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RESEARCH ARTICLE

Tillage and herbicide decrease soil biodiversity in olive orchards

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Abstract Olive growing is a centenarian activity in Andalusia, Southern Spain. Andalusia holds the largest olive tree growing area in the world. In spite of the relevance of olive growing for the Mediterranean economy, the influence of soil biodiversity on olive crops has been seldom studied. We hypothesized that soil diversity must be well preserved because the Andalusian olive groves are low-input and no-till systems. Soil depth should also help to structure soil diversity. We tested the effect of site features and soil management on nematode diversity, soil properties and soil food webs. Site features included use, position, orientation, radiation, slope, altitude and regional scale. Soil management included tillage and herbicide use. Results show that at the large scale, nematode abundance and soil food web structure were reduced under the tree canopy affected by herbicides by 57.9 and 14.2 %, respectively, in comparison to areas not treated with herbicides. Nematode abundance decreased by 47.7 %, taxa richness by 12.4 % and soil food web structure by 23.4 % in areas where herbicides are applied, in comparison to surrounding oak woodlands. The absence of vegetation in bare soils impacted the lower levels of the soil food web, depleting bacterial and fungal-mediated decomposition channels. This depletion spread up in turn to microbivore nematodes and nematodes in the upper level. We also found that nematode abundances decreased with soil depth on average by 73.2 % from the top soil layer (0–2 cm) to the deepest one (10–20 cm), irrespectively of the tillage practice.

Keywords Cover crops · Herbicide · Nematode · Soil food web · Soil-management system

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1 Introduction

Olive (*Olea europaea* L.) is probably one of the oldest Mediterranean crops, and it has been estimated that currently one third of European farmers are involved in olive growing (European Commission 2002). Spain holds the largest olive-growing area in the world, with 2.6 million ha (MAGRAMA 2012), most of them situated in Andalusia (Southern Spain). However, the Andalusian olive farming is considered eco-inefficient, owing to the soil erosion and lixiviation of agrochemicals caused by the soil-management system used that relies on heavy soil disturbance (Gómez-Limón et al. 2012).

Sustainable practices such as cover cropping interweaved within the rain-fed tree crops, and several no-tillage systems have been proposed to overcome these problems (Durán et al. 2008, 2009). The effects of these practices on physico-chemical soil properties is well documented (Nieto et al. 2012; Durán et al. 2013), but their effects on soil biodiversity are seldom studied.

Despite of the relevance of olive growing in European agriculture and the focus given to maintenance of soil health and soil biodiversity (e.g. European Commission 2010), little information is available on olive grove soil biodiversity. Only some studies on microbial activity (Moreno et al. 2009; Montes-Borrego et al. 2013) and soil arthropods (Castro et al. 1996) are accessible. Among soil nematodes, studies are partial and scattered; García-Ruiz et al. (2009) related the nematode community structure to the organic or conventional management in South-Spain olive groves and Castillo et al. (2010) summarized the composition of the plant-parasitic nematode community in Andalusian olive groves.

Nematode assemblages have been used as bioindicators of soil ecological conditions in numerous agricultural systems due to their ecological characteristics: ubiquity, abundance and taxonomical and functional diversities. Nematode diversity responds rapidly to agricultural management (Yeates and Bongers 1999) and serves as bioindicator of various soil functions due to the numerous roles that nematodes play within the soil food web (Ferris et al. 2001; Ferris 2010).

The objectives of this study were to (1) assess soil nematode diversity, abundances and vertical distribution in olive orchards under different soil managements (cover crop, herbicide use and tillage regimes) and (2) study the relationships between site features and soil properties, soil food web condition and ecological indices based on nematode assemblages. To fulfil such objectives, a double approach, including the assessment of the nematode community response to several factors both in experimental and field conditions, was undertaken. Under this double approach, the two main sources of environmental variability were taken into account; first, results from a landscape-scale approach in which the effects of natural environmental variability were studied is reported, followed by a local-scale experiment in which man-induced variability through different management practices was included.

2 Material and methods

2.1 Study areas and soil sampling

2.1.1 Study area 1

This area was selected to develop the soil biodiversity study and was located in the basin of the Víboras River, Valdepeñas de Jaén (Andalusia, Spain) (Table 1). Three mature (>50 years old) traditional rain-fed olive orchards of the cv. Picual in sloping lands with 70–100 trees per hectare and two dense shrubby oak (*Quercus ilex* L.) woodlands were selected to study the influence of the herbicide use and site features on soil nematode diversity and community structure. In olive groves, herbicides were applied on May under the canopy of the tree to control weeds (Fig. 1).

