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Soil enzymes, nematode community and selected physico-chemical properties as soil quality indicators in organic and conventional olive oil farming: Influence of seasonality and site features

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ABSTRACT

There is growing interest in the application of soil enzymes and nematode community indices as indicators of changes in soil quality under contrasting management practices. Although an abundant literature on this subject has arisen during the last 10 years, most of the studies have focused on measuring many soil quality indicators at a single or a few sampling times. However, soil enzyme activities show a natural temporal variability which could mask the variability due to the type and timing of soil management practices. In this study, we compared soil enzymes, nematode communities and physical–chemical soil properties in three pairs of organic and neighbouring conventional olive orchards. Dehydrogenase, β-glucosidase, arylsulfatase, acid and alkaline phosphatases activities, and potential nitrification were studied during an annual cycle, and variability due to sites, replicates within a site, management practices and seasonality has been accounted for. In addition, several nematode community indicators were also studied on one occasion. The geometric mean of enzymes activities (GMea), used as an integrating soil quality index, was validated through an independently performed principal component analysis (PCA). Seasonal variability of individual soil enzymes ranged from 29 to 71%, without a consistent temporal trend. Management system explained, on average, a maximum of 26.3 and 15% of the variability found for soil enzymes and nematode community indicators, respectively. Most of the variability found in both sets of indicators was due to different localities (up to 58 and 45% for soil enzyme and nematode community indicators, respectively) and replicates within a plot (up to 51 and 86%, respectively). Organic management resulted in significantly higher soil enzyme activities. However, differences were dependent on site and sampling. For nematode community indicators, the organic farms showed higher values only for one site. These results reveal the need for extensive comparative assessments to draw clear conclusions on the improvement of soil quality under sustainable management practices. The GMea was significantly higher in organic than in conventional managed plots, independently of the sampling and, moreover, showed significant correlation with the first axis of the PCA. In addition, the GMea, and scores on the first axis were highly correlated with some of the nematode indices. Therefore, the GMea was a suitable tool to condense the whole set of soil enzyme values in a single informative numerical value, which was more sensitive to management practices than nematode community indicators.

1. Introduction

Intensification of cultivation is widely recognized as one of the most significant human alterations to the global environment (Vitousek, 1994; Matson et al., 1997). Concerned by these issues, the European Commission has initiated agricultural policies promoting environmental-friendly practices by introducing in the subsidies policy environmental criteria, together with the traditional social and economic ones. In this context, the interest is now being focused on crop production systems, such as organic agriculture, that optimize yields while preserving soil and protecting the environment.

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A relatively high percentage (around 30%) of organic farming in the Andalucía region (southern Spain) is dedicated to organic olive oil production. Olive oil production is of enormous economic and social importance in Andalucía, where 2500 oil mills generate more than 30% of the world production of olive oil (information supplied by the Olive Oil Agency, Spanish Minister of Environment and Rural Development). According to these figures, the ecological impact of these crops is huge, with olive orchards being the dominant landscape throughout more than 2 millions hectares.

Consensus about soil quality standards is not yet well established, mainly since no single or combined biological and physico-chemical variable is available to reflect the many interacting processes responsible for soil quality (Puglisi et al., 2006). However, the implementation of environmental-friendly agricultural policies requires monitoring programmes, based on suitable soil quality indicators, to evaluate the success of specific agricultural practices.

Physical and chemical properties have been extensively used to measure soil quality (Parr and Papendick, 1997). However, these properties usually change on a time scale (decades) which is too long for management purposes. In contrast, soil properties based on biological and biochemical activities, such as soil enzymes and the nematode community, have been shown to respond to small changes in soil conditions, thus providing information sensitive to subtle alterations of soil quality (Pascual et al., 2000).

Soil enzyme activities have been suggested as suitable indicators of soil quality because of their intimate relationship with soil biology, ease of measurement, and rapid response to change in soil management. Many long-term studies have shown that soil enzyme activities are sensitive in discriminating among soil management practices, such as fertilisation by means of animal manure or green manures/crop residues (Dick et al., 1988; Martens et al., 1992) and municipal refuse amendment (Perucci, 1992), as well as among tillage treatments (Gupta and Germida, 1988). The response of soil enzyme activities to specific soil practices has been used to compare agricultural systems (combinations of soil practices) such as organic versus conventional farming (Benitez et al., 2006; van Diepeningen et al., 2006; Melero et al., 2006). However, in these studies, the emphasis has been set on the comparative assessments of many dependent variables measured at a single time in the selected systems, rather than on a time series dynamic assessment.

The natural intra-annual variability of soil variables is an important factor to consider in the applicability of a soil quality index, since broad ranges of temporal variability would mask the variability due to management practices, making difficult an accurate interpretation of the index. In addition, for monitoring and management purposes, soil quality indices with substantial natural variability are not suitable for a low sampling frequency regime. However, soil is a dynamic biological entity within a continuously changing environment. Seasonal fluctuation in, for instance, soil water potential, gas exchange and temperature, account for most of the microbiological variability, including production, survival and stabilization of soil enzymes within the soil matrix (Aon et al., 2001). Due to the diverse origins, locations within the soil matrix and functions of the different enzymes, their activities show diverse magnitude and temporal patterns of natural variability, within which perturbation effects might be masked.

In agro-ecosystems, the type and timing of management practice (tillage, fertilisation, irrigation, harvest, etc.) must be considered in addition to the above mentioned natural temporal variability in soil microbiological properties. Therefore, if soil enzyme activities are considered for use as soil quality indicators, their seasonal variability should first be tested.

Due to the complexity of soil structure and functionality a good soil quality indicator has to be integrative. Therefore, complex indices, calculated by means of algebraic combinations of different soil biochemical properties (Trasar-Cepeda et al., 1998; Nannipieri et al., 2002) or multivariate analysis (Blair et al., 1995), have been developed. In this respect, numerical techniques such as principal component analysis (PCA) (Sena et al., 2002) and factorial analysis (Shukla et al., 2006; Melero et al., 2006) have been proposed.

Aside from soil enzyme activities, there is interest in the soil nematode community, especially free-living species, as bioindicators of soil quality (Bongers and Bongers, 1998; Yeates and Bongers, 1999). Different nematode-based indices such as the ecological maturity index (Bongers, 1990), the plant-parasite index (Bongers et al., 1995), and indices related to diversity and species richness (Yeates and Bongers, 1999) have been proposed for monitoring soil quality under contrasting management practices. However, the effects of plant crop, nematode biogeography and soil characteristics on the distribution of particular nematode species have been reported as being responsible for contradictory results in studies comparing agro-ecosystems differing in soil management practices (Wardle et al., 1995).

We evaluated nematode community parameters and seasonal trends in selected soil enzymes, and a combined index of soil quality, in three independent, paired organic and conventional olive orchards. Intra-plot, among-plots and management-type variability were accounted for. Finally, we compared the results from principal component analysis, which included physico-chemical soil properties and a combined soil index (based on enzyme activities).

