice as high. Similar results were found when comparing the nodule dry weight, which was approximately five times higher on beans grown in the organic orchard compared with the beans grown in the conventional orchard, whereas in the vetch it was nearly

Table 5. Average number of nodules, nodule dry weight, most probable number (MPN) of *Rhizobia* and dry weight of nodulated *Vicia* species in the organic (O) and conventionally (C) managed plum orchards.

	Beans *(Vicia faba)**		Vetch (*Vicia sativa*)*	
	O	C	O	C
Nodules (number plant^{-1})	91.2 a	8.8 b	11.9 a	7.7 b
Nodule dry weight (mg plant^{-1})	157.33 a	33.58 b	21.17 a	13.08 b
Plant dry weight (g)**	119.54 a	48.00 b	nd	nd
Rhizobia (MPN) (number g^{-1} soil)	10,429 a	499 b	nd	nd

Values followed by the same letter were not significantly different ($p < 0.05$). * Values for beans *(Vicia faba)* Feb 2010 and vetch (*Vicia sativa*) Feb 2011 compared separately. ** Dry weight of above-ground parts of plant.

twice as high. The number of rhizobia in the soil (MPN) were also much higher in the organic treatment.

The rhizobium-legume symbiosis is a major contributor to soil fertility since it can provide over half of the biological source of fixed N (Tate 1995). It is well known that several environmental conditions and management practices are limiting factors for the growth and activity of the nitrogen-fixing plants. Mostly problematic are low moisture conditions (low rainfall, poor water-holding capacity), extreme temperatures, acidic soils with low nutrient status and high concentrations of NO_3 (Zahran 1999). Despite the importance of N fixation in organic systems, there are few studies reporting on the influence of organic management on the persistence of rhizobia and their ability to form successful *Rhizobium*-legume symbiosis. Grossman et al. (2011) found that the organically managed soybean fields contained a greater diversity of rhizobia isolates than conventionally managed fields. Most studies with organic amendments showed that the organic matter addition, such as manure, increased microbial activity, including rhizobial growth, and legume nodulation (Tagoe et al. 2008; Grossman et al. 2011). Herrmann et al. (2014) also reported that nodulation was positively affected by plant residues application. The results of this study were in line with those showing a population enrichment of rhizobia in the organic orchard and improved nodulation compared with that in the conventional orchard. These results have sometimes been explained by improved P availability for the crops (Tagoe et al. 2008) and by the soil environment in terms of physical and chemical properties for nitrogenase activity (Ghosh et al. 2004). In addition, other studies demonstrated that the availability of NO_3 inhibited nodule formation on legumes, whereas the slow mineralisation of N of organic fertilisers improved nodulation (Otieno et al. 2009), as probably occurred in the conditions of this study reported here.

Mycorrhizal studies

Although there are different types of mycorrhizal fungi, roots of most herbaceous and horticultural species, including plums, establish mycorrhizal symbiosis with a similar type of endomycorrhizal fungi; vesicular arbuscular mycorrhizal fungi (Smith and Reed 1997). Therefore, the degree of mycorrhizal colonisation in roots of the plum trees and herbaceous species (which are undoubtedly easier to process) were analysed. Overall, a high degree of mycorrhized roots in the herbaceous species and in the plum trees in both types of management systems were observed, but the only significant difference was observed in *Vicia faba*, for which it was higher in the organic plot (Table 6). For plants, mycorrhizal symbiosis is a strategy for improving the uptake of nutrients and water from the soil and is particularly relevant for the acquisition of phosphorus, when this element is scarce (Smith et al. 2011). The high degree of mycorrhization observed in this study correlated with the low level of phosphorus in the soil in both treatments.

Table 6. Mycorrhization degree in roots of herbaceous species and plum cultivars from two orchards under organic (O) and conventional (C) management.

	Mycorrhization (%)	
Species (year)	O	C
Conyza bonariensis (2012)	77.66 a	71.33 a
Vicia faba (2012)	63.00 a	24.66 b
Cultivar 'Souvenir' (2012)	52.00 a	66.50 a
Cultivar 'Souvenir' (2013)	75.67 a	66.00 a
Cultivar 'Showtime' (2013)	74.33 a	63.33 a

Values, in rows, followed by the same letter were not statistically different ($p < 0.05$).