At each site, composite soil samples were taken from six randomly selected trees. In olive groves, three soil samples were taken in the area treated with herbicides (under the canopy) and three soil samples were taken in the non-treated area (outside the canopy), and samples from each area were composed into a single sample. In the oak woodlands, three soil cores were taken from each tree, under the canopy at 1.5 m from the tree trunk and composed into a single sample. Soil cores were taken with an auger soil sampler at 0–20-cm depth. Three samplings were performed: i) March 2010 (2 months before the application of herbicides), ii) July 2010 (2 months after the application of the herbicides) and iii) October 2010 (5 months after the application of the herbicides). Therefore, 144 soil samples were taken (three olive groves × six trees × two areas × three samplings) + (two oak woods × six trees × three samplings). At the time of the first soil sampling, a GPS was used to record the position (latitude and longitude) and altitude of each sampling site.

2.1.2 Study area 2

This area was used to assess the vertical nematode distribution under different management systems and consisted of an experimental olive grove located in Arquillos (Andalusia, Spain) (Table 1). The plantation consisted of rain-fed olive trees of the cv. Picual with two to three trunks, planted in 1976 at a density of 70–100 trees per hectares. The experimental design was a randomized complete block design of 35 elementary plots (five treatments per block × seven blocks); therefore, each treatment had seven repetitions. Each elementary plot was composed by 16 trees (four rows × four columns); the central section (four trees, in two rows × two columns) was sampled while the outer zones were considered buffer zones among treatments.

In February 2010, composite soil samples were gathered before tillage from two sampling points in the middle of each elementary plot. At each sampling point, 100 × 50 cm trial pits were opened and soil cores were taken at depths of 0–2, 2–5, 5–10 and 10–20 cm. A modified soil sample ring kit (Eijkelkamp 07.53.SE model E), which includes a set of cylinders of 2, 3, 5 and 15 cm specifically manufactured for this purpose, was used. Soil cores from the same depth and from the two sampling points at the same elementary plot were combined into one single soil sample. Therefore, 140 soil samples were taken (5 treatments × 7 blocks × 4 depths). Soil samples were divided into two subsamples for nematode and physico-chemical analyses.

2.2 Soil nematode analyses

Fresh soil subsamples for nematode extraction were stored at 6–10 °C for no more than 1 week and then processed. Soil nematodes were extracted by a modification of the Baermann funnel method (Barker 1985). Nematodes were extracted after soaking fresh soil overnight from 400 g of fresh soil in the samples collected in the study area 1 and for 40 g of fresh soil in the samples collected in the study area 2. In both cases, the total number of nematodes per sample was counted under the

Table 1 Soil-management systems, location and site features of the study areas (S Spain)

		Location	Surface (ha)	Altitude (m a.s.l.)	Weed management	Slope (%)	Orientation (degrees)	% North facing	Solar radiation (kWh m^{-2})
Study area 1	Grove 1	37° 36′ 29″ N 03° 49′ 17″ W	0.59	1045	Gly 36 %+Gly 36 %+MCPA 18 %	23.1	61.4	73.71	1447
	Grove 2	37° 35′ 60″ N 03° 49′ 45″ W	1.92	1131	Gly 36 %	34.6	205.7	6.7	1476
	Grove 3	37° 36′ 39″ N 03° 47′ 42″ W	5.9	992	Gly 36 %+mowing	16.3	272.9	89.7	1330
	Oak woodland 1	37° 36′ 04″ N 03° 49′ 46″ W	–	1168	–	49.4	213.5	8.3	1432
	Oak woodland 2	37° 37′ 51″ N 03° 49′ 21″ W	–	1205	–	33.4	231.8	19.3	1550
Study area 2	T	38° 12′ 36″ N 03° 23′ 01″ W	4.4	388	Cultivator or disk harrow	2.5	31	–	1807
	NTH	38° 12′ 36″ N 03° 23′ 01″ W	4.4	388	Sim 90 %, Diu 80 %+Gly 36 %	2.5	31	–	1807
	CH	38° 12′ 36″ N 03° 23′ 01″ W	4.4	388	Cover crop+Gly 36 %	2.5	31	–	1807
	CM	38° 12′ 36″ N 03° 23′ 01″ W	4.4	388	Cover crop+mowing	2.5	31	–	1807
	CMD	38° 12′ 36″ N 03° 23′ 01″ W	4.4	388	Cover crop+mowing+disk harrow	2.5	31	–	1807

T tillage, *NTH* no tillage+herbicide, *CH* cover crop+herbicide, *CM* cover crop+mowing, *CMD* cover crop+mowing+disk harrow, *Diu* diuron, *Gly* glyphosate, *MCPA* 2-methyl-4-chlorophenoxyacetic acid, *Sim* simazine

binocular microscope and at least 100 nematodes were identified to genus or family level under the compound microscope, but different methodologies used impeded direct comparisons of results. Nematode abundances were expressed as total number of individuals per 100 g of dry soil.