2. Materials and methods

2.1. Field sites and experimental design

The study was conducted in the province of Jaén (southern Spain), the largest olive oil producer in the world on an area basis. The field sites were situated less than 10 km from the town of Jaén, where climatic conditions correspond to a Mesic moisture and temperature regimes, with mean annual temperature and precipitation of 16 °C and 560 mm, respectively.

Three organic olive oil farms (O) differing in soil and landscape characteristics were chosen from the database supplied by the Andalusian Committee of Organic Farming (CAAE; Junta de Andalucía). The organic farms were selected at Pegalajar (PG), Puente Tablas (PT) and Puente Sierra (PS). All the farms had trees of similar age (between 35 and 60 years) and a plantation density of 70–90 trees ha^{-1}. Nutrient inputs, at a biannual rate of approximately 25–35 tons per ha, consist of organic fertilisation with a mixture of sheep and hen manure from extensive livestock. Crop and spontaneous weed residues were left on the soil surface. Weeds and pests have been controlled, at least during the last 5 years, by tillage and/or cutting, and by natural biological control, respectively.

To minimize the effects of differences in soil, climate and landscape features the three organic olive oil farms were paired with three conventional olive oil farms (C) which were chosen within a distance of tens of meters of the organic farms. Differences between pairs of sites were attributed primarily to a set of management practices. In the three conventional farms, cropping practices consist of fertilisation by annual additions of ammonium nitrate or urea, at an approximate rate of 1.5 kg N $tree^{-1}$ (95–120 kg N ha^{-1}), and weed control by intensive tillage and/or weed and pest suppressing pesticides.

Table 1 shows the general characteristics of the organic and conventional olive oil farms chosen for this study.

Table 1
General characteristics and management practices of the paired comparable organic and conventional olive oil orchards located at three selected olive oil farming sites (PG, PT and PS stand for Pegalajar, Puente Tablas and Puente Sierra sites).

Site	Agricultural system	Years since organic certification[a]	Soil management practices	Fertilisation	Farm area (ha)/slope[b]	Tree density (ha^{-1})/age (years)
PG	Organic	5	No tillage. Summer irrigation. Weed under tree canopy harvested	Sheep manure	1.5/light	90/60
	Conventional	–	No tillage. No irrigation. Chemical weed control	Urea	6/moderate	80/45
PT	Organic	4	High tillage frequency and intensity. Summer irrigation	Hen manure	8/light–moderate	70/35
	Conventional	–	Shallow tillage. Chemical weed control	NH_4NO_3	12/light–moderate	65/8
PS	Organic	5	No tillage. Summer Irrigation. Weed under tree canopy harvested	Hen manure	4/moderate	70/40
	Conventional	–	No tillage. Summer irrigation. Chemical weed control	NH_4NO_3	12/moderate	70/40

[a] Years since organic certification include 3 years of conversion from conventional to organic management practices.
[b] Light, light–moderate and moderate slopes stand for slopes between 2 to 5%, 5 to 10% and higher than 10%, respectively.

2.2. Sampling design

Soil samples were collected from the top 10 cm every 2 months from April 2003 to July 2004, with a 5-cm diameter corer. At each sampling, 5 trees per farm were randomly chosen. Two soil samples, each a composite of four cores, were collected under the canopy of the selected trees. In the organic olive oil plots, extreme care was taken to avoid collection of distinguishable manure remains. Soil samples were transported to the lab and fresh-sieved (<2 mm) on the same day. Soil enzyme activities and physico-chemical properties were examined on one of the composite soil samples. The nematode community was studied in the other subsample only in the May 2003 sampling.

2.3. Physico-chemical soil properties

The soil pH was determined in fresh soil subsamples in a slurry with $CaCl_2$ 0.01 M (1:1; soil:$CaCl_2$) following McLean (1982), the water content by gravimetry and the organic matter content by weight loss after ignition (Nelson and Sommers, 1982). The water-holding capacity (WHC) at −33 kPa was determined in a Richard's membrane-plate extractor (Klute, 1986), and particle size distribution by means of the pipette method (Gee and Bauder, 1986). Laboratory (20–22 °C) air-dried subsamples were used to analyse exchangeable Ca, Mg, K and Na following Grant (1982). Active carbonate and labile phosphorus contents were determined following the methods of Nelson (1982) and Olsen and Sommers (1982), respectively. Cation exchange capacity was analysed according to Rhoades (1982). Total carbon and nitrogen was analysed on oven-dried samples in a Leco CNH-932 analyser.

2.4. Enzyme activities and soil biological properties

Enzyme activities were analysed for 1 g air-dried soil samples. Zornoza et al. (2006) have shown that β-glucosidase and acid phosphatases activities on air-dried soils were not significantly different from those obtained from fresh soil analyses for two Mediterranean locations and different seasons. Acid phosphatase (EC 3.1.3.2, orthophosphoric-monoester phosphohydrolase, acid optimum), alkaline phosphatase (EC 3.13.1 orthophosphoric-monoester phosphohydrolase, alkaline optimum), arylsulfatase (EC 3.1.6.1., arylsufate sulfohydrolase) and β-glucosidase (EC 3.2.1.21, β-D-glucoside glucohydrolase) activities were determined according to Tabatabai (1982) and reported as μg *p*-nitrophenol (*p*NP) $g^{-1}\ h^{-1}$. Control assays were performed in all cases by adding the substrate after the reaction was stopped and before filtration of the soil suspension. Dehydrogenase was determined as described by Casida et al. (1964) applying the following modification: 1 g soil was mixed with 0.01 g $CaCO_3$ and incubated in the presence of 1 ml 3% 2,3,5-tri-phenyl tetrazolium chloride (TTC) and 3 ml water at 37 °C in darkness. After 6 h, 10 ml methanol was added, and the suspension homogenized, filtered and washed with methanol until the reddish colour caused by the reduced TTC (triphenyl formazan) had disappeared from the soil. The optical density at 485 nm was compared to that of triphenyl formazan standards. The potential rate of soil ammonium oxidation was analysed following the method described by Kandeler (1995). Briefly, soil samples were incubated with a solution containing ammonium and sodium chloride (NaCl; an inhibitor of the nitrite oxidation to nitrate) at 25 °C for 5 h. The soil nitrite concentration was analysed after the incubation and compared with control samples which received ammonium and sodium chloride but were frozen until the end of the incubation period. The values of activities and rates are reported on an oven-dried soil weight basis. For each soil sample, the geometric mean of enzyme activities (GMea) was calculated as:

$$\mathrm{GMea} = \sqrt[6]{\mathrm{AcP} \times \mathrm{AlP} \times \mathrm{Glu} \times \mathrm{Ary} \times \mathrm{Dehy} \times \mathrm{PN}},$$

where AcP, AlP, Glu, Ary, Dehy and PN stand for acid phosphatases, alkaline phosphatases, β-glucosidase, arylsulfatase and dehydrogenase activities, and potential rate of soil ammonium oxidation, respectively. This algorithm has been shown to adequately indicate soil quality in plots receiving different dose of heavy-metal enriched sludge (Hinojosa et al., 2004) and is applied in this work as an overall indicator of soil quality.