Table 7. Number and biomass of earthworms per m^2 of soil in the organic (O) and conventionally (C) managed plum orchards ($n = 5$).

	Number of earthworms		Biomass (g m^{-2})	
Year	O	C	O	C
March 2007	17.80 a	7.27 b		
Nov 2007	20.33 a	4.13 b		
March 2011	119.54 a	48.00 b	48.77 a	29.75 a
April 2012	32.44 a	20.00 a	3.87 a	3.00 a
November 2012	92.00 a	45.33 b	37.50 a	13.68 b
March 2014	41.33 a	81.33 a	25.33 a	60.00 a

For each date, values in rows followed by the same letter were not statistically different ($p < 0.05$)

Earthworm populations

At most times of sampling, the number and the biomass of earthworms were higher in the organic plots, but the biomass of the earthworms were only significantly higher on one occasion (November 2012). Earthworm abundance ranged from 17.8 to 119.5 individuals m^{-2} in the organic plots and from 4.1 to 48.0 m^{-2} in the conventional plots, with the exception of the last year of the study when a contradictory trend (though not significant) was found (Table 7). The biomass of the worms ranged from 3.87 to 48.77 g m^{-2} in organic plots and from 3.0 to 29.75 in conventional plots. The application of organic manure has been shown to influence the number of earthworms in the soil, with numerous comparative investigations showing that the abundance and biomass of earthworms were considerably greater under the organic system than conventional systems (Scullion et al. 2002; Bengtsson et al. 2005). In addition, some studies performed in perennial crops such as orchards have revealed similar results (Hole et al. 2005). In a review, Hansen et al. (2001) reported a 20-fold increase in the population of earthworms as a consequence of converting a conventional system to organic farming. In a Mediterranean-type ecosystem the OM and earthworm abundance were highest at the organic orchard sites compared to the conventional sites (Walmsley and Cerdà 2017). Greater input of composts and manures provided more food sources for the earthworms thus promoting growth and reproduction. Nevertheless, the increase depended on the type of fertilisers used. Pfiffner and Mäder (1997) showed that the biomass and abundance of earthworms were 1.3 to 3.2 higher in organic compared with conventional plots and it was attributed to the inclusion of clover leys in the rotations avoiding adverse effects associated with chemicals. Scullion et al. (2002) indicated that earthworm biomass was often higher on organic farms compared with similar phases on conventional farms. However, these differences were not consistent and in some cases the populations were higher on the conventional farms. They also indicated that the combination of absence of ploughing and presence of clover under-storey increased earthworm populations greatly. In this study reported here, a minimum tillage practice (tillage undertaken when necessary) was used in both treatments. The role of earthworms in enhancing soil fertility is well known because they contribute to physical, chemical and biological soil processes (Edwards 1998) and it seems clear that organic management practices can improve earthworm populations compared with conventional practices, as was demonstrated in this work.

Overall, these findings of this study were in agreement with the conclusions of the European Investigation System (Niggli et al. 2008), which indicated that organic systems that included cover crops, provided enhanced eco-system services, consistent with the Sustainable Development Goals that are the target of current EU policies (European Commission 2014).

Conclusion

This comprehensive study indicated that the organic management regime resulted in improved soil quality in terms of the chemical and biological characteristics of the soil, with increased concentrations of organic matter, macro and micronutrients, increased microbial populations, increased legume nodulation, mycorrhizal colonisation and numbers of earthworms. The results of the study suggested that in Mediterranean conditions, organic management that include soil management practices, such as the use of cover crops and compost application, had a strong effect on different indicators of soil quality and fostered biotic interactions between above and below-ground components, thereby improving the sustainability of the farming system and confirming soil quality as a necessary indicator of sustainability land management. The outcomes presented will strengthen the significance of organic management in Mediterranean fruit tree ecosystems and can be considered as a specific kind of nature-based solution. The adoption of organic farming could be a key land-use policy strategy to maintain soil quality, soil functions and services to achieve sustainability. However, in order to be truly sustainable, the system must maintain levels of soil fertility sufficient for economic crop production in the long term whilst also protecting the environment. Nevertheless, organic farm management strategies are diverse, and the sustainability of the systems depend more on the choice of management practices than on the general farming system and more studies are needed to confirm this.

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Disclosure statement

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