Abundance of nematodes, taxa richness, Shannon Diversity Index (Shannon 1948), Maturity Index (Bongers 1990) and soil food web indices (Ferris et al. 2001) were calculated for each sample. The Maturity Index (Bongers 1990) weights the abundance of different nematode taxa belonging to five colonizer-persistent (cp) groups, a scale that classifies nematode families into five groups from cp-1 (enrichment-opportunistic nematodes) and cp-2 (nematodes with short life cycles, most microbial and plant feeders, relatively resistant to environmental perturbations) to cp-5 (mostly predators and omnivores, with long life cycles and sensitive to environmental disturbance). Soil food web indices (Ferris et al. 2001) are based on nematode guilds (a combination of the cp groups and nematode trophic habits) and include the Basal Index, indicator of soil food web perturbed condition; the Enrichment Index, indicator of enrichment conditions dominated by fast-growing bacterivore organisms; the Channel Index, indicator of the predominance of fungal-mediated organic matter decomposition and the Structure Index, indicator of structured soil food webs and enhanced ecological functions developed by higher trophic links. The Enrichment Index and Structure Index allow the diagnosis of the soil food web condition (Ferris et al. 2001).

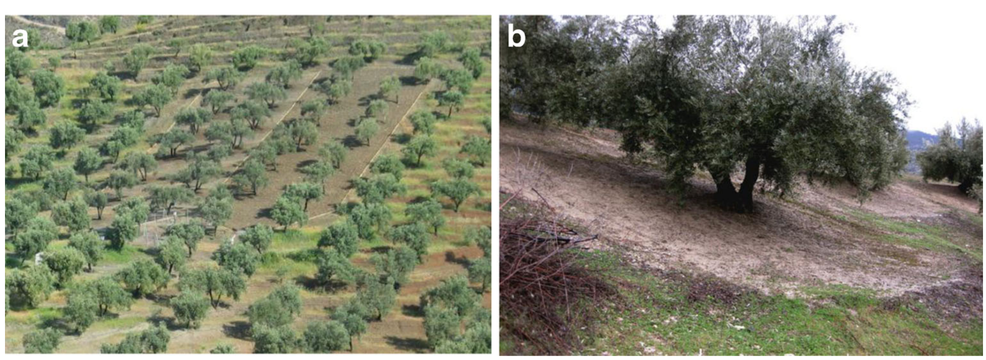

Fig. 1 Olive plantations in sloping lands (**a**) and olive trees under conventional management showing the area under the canopy, treated with herbicides, and the area outside the canopy, covered with weeds (**b**)

2.3 Soil physico-chemical analyses

Soil subsamples for physico-chemical analyses were air-dried, ground and sieved through a 2-mm sieve and used to analyze soil texture, pH, soil organic carbon (SOC), total nitrogen (N), available phosphorus (P), potassium (K), electrical conductivity, cation exchange capacity and soil moisture. All soil parameters were determined following the official analysis methods published by the Spanish Ministry of Agriculture (MAPA 1994).

2.4 Statistical analyses

ANOVAs were used to test the effect of sampling date on the nematode community within each habitat in the diversity study data set and to detect differences on the soil nematode community and soil properties in the study area 2 (data set 2). Factorial ANOVAs were used to assess the significance of the combined effect of tillage and weed control technique in data set 2. Tukey tests were used as post hocs.

Canonical analyses were used to infer associations between site features, soil properties and the nematode community and the soil food web condition. Two analyses were performed. The first one was performed on data set 1 and related ecological indices to site features (latitude, longitude, altitude, slope, direction, percentage of north facing and estimated solar radiation) and the presence of native undisturbed soils. The second one was performed on data set 2 and related ecological indices to soil properties (clay, sand, silt, pH, bulk density, SOC, total N, P, K, cation exchange capacity, available water capacity), soil depth and soil management systems. In the resulting ordinations, variables scoring further from the graph centre present higher factor scores in the definition of the roots. To facilitate interpretation, independent variables were marked with arrows. All data were log-transformed before analysis.

3 Results and discussion

3.1 Biotic parameters in relation to soil-management systems

Forty-eight and 33 nematode taxa were found in the study areas 1 and 2, respectively. In area 1, 17 taxa were bacterial feeders, five fungivores, 17 herbivores, two omnivores and seven predators, while in area 2, 13 taxa were bacterivores, five fungivores, 11 herbivores, three predators and one was an omnivore. Most nematodes associated to olive orchards in both areas were bacterivores, often the most diverse trophic group in agricultural fields (Ferris et al. 2004), and herbivores, frequently abundant in perennial agricultural systems (Neher and Campbell 1994). Although nematodes can be important pests of olive trees (Castillo et al. 2010), no relevant abundances of pest taxa were found in any area.