2.5. Nematode analysis

Nematodes were extracted from the second replicate soil sample of the May 2003 sampling by the flotation method of Flegg (1967), using the modified funnel technique of Baermann (1917). The specimens were killed by heat (65–70 °C), and preserved with 4% formalin and glycerin mounting in permanent microscopic preparations following Siddiqi (1964). Specimens were identified by measuring several measurements with an ocular micrometer and/or a camera lucida. Nematodes were identified to species level, when possible, by means of compound optical microscopy and according to different monographs (Andrássy, 1983, 1984; Jairajpuri and Ahmad, 1992; Siddiqi, 2000). The maturity index (MI) was calculated following Bongers (1990) with the modification of substituting relative abundance (percentage of individuals of each species relative to the total number of individuals) for relative frequency (percentage of samples in which a species was found). Nematode species richness was determined for each

trophic group according to the classification of Yeates et al. (1993). Feeding habits were plant, hyphal, bacterial and unicellular eucaryote feeding, animal predation, substrate ingestion and omnivory. The Shannon–Weaver diversity index (H′) was calculated as: $H' = -\sum pi(\ln pi)$, where pi is the proportion of nematodes in the trophic group i present in the total nematode community.

2.6. Statistical analyses

Significant differences in soil physico-chemical properties, number of nematode species within each of the seven trophic groups, total number of nematodes species, MI and H′ between organic and conventional olive orchards were evaluated by means of two-way ANOVA. Differences among levels of factors (organic and conventional for agriculture system and PG, PT and PS for sites) were tested using the Fisher Least Significant Difference (LSD) test. Differences in soil enzyme activities and potential rate of soil ammonium oxidation between organic and conventional plots, at the different sampling times, were evaluated by means of repeated-measures two-way ANOVA, and a post hoc Fisher LSD test was applied to compare individual sets of means. The variance of the soil enzyme activities, potential rate of soil ammonium oxidation, nematode community indicators and GMea attributable to the different sources of variation (management practices, sites and variability due to replicates within a site) was tested using a component-of-variance analysis. Assumptions of analysis of variance (homocedasticity and normality) were tested and assured by using transformed data sets [log(dependent variable value + 1)] when necessary (Zar, 1999). Significance was accepted at $P < 0.05$ in all cases. Principal components analysis (PCA) was applied using data for soil physical and chemical properties and soil enzyme activities. Nematode community indicators were not included in the PCA because sampling for nematode analyses was different to that of soil physico-chemical variables. GMea was not included in the PCA since it was calculated from the values of soil enzyme activities. The two first coordinates (PC1 and PC2) were selected for further interpretation of the results. Pearson product-moment correlations were calculated between soil physico-chemical properties, soil enzyme activities, potential rate of soil ammonium oxidation and scores on PC1 and PC2, for the interpretation of the new axes. Product-moment Pearson correlations between scores of the different locations and managements at the PC1, GMea, and nematode indicators were also done. Statistical analyses were performed using STATISTICA 6.0 scientific software (StatSoft Inc., 2001).

3. Results

3.1. Soil physico-chemical properties

Exchangeable Mg, labile P and organic matter content showed significantly higher values ($P < 0.05$) in soils under the organic than under the conventional management for all the sites (Table 2). Soil total carbon content showed higher values in the organic farms at the PT and PS sites, while soil exchangeable Ca and K contents were higher in the PT and PG organic farms, respectively. Soil exchangeable Na, carbonate and total N content, pH and cation exchangeable capacity showed no significant differences between organic and conventional farms (Table 2).

The highest contents of soil exchangeable Ca and Mg, labile P, and clay percentages were found at the PT site. Soil cation exchangeable capacity, organic matter content and total N were highest at the PG site, and soil carbonate at PS. Finally, silt percentage, exchangeable Na and pH did not differ significantly among sites.

3.2. Soil enzyme activities

Mean annual values (2003 data) of acid phosphatase activity in soils under organic management were 134, 192 and 251 μg *p*NP h^{-1} g^{-1} at the PT, PS and PG sites, respectively. During the year, values were relatively high during spring and early summer, and showed a decrease from mid to late summer, except for those from the soil managed by organic practices at the PG site (Fig. 1a). When comparing organic versus conventional soil managements, values of acid phosphatase activity were significantly higher in the former than in the latter (Table 3), although this result depended on sampling time and location. Thus, at the PG and PS sites, acid phosphatase values were higher in the soil from the organic practices for the whole period, except for mid summer, but only during the spring time at the PT site. Soil alkaline phosphatase activity showed an increasing trend from spring to fall in all the plots (Fig. 1b). For this soil enzyme activity, significant differences were found when comparing soils managed by either organic or conventional practices, and among locations (Table 3).

Table 2
Soil physico-chemical properties of the organic (O) and conventional (C) olive oil farms at the selected sites (PG: Pegalajar; PT: Puente Tablas; PS: Puente de la Sierra). ANOVA probability values for the effects of management, site and their interaction on the soil physico-chemical properties are also shown. NS: no significant effect at $P = 0.05$. Mean comparisons between management practices and among sites are based on LSD ($P < 0.05$).

Properties	PG		PT		PS		Management practices (M)	Site (S)	M × S
	Organic[a]	Conventional[a]	Organic	Conventional	Organic	Conventional			
Clay[b]	32.2	25.3	36.8	46.0	15.7	26.6	0.14^{NS}	<0.01; PS = PG > PT	<0.01
Silt[b]	48.9	60.1	41.4	37.2	66.5	58.9	0.94^{NS}	<0.01; PS = PG > PT	<0.01
Sand[b]	17.6	24.5	21.5	16.6	17.7	14.4	0.06^{NS}	<0.01; PT > PG = PS	0.051^{NS}
Exchangeable[c] Ca	76.3	77.6	122	59.7	61.1	65.2	<0.01; O > C	<0.01; PS > PT = PG	<0.01
Exchangeable[c] Mg	8.2	1.22	5.4	2.3	2.0	0.85	<0.01; O > C	<0.01; PT > PT = PG	0.34^{NS}
Exchangeable[c] K	3.1	1.58	2.5	2.6	1.04	0.6	0.10^{NS}	<0.01; PG = PT > PS	0.24^{NS}
Exchangeable[c] Na	0.07	0.07	1.3	0.09	0.46	0.17	0.09^{NS}	0.20^{NS}	0.20^{NS}
Labile[d] P	61.3	19	105	46.6	15.3	9.6	<0.01; O > C	<0.01; PT > PG = PS	0.07^{NS}
Carbonate[b]	54.8	57.2	33.0	31.2	70.2	69.6	0.99^{NS}	<0.01; PS > PG > PT	0.89^{NS}
CEC[c]	19.8	17.1	15.5	12.3	10.1	8.2	0.049; O > C	<0.01; PG > PT > PS	0.90^{NS}
Loss-on-ignition[c]	8.4	5.3	5.0	3.6	4.7	3.3	<0.01; O > C	<0.01; PG > PT > PS	0.20^{NS}
pH	7.8	7.9	7.6	7.6	7.8	7.6	0.89^{NS}	0.62^{NS}	0.87^{NS}
Total C[b]	8.2	7.9	5.6	4.6	8.9	7.8	0.02; O > C	<0.01; PG = PS > PT	0.05^{NS}
Total N[b]	0.33	0.26	0.22	0.15	0.21	0.13	<0.01; O > C	<0.01; PG > PT = PS	0.19^{NS}

[a] Values in the organic and conventional columns are the mean of three replicates. The coefficient of variation of the mean was always lower than 37%.
[b] %.
[c] mequiv. 100 g^{-1} dry soil.
[d] μg P g^{-1} dry soil.

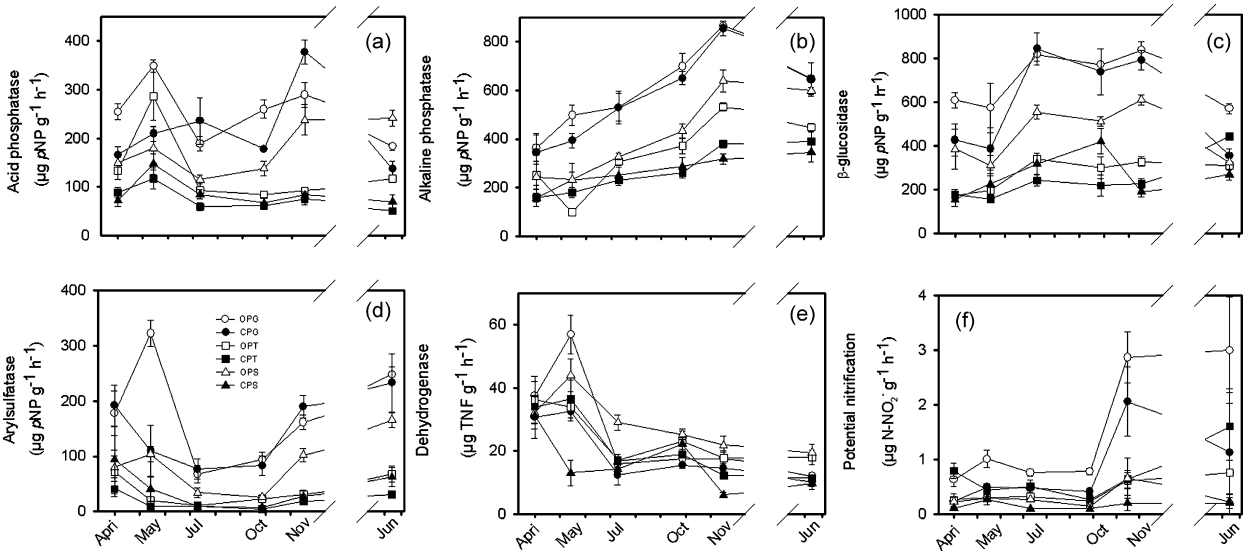

Fig. 1. Temporal patterns of soil (a) acid and (b) alkaline phosphatase, (c) β-glucosidase, (d) arylsulfatase, and (e) dehydrogenase activities, and (f) potential rate of ammonium oxidation, during the study period (April 2003 to June 2004). The variables were measured in organic (open symbols) and comparable conventionally managed (full symbols) olive oil crops at the different sites: PG (Pegalajar; circles), PT (Puente Tabla; squares) and PS (Puente Sierra; triangles). Data are the means of five replicates and bars represent the standard errors of the means.

Table 3
Probability levels of the effects of management practice (organic O versus conventional©), site (PG, PT and PS) and sampling time on the assayed soil enzyme activities and the geometric mean (GMea) as determined by means of a repeated-measures ANOVA. NS: no significant effect at $P = 0.05$. T1, T2, T3, T4, T5 and T6 stand for the 6 samplings arranged in chronological order.

	Acid phosphatases	Alkaline phosphatases	β-Glucosidase	Arylsulfatase	Dehydrogenase	Potential nitrification	GMea
Management practices (M)	<0.001 O > C	<0.001 O > C	<0.001 O > C	<0.001 O > C	<0.001 O > C	0.067^{NS} O = C	<0.001 O > C
Site (S)	<0.001 PG > PS > PT	<0.001 PG > PS > PT	<0.001 PG > PS > PT	<0.001 PG > PS > PT	0.116^{NS} PG = PT > PS	<0.001 PG > PT = PS	<0.001 PG > PS = PT
Sampling (Sp)	<0.001 T5 > T2 > T3 = T1 = T4 = T6	<0.001 T5 > T6 > T4 > T3 > T1 = T2	<0.001 T3 = T4 = T5 > T6 > T1 = T2	<0.001 T6 = T1 = T2 > T5 > T4 = T3	<0.001 T2 = T1 > T4 = T3 > T5 = T6	<0.001 T6 > T5 > T2 = T1 = T3 = T4	<0.001 T5 > T6 > T2 = T1 > T4 = T3
M × S	0.03 OPG = CPG OPT > CPT OPS > CPS	0.002 OPG = CPG OPT = CPT OPS > CPT	0.004 OPG > CPG OPT = CPT OPS > CPS	0.915^{NS}	0.001 OPG > CPG OPT = CPT OPS > CPS	0.001 OPG > CPG OPT = CPT OPS = CPS	0.029 OPG > CPG OPT = CPT OPS > CPS
M × Sp	<0.001	0.023	0.079^{NS}	0.056^{NS}	0.021	0.441^{NS}	0.118^{NS}
S × Sp	<0.001	<0.001	<0.001	0.015	<0.001	<0.001	0.003
M × S × Sp	<0.001	0.45^{NS}	0.003	0.042	0.008	<0.001	0.025

Significant effects were found for all the possible interactions among factors for the alkaline phosphatase activities, except for the triple interaction (management practices × site × sampling).

For the whole study period and the PS and PG sites, β-glucosidase activity showed higher values in the soils managed by organic than by conventional practices (Table 3). However, no common time trend was found when comparing sites. However, the comparison between locations showed a trend from the highest values at the PG site to the lowest at the PT site (Fig. 1c; Table 3). Arylsulfatase activity in soils managed by organic practices was significantly higher than that in soils managed conventionally, independently of the site or sampling time (Table 3). Soil dehydrogenase activity also showed significantly higher values under organic practices although, in this case, the differences depended on the site (only at PG and PS sites) and sampling time (Table 3). Finally, the potential ammonium oxidation rate varied significantly between sites and sampling times, but not with management practice (Fig. 1f; Table 3).