In the study area 1, nematode abundances were high in summer, although temporal dynamics were not strong and no significant differences on nematode abundances were found across sampling dates (Table 2). Nematode abundances often vary among seasons, since their metabolic activity and survival strategies largely depend on temperature and soil moisture. Such seasonal effects were almost absent within the olive orchards, probably because limited resources throughout the year do not allow populations' build up when environmental conditions are favourable.

Oak woodlands showed higher nematode richness, higher Maturity Index and lower Channel Index than the olive groves (Table 3), indicating more structured soil food webs than olive groves, and thus being a reservoir of soil diversity and presumably to their associated ecological services at the landscape level. Maintaining oak patches in olive-growing areas has been suggested as crucial for wildlife habitat restoration (Nekhay et al. 2009). In our study, we have shown that oak patches are also important for maintenance of soil biodiversity. The extremely high values of the Channel Index in olive groves are, however, not common in agricultural systems, indicating the prevalence of slow organic matter decomposition mediated by fungi, typical from mature forests.

Abundance of nematodes, taxa richness and the Structure Index were lowest within the area treated with herbicides, indicating a reduced soil food web complexity. Since herbicides are often harmless to soil fauna (Santos et al. 2011), effects of herbicide application on nematodes have to be mostly indirect, mediated by the effects on the diversity and the composition of the plant community (Parfitt et al. 2010). Soil food web condition in the study area 1, indicated by the values of the Enrichment and the Structure Indices, ranged from degraded in the soil under the influence of herbicides to a structured condition in the oak woodlands (after Ferris et al. 2001), with intermediate values in the olive grove soils non-treated with herbicides.

Soil treatments also affected the nematode community in the study area 2 (Table 3). In the no-tillage+herbicide or bare soil treatment, the nematode abundance, nematode richness, Shannon Diversity Index and Channel, Enrichment and Structure indices were significantly lower than under any other treatment. Structure and the Channel Indices showed intermediate values in the cover+herbicide treatment and in the tillage and cover+mowing treatments, respectively (Table 3). Tillage and herbicides affected less biological indices than the combination of both; Channel Index only responded to the combination of tillage×herbicide, while nematode abundance responded to herbicide application but not to tillage. Enrichment and Channel indices are indicators of the prevalence of organic matter decomposition mediated by bacteria and fungi, respectively. Consequently, their low values in the bare soil

Table 2 Values of nematode abundances, taxa richness, ecological indices, tardigrade abundance, and physico-chemical soil properties at different sampling dates and habitats in study area 1

	Sampling date			Habitat		
	March	July	Oct.	H	NH	Oak
Tot.	317.20	657.08	340.71	251.60a	596.86b	480.60b
	±29.11	±105.83	±32.64	±40.65	±78.32	±79.77
S	12.24ab	12.00a	13.18b	11.82a	12.42ab	13.50b
	±0.37	±0.37	±0.35	±0.34	±0.29	±0.49
H’	1.85	1.87	1.97	1.87	1.90	1.93
	±0.05	±0.04	±0.04	±0.05	±0.03	±0.05
MI	1.83a	1.80a	1.45b	1.68ab	1.60a	1.87b
	±0.06	±0.06	±0.07	±0.06	±0.07	±0.07
EI	24.40	26.01	30.65	26.45	27.88	26.40
	±1.80	±1.43	±2.24	±1.47	±1.78	±2.62
SI	38.65	45.56	51.97	39.82a	46.40b	51.98b
	±3.56	±3.57	±3.11	±3.54	±3.22	±3.43
CI	73.93	86.20	87.35	94.98a	85.40a	59.20b
	±4.88	±3.61	±3.75	±1.56	±3.45	±6.30
BI	48.94a	43.42ab	37.72b	47.03	42.46	39.43
	±2.47	±2.38	±2.08	±2.26	±2.30	±2.57
Tard.	6.06a	30.73ab	37.83b	4.11a	41.50b	29.87b
	±1.92	±18.24	±15.35	±1.33	±19.01	±12.70
pH	7.92	7.70	7.90	7.83	7.91	7.75
	±0.05	±0.05	±0.04	±0.04	±0.05	±0.04
SOC (%)	3.22	3.21	3.24	1.89a	1.72a	7.56b
	±0.49	±0.41	±0.42	±0.05	±0.16	±0.45
N (%)	0.25	0.27	0.25	0.15a	0.18a	0.54b
	±0.03	±0.03	±0.03	±0.01	±0.02	±0.03
EC (µS cm^{-1})	366.35a	256.34b	288.22ab	330.76ab	236.40a	366.82b
	±29.45	±31.60	±29.76	±34.45	±18.42	±38.13
SM (gH_2O per g dry soil)	0.14b	0.02a	0.15b	0.10ab	0.08a	0.14b
	±0.01	±0.00	±0.01	±0.01	±0.01	±0.01