While marked time changes in the geometric mean of the assayed soil enzyme activities (GMea) were found in the soils under the organic practices at the PG and PS sites (Fig. 2a and c, respectively), no changes along time were found in the soil with the same management at the PT site (Fig. 2b). Seasonal changes found in the GMea in the conventionally managed soil showed a similar trend to that of the soil under organic practices at all the sites, although showing smaller amplitude.

Significant differences due to the individual factors "management practices", "site" and "sampling time" were found for the GMea values (Table 3). Furthermore, significant effects of the interactions "management practices × site" and "site × sampling time" were also found (Table 3). When pooling data from all the locations (Fig. 3; Table 3), significantly higher values were found in the GMea of the soils managed by the organic practices, no matter which sampling time was considered.

The variability explained by the type of agricultural practice was generally low and ranged between 2.0 and 26.3% for the

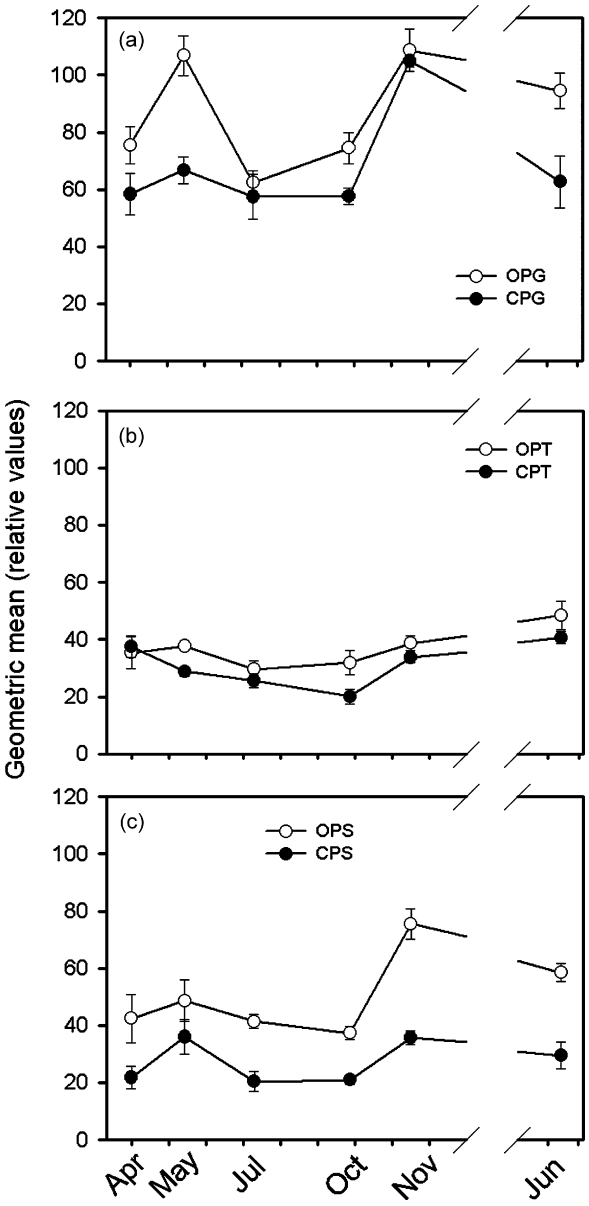

Fig. 2. Temporal patterns of the geometric mean of assayed enzymes determined in organic (open circles) and comparable conventional (full circles) farms at different sites; (a) PG, (b) PT and (c) PS. Data are the means of five replicates with bars representing the standard errors of the means.

potential soil ammonium oxidation rate and soil acid phosphatase (mean annual variability), respectively (Fig. 4). On the other hand, variability due to location was highest for the soil arylsulfatase activity (58.4% mean variability). Finally, for the GMea, the agricultural system and the location explained, on average, 16.3 and 55.6% of the variability, respectively.

3.3. The nematode community

Number of species for each trophic group, mean total richness, MI and H′ indexes were highly dependent on the sampling site (Table 4). Site and replicate within a site for the whole set of nematode indicators accounted for a maximum of 42 and 86.3% of the total variability, respectively (data shown only for the total number of nematode species, Fig. 4). At the PG site, the number of plant, hyphal, bacterial and unicellular eucaryote feeding nematodes, omnivorous nematodes and the richness and the H′ diversity indices were highest, whereas hyphal and bacterial feeders

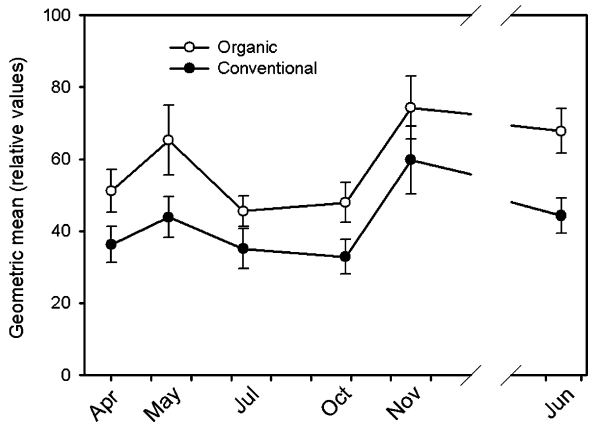

Fig. 3. Temporal pattern of the GMea determined in organic (open circles) and conventional (full circles) olive orchard farms. Data are the means of 15 soil samples (grouped data from five replicates per plot in three plots) and bars represent the standard errors of the means.

showed the lowest values at the PS site, with predators showing the lowest values at the PT site. Management practices only explained a maximum of 15% of the variability in nematode community parameters (Fig. 4). Differences due to the management for mean richness, plant and unicellular eucaryote feeding and omnivorous and H′ were only found at the PS site (Table 4). Mean MI values ranged from 2.7 to 3.5 in soils of the organic plots and from 2.5 to 3.3 in those conventionally managed, with no significant differences among management types and sites (Table 4).

3.4. Principal components analysis

Eigenvalues from the PCA analysis indicate that the first two principal components (PC) accounted for 65.5% of the variance of data (PC1: 36%, PC2: 29.5%). Soil cation exchange capacity, loss on ignition, total N, and alkaline and acid phosphatases, β-glucosidase and arylsulfatase activities were all significantly and positively

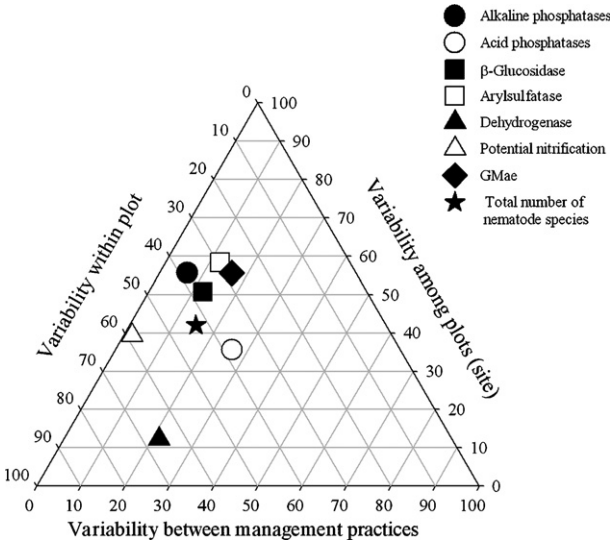

Fig. 4. Percentage variation in soil microbial enzyme activities, GMea and nematode numbers in olive oil farms with different management practices (organic versus conventional), sites with a specific management practice (PG, PT and PS), and different soil samples collected within the same plot at different sampling times. Symbols show the mean variability of the different sampling times for soil enzymes and GMea and the variability in one sampling for the total number of nematode species.

Table 4
Number of nematode species for each of the trophic groups (*sensu* Yeates et al., 1993), total number of nematode species, maturity and Shannon diversity indexes for organic and conventional olive farming at PT, PG and PS sites. Data are the mean (±S.D.) of three replicates. Probability values for the effects of site, management and their interaction on the number of nematode species at each of the trophic groups, nematode richness, MI (Maturity index) and H′ (Shannon–Weaver diversity index) are shown below. PS_O and PS_C stand for organic and conventional farming at PS site, respectively. PT, PG and PS, stands for Puente Tablas, Pegalajar and Puente Sierra sites.

	Plant feeding	Hyphal feeding	Bacterial feeding	Substrate ingestion	Animal predation	Unicellular eucaryote feeding	Omnivorous	Richness	Total[a] richness	MI	H′
PG											
Conventional	7.6 ± 1.2	2.3 ± 0.4	7.6 ± 2.4	1.0 ± 0.0	4.0 ± 0.8	0.6 ± 0.4	4.0 ± 1.6	27.3 ± 4.9	53	3.3 ± 0.06	0.77 ± 0.09
Organic	5.3 ± 0.4	2.6 ± 0.9	9.3 ± 1.0	0.6 ± 0.4	6.0 ± 3.2	0.3 ± 0.4	4.0 ± 0.8	28.3 ± 3.3	50	3.5 ± 0.2	0.77 ± 0.06
PT											
Conventional	4.6 ± 0.4	2.3 ± 1.2	8.3 ± 0.4	0.3 ± 0.4	1.3 ± 1.2	0.0 ± 0.0	1.3 ± 0.4	18.3 ± 1.7	31	2.5 ± 0.3	0.53 ± 0.04
Organic	4.6 ± 3.0	1.6 ± 0.9	6.0 ± 2.9	0.3 ± 0.4	2.3 ± 0.4	0.0 ± 0.0	1.3 ± 1.2	16.3 ± 6.9	32	2.7 ± 0.2	0.48 ± 0.16
PS											
Conventional	2.8 ± 1.3	0.2 ± 0.4	3.6 ± 1.9	0.2 ± 0.4	3.2 ± 1.1	0.0 ± 0.0	1.0 ± 0.6	11.0 ± 2.1	31	3.1 ± 0.3	0.36 ± 0.05
Organic	5.6 ± 0.9	1.6 ± 0.4	4.0 ± 2.1	0.6 ± 0.4	4.6 ± 1.7	1.3 ± 0.9	4.0 ± 0.0	22.0 ± 5.7	34	3.4 ± 0.3	0.67 ± 0.13
Site	0.09	0.02 PS < PT = PG	0.01 PS < PT = PG	0.21	0.04 PT < PS = PG	0.10	0.004 PG > PS = PT	0.03 PG > PS = PT	–	0.01 PG = PS > PT	0.001 PG > PS = PT
Management	0.83	0.38	0.86	0.84	0.12	0.19	0.06	0.19	–	0.6	0.10
Site × management	0.06 $PS_O > PS_C$	0.14	0.32	0.35	0.90	0.02 $PS_O > PS_C$	0.03 $PS_O > PS_C$	0.07 $PS_O > PS_C$	–	0.4	0.02 $PS_O > PS_C$

[a] Sum of the different species found in the three soil sample from each location.

correlated with PC1 scores (Table 5). Soil carbonate content, total C sand percentage labile P and exchangeable Mg were significantly correlated with PC2 scores (Table 5). Fig. 5a shows the position of the organic and conventional sites in the orthogonal space defined by the two first PCs, and Fig. 5b the scores of the sites at PC1. There were significant differences (two-way ANOVA; $P < 0.005$) due to site (PG > PT = PS) and management practices (O > C). Finally, the effect of management practices was independent of the site ($P = 0.78$ interaction management practices × site).

3.5. Correlation among soil physico-chemical and biological variables

A significant positive correlation was found between GMea, calculated from values of soil enzyme activities, and the scores of PC1, calculated from both physico-chemical and biological soil properties (Table 6). The numbers of predatory and omnivorous nematodes were highly correlated with the PC1 scores, GMea and organic matter (Table 6). Nematode community indices such as MI, richness and H′ were also correlated with GMea and the PC1 scores (Table 6).

Table 5
Pearson product-moment correlation coefficients between soil physico-chemical properties, enzyme activities and potential rate of soil ammonium oxidation and scores of the PC1 and PC2, which explained, respectively, the 36 and 29% of the variance in the PCA analysis.

	PC1	PC2
Clay	−0.02	0.63
Silt	0.27	0.60
Sand	−0.06	−0.73*
WHC	0.49	−0.54
Exchangeable Ca	0.26	0.61
Exchangeable Mg	0.26	0.73*
Exchangeable K	0.65	0.54
Exchangeable Na	−0.02	0.49
Labile P	0.38	0.75*
Carbonate	−0.14	−0.90**
CEC	0.84**	0.23
Organic matter	0.87**	0.16
Total C	0.18	−0.89**
Total N	0.97**	−0.04
Alkaline phosphatases	0.90**	−0.27
Acid phosphatases	0.88**	−0.26
β-Glucosidase	0.85**	−0.47
Arylsulfatase	0.88**	−0.37
Dehydrogenase	0.29	−0.18
Potential rate of soil ammonium oxidation	0.70*	0.12

* Significant correlation coefficients at a significant levels of 0.05.
** Significant correlation coefficients at a significant levels of 0.01.

4. Discussion

The implementation of environmental criteria through agrarian policies requires the selection of soil quality indicators to measure the success of specific agricultural practices. Suitable indicators should ideally be responsive to different management practices (Bandick and Dick, 1999), but at the same time, should show relatively low intra-annual variability, in order to minimize the need for repeated sampling to separate the effects of seasonality and management practices.