Data are expressed as mean±standard error of the mean

Mean values for sampling dates or habitats in each row followed by the same letter do not differ significantly at $p<0.05$ according to Tukey tests

H grove soils with herbicides (under the canopy), *NH* non-treated grove soils (rows between tress), *Oak* oak woodlands, *Tot.* nematode abundance (number of nematodes/100 g of dry soil), *S* nematode taxa richness, *H’* Shannon Diversity Index, *MI* Maturity Index, *EI* Enrichment Index, *SI* Structure Index, *CI* Channel Index, *BI* Basal Index, *Tard.* tardigrade abundance (number of tardigrades/kg of dry soil), *SOC* soil organic carbon (%), *N* total nitrogen content, *EC* electrical conductivity and *SM* soil moisture

treatment may be due to depleted microbial functional diversity and reduced enzymatic activity, as previously reported by Moreno et al. (2009). These authors suggested that the presence of a vegetation cover was the main determinant of microbial performance and this effect may spread up to the next soil food web level, composed by enrichment-opportunistic bacterial-feeding and fungivore nematodes. Besides, the effects detected on soil food web complexity show that also nematodes at the upper levels of the soil food web, especially sensitive to environmental perturbation and chemical stress (Bongers and Bongers 1998), were affected by the bare soil treatment. Such changes on the soil biota may be due to the decrease on soil nutrients and increased soil compaction in bare soils (Moreno et al. 2009).

3.2 Soil depth vs. nematode trophic groups

Figure 2 shows that the abundances of nematode trophic groups were heavily affected by soil treatment and depth. Total nematode abundance and all trophic groups except predators were significantly affected by treatment and depth, while their interaction significantly affected total nematode abundance, bacterivores and herbivores ($p<0.05$, data not shown).

Table 3 Values of nematode abundances, taxa richness, ecological indices, tardigrade abundances and soil properties in different soil treatments in study area 2

	T	NTH	CH	CM	CMD	Till.	Herb.	Till.×Herb.
Tot.	213.70a	102.72b	497.50a	591.57a	242.77a		**	**
	±22.52	±36.17	±75.22	±122.50	±37.25			
S	8.19a	4.65b	7.43a	7.48a	6.82a	*	**	**
	±0.35	±0.60	±0.39	±0.35	±0.37			
H'	1.66a	1.11b	1.36a	1.52a	1.52a	**	**	**
	±0.04	±0.12	±0.06	±0.05	±0.06			
MI	2.19	2.25	2.19	2.24	2.22			
	±0.05	±0.19	±0.05	±0.07	±0.05			
EI	56.04a	37.27b	50.78a	51.38a	51.00a	*	**	**
	±2.12	±5.60	±2.50	±3.83	±2.14			
SI	34.96a	23.89b	24.36ab	29.13a	31.17a	**	**	**
	±3.45	±7.28	±3.68	±4.97	±4.46			
CI	52.53ab	47.63b	69.49a	53.99ab	69.60a			**
	±4.95	±8.40	±5.14	±6.23	±5.15			
BI	34.20	40.71	41.49	36.02	38.62			
	±1.66	±5.07	±2.31	±3.12	±2.43			
Clay (%)	23.23a	15.22b	14.73b	18.00b	15.05b	*	*	**
	±1.54	±1.97	±1.53	±2.19	±1.74			
Sand (%)	60.74a	70.08ab	69.83ab	65.14ab	70.58b			*
	±2.04	±2.69	±1.94	±2.60	±2.05			
Silt (%)	16.03	14.70	15.43	16.86	14.37			
	±1.25	±1.00	±0.71	±0.82	±0.45			
pH	7.03a	6.27b	6.20b	6.62ab	6.36b	**	**	**
	±0.11	±0.08	±0.13	±0.16	±0.12			
BD (g cm^{-3})	1.52ab	1.66a	1.64a	1.56ab	1.49b	**	**	**
	±0.03	±0.03	±0.04	±0.05	±0.02			
SOC (%)	1.01ab	0.63a	1.02ab	1.35b	1.29b	**	**	**
	±0.07	±0.08	±0.20	±0.29	±0.06			
N (%)	0.10ab	0.07a	0.09b	0.14b	0.16b	**	**	**
	±0.00	±0.00	±0.01	±0.03	±0.01			
P (mg kg^{-1})	4.34a	14.03b	11.28b	7.07a	5.04a	**	**	**
	±0.61	±2.35	±1.13	±0.91	±0.37			
K (mg kg^{-1})	79.24a	52.33a	110.21b	114.23b	86.24ab			**
	±6.11	±5.18	±7.44	±11.63	±5.88			
CEC (cmol kg^{-1})	6.93ab	5.03a	9.38b	10.69b	10.05b	*	*	**
	±0.46	±0.24	±0.73	±1.24	±0.74			