We assume that soil enzymes activities measured in our air-dried soil samples resemble those under field-moist condition. Bandick and Dick (1999) and Li and Sarah (2003) found that soil enzyme activities measured in air-dried samples were representative of those obtained under field-moist conditions, and concluded that soil enzyme measured in air-dried soils were sensitive enough to be used as part of a soil quality index.

In this study, soil enzyme activities varied significantly during the whole period, both in the organic and conventional farming. Average coefficients of variation were higher than 29% for the assayed soil enzymes and potential rate of soil ammonium oxidation. Bolton et al. (1985) showed intra-annual variability (three samplings within 1 year) as low as 2–7% for phosphatase and urease activities. However, our results agree well with those found by Aon and Colaneri (2001), who reported coefficients of variation typically around 50% for acid and alkaline phosphatases, dehydrogenase and β-glucosidase (three sampling times during the growing season of a soybean crop at Salado river basin), or with those reported by Debosz et al. (1999) who found more than 50% temporal variation in microbial C biomass and β-glucosidase, cellobiohydrolase and endocellulase activities based on nineteen samplings over 2 years in Denmark. Our results also agree with those of Sinsabaugh et al. (2003) who found temporal coefficients of variation higher than 45% for four out of ten soil enzymes in a sweetgum forest.

Relatively high intra-annual variability in soil activity, as reported in this work for soil enzymes, is to be expected under a

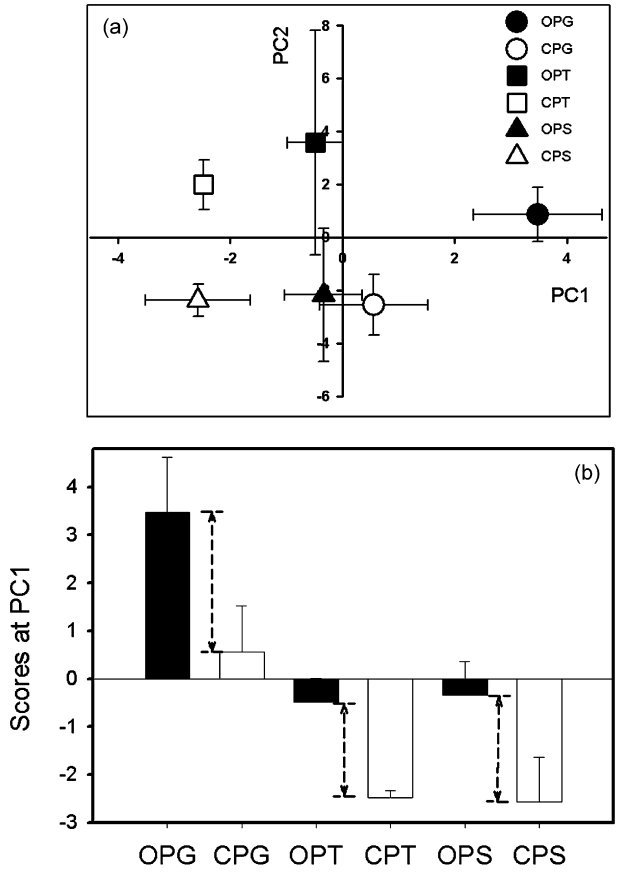

Fig. 5. Ordination of the different olive oil farms in the space defined by the PC1 and PC2 axis of the PCA analysis carried out with physico-chemical and biochemical soil properties (a). Coordinates are the mean of three replicates and bars represent the standard deviation of the mean. Full and open symbols indicate organic and comparable conventional olive orchard farms, respectively. Circle, square and triangle stand for the Pegalajar (PG), Puente Tablas (PT) and Puente Sierra (PS) sites, respectively. (b) Scores at the PC1 of the organic (O) and conventionally (C) managed farms at the PG, PT and PS sites. Values are the means of three replicates and bars represent the standard deviations of the means. Short dashed lines illustrate the differences in the values at the PC1 between each pair of farms at each site.

Mediterranean-type climate with high seasonal variability in, for instance, soil water content and temperature. However, despite the importance of analysing intra-annual variability into assess the potential use of soil enzymes as indicators of soil health/quality, the number of studies evaluating their temporal variability is still relatively low.

We found no consistent temporal trends among individual soil enzyme activities. However, when grouping data from the three organic and conventional olive orchard farming systems, values of soil enzyme activities were generally higher in spring and early autumn. This general trend has previously been described both in agro-ecosystems (Aon et al., 2001; Aon and Colaneri, 2001; Schloter et al., 2003) and forest ecosystems (Rastin et al., 1988), and is likely related to the influence of optimal environmental conditions for microbial growth and activity in these seasons. In general, the temporal patterns for individual enzyme activities were similar in comparable organic and conventionally managed farms, suggesting that timing of application of specific soil practices (irrigation, harvesting, tillage, organic or inorganic fertilisation) did not have a large influence on temporal variations in the assayed soil enzymes, at least within the time frame of this study.

Variability in soil enzyme activities was mainly due to sites and samplings within a site. Similar results have been found by van Diepeningen et al. (2006) and Girvan et al. (2003), who concluded that soil type was much more important in determining microbial structure and function than management type, and with those of Sinsabaugh et al. (2003) who recommended a high number of replicates to minimize the variability within an experimental field. As for soil enzyme activities, most of the differences in the distribution of nematode species in the various trophic groups, total nematode richness and the MI and H′ were due to sites rather than to soil management practices.

Soil physico-chemical properties, except for exchangeable Na, were different among organic farms. It has previously been reported that an inherent characteristic of most soil properties, including soil enzymes and nematode community, is that they vary as a function of soil type (Dick et al., 1996). This result suggests that when using soil enzyme activities and nematode community indices as assessment tools to account for favourable environmental effects of organic agricultural management practices, a high number of plots showing a wide range of soil properties should be investigated. For instance, in one of the organic farms, the PG site, we found the highest content of soil organic matter, soil enzyme activities, number of nematode species within each of the trophic groups, total nematode richness, MI and H′ values. However, at the organic PT site, despite higher exchangeable Ca, Mg, K and labile P, we found the lowest organic matter content, soil enzyme activities, nematode species richness and MI values. Since soil enzyme activities and the nematode community may be responsive to tillage treatments, the high intensity of tillage at this

Table 6
Correlation coefficients (Pearson product-moment) between number of nematode species within each trophic group, total nematode richness, MI and H′ indices and PC1 scores, soil organic matter content and the geometric mean of enzyme activities (GMea).