Data are expressed as mean±standard error of the mean. Mean values in each row followed by the same letter do not differ significantly at $p<0.05$ according to Tukey tests. The significance of the effects of tillage (Till), herbicide (Herb.) and their combination (Till.×Herb.) is indicated as $p<0.05$ (*) or $p<0.01$ (**)

T tillage, *NTH* no tillage+herbicide, *CH* cover crop+herbicide, *CM* cover crop+mowing, *CMD* cover crop+mowing+disk harrow, *Tot.* nematode abundance (number of nematodes/100 g of dry soil), *S* nematode taxa richness, *H'* Shannon Diversity Index, *MI* Maturity Index, *EI* Enrichment Index, *SI* Structure Index, *CI* Channel Index, *BI* Basal Index, *BD* bulk density, *SOC* soil organic carbon, *CEC* cation exchange capacity

In the tillage, cover+herbicide and cover+mowing treatments, total nematode abundance was significantly reduced at the deepest layer, due to the reduction in the abundance of bacterivores, fungivores and herbivores. In the no-tillage+herbicide and cover+mowing+disk treatments, the effect of depth was not significant (Fig. 2). Predatory nematode abundances were low and presented high deviations, so no effects of depth was found in any treatment for the predator trophic group. The soil food web in the study area 2 either was degraded (soils 2–20-cm depth) or disturbed (all treatments