	Plant feeding	Hyphal feeding	Bacterial feeding	Substrate ingestion	Predatory	Unicellular eucaryote feeding	Omnivorous	Richness	H′	MI	Scores at PC1	GMea
Hyphal feeding	0.60**											
Bacterial feeding	–	–										
Substrate ingestion	–	–	–									
Predatory	–	–	–	–								
Unicellular eucaryote feeding	–	–	–	–	0.53*							
Omnivorous	0.55*			–	0.56*	–						
Richness	0.74**	0.63**	0.65**	–	0.59*	–	0.78**					
H′	0.77**	0.63**	0.55*	–	0.60**	0.52*	0.82**	0.99**				
MI	0.51*	–	–	–	0.76**	0.67**	0.76**	0.64**	0.72**			
Scores at PC1	–	0.47*	–	–	0.66**	–	0.71**	0.75**	0.76**	0.55*		
GMea	–	–	–	–	0.71**	–	0.75**	0.78**	0.79**	0.64**	0.94**	
Organic matter	–	0.56*	0.61**	–	0.54*	–	0.55*	0.75**	0.70**	–	0.82**	0.77**

* Significant correlation coefficients at a significant levels of 0.05.
** Significant correlation coefficients at a significant levels of 0.01.

site (up to five times per year) might explain these low values. In this sense, Gupta and Germida (1988) showed that tillage depressed phosphatase and arylsulfatase activities by 49 and 65%, respectively, after several decades, and Wander and Bollero (1999) showed an important decrease in soil quality under tillage. Tillage has also been reported as having a high impact on the soil nematode community. For instance, Freckman and Ettema (1993) found a high impact of tillage on omnivorous, bacterivorous and fungivorous nematode. Therefore, our results indicate that soil health/quality inferred from soil enzyme activities and the nematode community can be significantly lower in the "nominally" organic farms if subjected to intensive tillage.

A number of long-term studies have shown that soil enzyme activities have the ability to discriminate among soil management practices (see Dick, 1994). In our study, despite the relatively low proportion of the variability of the soil enzyme activities explained by management practices, differences between organic and conventional farms were significant. Soil acid and alkaline phosphatases, β-glucosidase, arylsulfatase and dehydrogenase activities were all higher under organic than conventional management, but this was not always true when comparing values for pairs of organic and conventional olive oil orchards at each of the three sites. For instance, at the PS site, the assayed soil enzymes were higher in the organic farm, but at the PG and PT sites, only 4 and 3 of the assayed soil enzyme were clearly higher, respectively. Overall, organic management did not significantly increase the various nematode community indicators, although at the PS site values were higher than in the conventional farm. The overall lack of significant differences in nematode indices between the organic and the conventionally managed plots in this study (except for the PS site) do not agree with previous findings that report a higher diversity of soil nematodes under organic management (Asteraki et al., 2004; Mäder et al., 2002). However, results similar to ours were found by van Diepeningen et al. (2006) when comparing 13 organic with 14 conventional farms, and by Yeates and Bongers (1999) for three organically managed and comparable conventional grassland soils and those found by Neher (1999) when comparing 5 organically with 5 comparable conventionally managed fields. In general, our results agree with Wardle et al. (1995), who found, in an extensive bibliographic review of 63 studies, that in 60% of the studies no differences were found for either nematode abundance or biomass between organic and conventional farms. In our case the lack of differences in the soil nematode community between the organic and the conventional olive orchards might be explained by: (i) tillage and fertiliser type applications affect some nematode genera in opposite ways (Fiscus and Neher, 2002) and, therefore, MI, H′ and the number of species per sample might not show significant differences, since changes in absolute abundance of a particular group can be masked by changes in abundance of others groups. (ii) The relatively slow colonization rates of nematodes (Prot and Netscher, 1978; Pinkerton et al., 1987 reported, respectively, migration rates of 4.5 and 8 cm d^{-1} for different nematode species of the genus Meloidogyne) compared with the relatively short time over which organic management practices have been applied in the organic farms, and the degree of isolation of organic farms (except at PG) from potential source of nematode species. However, the nematode community was only analysed in one sampling and, therefore, the lack of significant differences between soils managed organically and conventionally should be viewed with caution.

A large number of physical, chemical and biochemical properties that determine soil processes and their spatial and temporal variability, contribute to define soil quality *sensu* Doran and Parkin (1994). Consequently, individual soil properties may fail to give an appropriate estimation of soil quality. In this work the GMea values in the soils under organic practices were significantly higher than in the soils under conventional practices and this was not dependent on the sampling date. On average, GMea increased 18, 25 and 84%, in the soils under organic practices with respect to the conventionally managed ones, at the PT, PG and PS sites, respectively. Nevertheless, the magnitude of the differences depended on the site. At the PT sites, as discussed above, the high tillage intensity and frequency might explain the low differences in soil functional quality. The GMea values calculated from the assayed soil enzymes showed lower temporal variability (between 25 and 38%) than those for the individual enzyme activities and, therefore, a complex index such as GMea, would be more suitable for assessing soil quality (Puglisi et al., 2006).

In addition, GMea was highly correlated with some functional indicators of the nematode community. The abundance of predatory and omnivorous nematodes, general nematode richness and MI showed positive correlations with GMea, and correlations were mainly due to the location rather than to the type of managements. Higher values of those indicators were found at the sites with higher soil GMea and organic matter values while sites with lower values of soil enzyme activities also showed lower values of nematode indicators. This might be explained when considering that external organic matter inputs from organic fertilisers and cover crops increase energy availability for soil microbes, thereby enhancing microbial activity and biomass, and bacterial feeding, predatory and omnivorous nematodes which, in this work, contributed significantly to the mean and total nematode richness, MI and H′.

On the other hand, the calculation of GMea is based only on soil enzyme activities, and specific soil physical and chemical properties are not directly included. Therefore, we performed a PCA to reveal any possible relationship between the GMea and the results of the PCA which included diverse physico-chemical properties. The PCA in this work showed a clear discrimination of paired locations according to management practices along the first principal component (PC1), and a less clear ordination was found along PC2 by means of soil properties. The scores assigned to the locations at the PC1 was highly correlated with soil enzyme activities ($r > 0.85$) and organic matter ($r = 0.87$). In addition, this axis was significantly correlated with GMea values, predatory and omnivorous nematodes, and nematode richness and MI, which had not been included in the PCA analyses. Therefore, the use of an index based only on selected soil enzyme activities has proven to be integrative enough to avoid analysis of physical and chemical properties, which usually show changes in the long-term (Parr and Papendick, 1997).

Furthermore, the complete set of biochemical properties on which the GMea is based can be easily determined at one time in batch procedures, and is directly related to important aspects of soil functional quality (Bandick and Dick, 1999; Dick, 1997) including some nematode community indicators. The usefulness of GMea as a soil quality indicator has also been shown by Hinojosa et al. (2004) for assessing the impacts of pyrite sludge load enriched on heavy metals on soil, and more recently, Puglisi et al. (2006) have also pointed out the utility of GMea for evaluating soil quality.

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