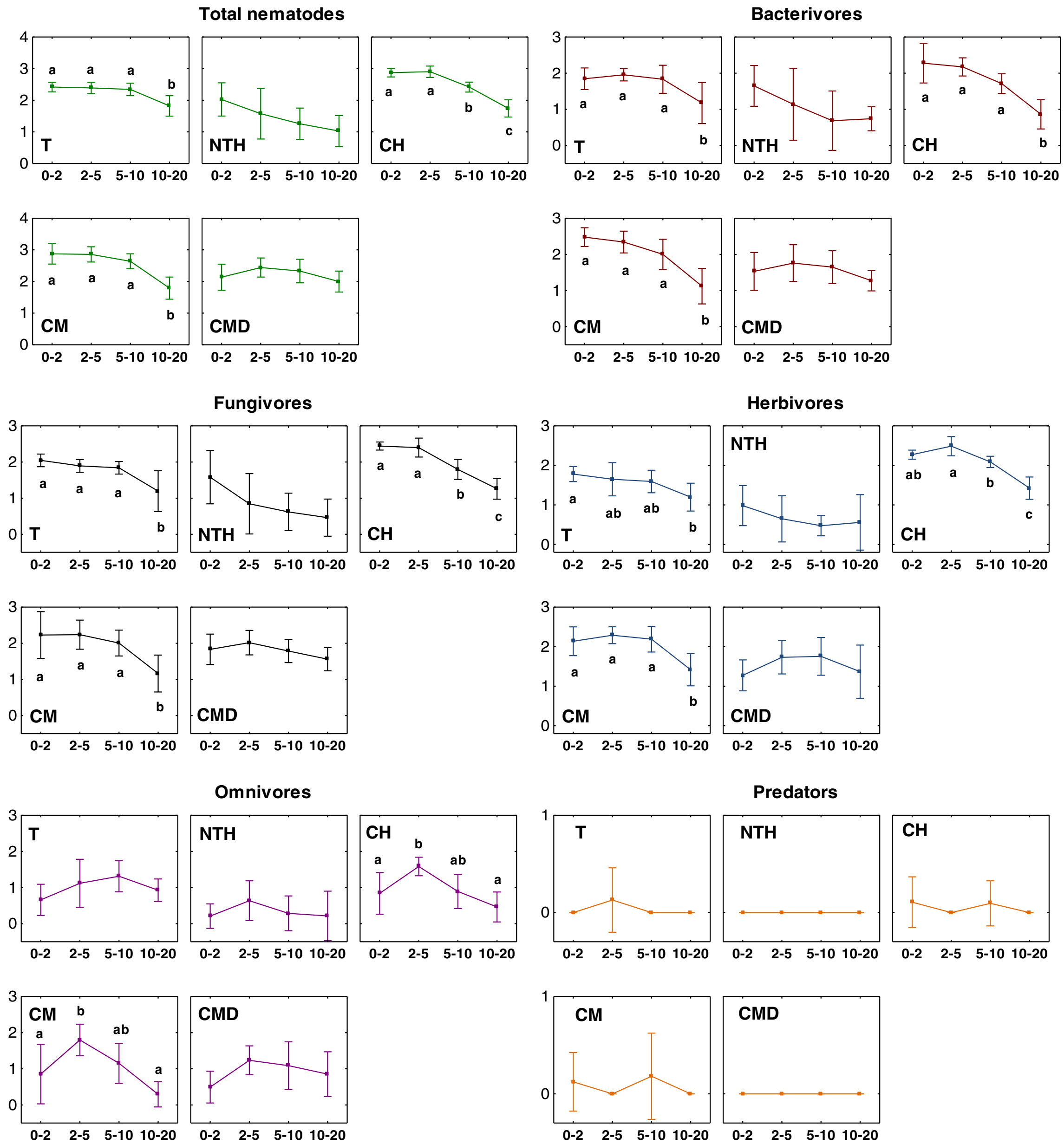

Fig. 2 Mean abundances of all nematodes, bacterivores, fungivores, herbivores, omnivores and predators (in 100 g of dry soil) across soil treatments at four soil depths (0–2 cm, 2–6 cm, 6–10 cm, 10–20 cm). *T* tillage, *NTH* no tillage+herbicide, *CH* cover crop+herbicide, *CM* cover crop+mowing, CMD cover crop+mowing+disk harrow. *Different letters* below/over SE bars indicate significant differences at $p<0.05$ according to the Tukey test. Nematode abundances were log-transformed before analyses

with covered crops and soils from the upper layer) (after Ferris et al. 2001).

While the soil food web was well structured in the oak woodlands, indicator of complex, mature and suppressive biota (Ferris et al. 2001), soil food web condition was degraded or disturbed in olive groves. Degraded food webs are characterized by low fertility and low suppressiveness, as main services provided by organisms in the lower and upper guilds of the food web, while disturbed webs present low fertility but higher complexity (Ferris et al. 2001). The worst soil food web condition was found in the bare soil experimental treatment, confirming the strong effects of the absence of vegetation on the olive grove's soil food web. As could be expected, the soil food web was enriched in the upper soil layers, while no differences were found among the other soil depths.

As it has been previously reported by Minoshima et al. (2007), our results show a general decrease of nematode abundance with increased soil depth. Effects of tillage on nematodes are sometimes idiosyncratic, e.g. stimulating bacterial-feeding nematodes (Sánchez-Moreno et al. 2006) and affecting differently at varying soil depths (Van Capelle et al. 2012). In this sense, soil tillage may present unclear effects on nematodes; in previous studies, less than 5 % of the variability of nematode taxa abundances was explained by the tillage practice in experimental conditions (Zhang et al. 2012), while up to 84 % of arthropod abundance and diversity may be explained by tillage (Greenwood et al. 2011). In some cases, the intensity of tillage operation has been directly correlated to the abundances of plant-parasitic nematodes as those belonging to the genus *Heterodera* (Westphal et al. 2009). Our results from study area 2 show that total nematode abundance, abundance of bacterivores, fungivores and herbivores, was significantly reduced at 10–20-cm depth in three of the treatments, irrespectively of the tillage regime.

3.3 Relationships between site features, soil properties and ecological indices

The canonical analysis relating ecological indices and site features extracted 20 and 18 % of the variability of these site feature variables (roots 1 and 2, respectively) and 18 and 16 % of the variability of the indices (roots 1 and 2, respectively). Ordination of variables along root 1 shows a positive association between higher latitudes and soils undisturbed by agricultural practices with nematode abundance and Structure Index values (Fig. 3). Longitude, altitude and solar radiation were the main determinants of root 2, which scored oppositely to the Basal Index, the Channel Index, and the Enrichment Index.

The second canonical analysis related ecological indices and soil properties and management and extracted 19 and 12 % of the variability of ecological indices (roots 1 and 2, respectively) and 16 and 10 % of the variability of soil properties and management variables (roots 1 and 2 respectively). Root 1 was mainly defined by soil organic C, low soil depth and low bulk density, followed by high cation exchange capacity and N (Fig. 3), and was associated to nematode abundance and the Enrichment Index. The second root was mainly defined by the absence of herbicides, and high P, and was associated with the Maturity Index, the Structure Index and the Channel Index.

In previous studies, we found that site feature properties were associated to the nematode community as closely as soil properties, and elevation and slope displayed a good indicator value of soil disturbance mediated by human intervention (Sánchez-Moreno et al. 2011). The presence of such geographical patterns has recently been found for nematodes at larger spatial scales (Porazinska et al. 2012). In this study, the associations inferred by canonical analysis showed that native soils, present at higher latitudes, were associated to nematode abundance, nematode richness and food web complexity. Nematode diversity, higher in the natural areas, was associated to northern orientations, irrespectively of soil use. Opposite in previous reports (Sánchez-Moreno et al. 2011), slope was not a main determinant of nematode diversity. Our results indicate, in general, that the influence detected by site feature variables on ecological descriptors was mainly mediated by soil use. Commonly, nematodes are more abundant in upper soil layers (Scharroba et al. 2012), and, together with soil depth, the use of herbicides negatively affected nematode diversity and abundance, which were associated to higher soil N and C, strongly depleted in vegetation-free experimental treatments.

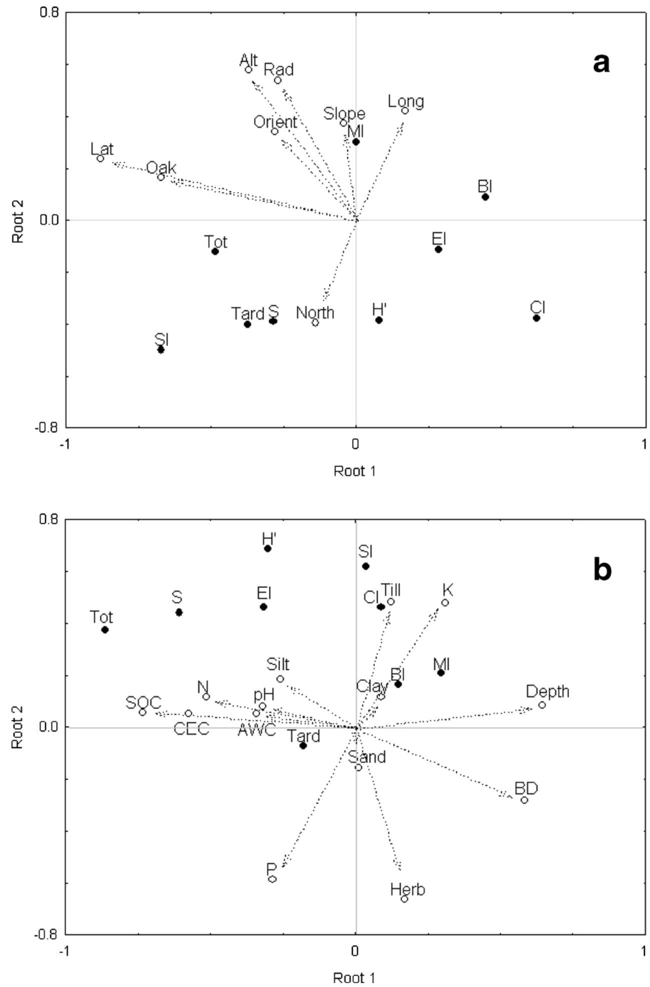

Fig. 3 Ordination resulting from two canonical analyses relating ecological indices to site feature variables (**a**) and soil properties (**b**). *Tot.* nematode abundance (number of nematodes/100 g of dry soil), *S* nematode taxa richness, *H'* Shannon Diversity Index, *MI* Maturity Index, *EI* Enrichment Index, *SI* Structure Index, *CI* Channel Index, *BI* Basal Index, *Tard.* tardigrade abundance (number of tardigrades/kg of dry soil), *SOC* soil organic carbon (%), *N* total nitrogen content, *EC* electrical conductivity, *SM* soil moisture, *Lat* latitude, *Long* longitude, *Alt* altitude, *Rad* annual solar radiation, *Slope* slope, *Orient* orientation, *Oak* woodland soils, *North* % north facing, *BD* bulk density, *Herb* herbicide use, *Till* tillage, *CEC* cation exchange capacity, *AWC* available water capacity

4 Conclusion

Our results confirm the large effect of vegetation on soil biota. Nematode abundances and diversity presented lower values in bare soils treated with herbicides, intermediate values in non-herbicide areas and higher values at oak woodlands. Besides, treatments combining tillage and herbicide use, which maintained the soil free of vegetation, were the most disturbing in terms of the ecological descriptors of the nematode community and the soil food web. These effects primarily affected the lower levels of the soil food web and depleted both bacterial- and fungal-mediated decomposition channels and then spread up through the soil food web reaching both microbivore guild and predator and omnivore guilds in the upper levels of the soil food web. The effects of site feature variables on the soil biota were mediated by soil use and management. In addition, although the effects of soil depth on soil organisms are often mediated by the effects of tillage, our results showed that nematode abundance decreased with soil depth, irrespectively of the tillage regime. Thus, based on our findings, we conclude that the traditional soil-management systems affected negatively on soil biodiversity as well as on soil properties. A minimum soil disturbance combined with plant covers could be at least partially offset this impact by providing a viable option to conserve soil quality and biodiversity with opportunities to increase overall land productivity as well as sustainable agro-environmental measures.

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