

# Temporal effects dominate land use as factors affecting soil nematode communities in Mediterranean oak woodlands

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**Abstract** We analysed the soil nematode community within three different montados (agricultural, pastoral and forestry uses), focusing on temporal variation. Nematodes were classified into trophic groups (bacterivores, fungivores, omnivores, predators and plant-parasitic nematodes (PPN)) and we calculated the maturity index for free-living taxa (MI), maturity index for plant-parasitic taxa (PPI) and the nematode channel ratio (NCR). Temporal variations were most evident during winter when there was a rise in the abundance of the five functional groups. Concordantly, there was a simultaneous increase of soil moisture and organic matter, due to litter decomposition. Fungivore abundance was highest in the forest and the temporal occurrence and abundance of many PPN genera was largely determined by land use. Land management was responsible for differences in plant community structure and composition, thus plant diversity increased from the agricultural to the forestry use. Because the montado shows great temporal variability in vegetation structure our results of MI, PPI and PPI/MI ratio explain the significant changes in the nutritional status over time, with the highest values recorded in the spring. NCR is a good indicator of energy efficiency in the soil decomposition process. Its lowest values were attained in the

forest, where the slow-growing plant species favoured a lower activity of the bacterial energy channel and a prevalence of the fungal-based decomposition energy channel. Nematode composition reflected plant succession, changes in decomposition in the soil food web and temporal variations in the structure of soil.

**Keywords** Cork oak · Montado · Soil nematodes · Environmental indicators

## Introduction

In the southern Iberian Peninsula, Mediterranean forests were dominated by cork oak (*Quercus suber*) and holm oak (*Q. ilex*) trees. However, since the late Middle Ages and especially between 1850 and 1950, many of these forests have been lost by human activities such as shrub clearing, ploughing, tree thinning and conversion to agricultural land (Díaz et al. 1997). Presently, these ecosystems, known as *montados* in Portugal, consist of an open tree layer with a shrub or annual herbaceous understory and are characterized by a systematic combination of agricultural, pastoral, and forestry uses (Pinto-Correia 1993; Joffre et al. 1999; Costa et al. 2009). Cork oaks trees are of major economical importance in *montados* as their bark (i.e., cork) can be harvested every 9 years, a process that does not harm the trees because the cork layer regenerates after extraction (Pausas et al. 2009).

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Moreover, *montados* are protected habitats within the EU Habitats Directive (92/43/EEC) and are considered high nature value farming systems, according to the classification proposed by the European Environmental Agency (Paracchini et al. 2008). This ecosystem has evolved over centuries as a consequence of management activities but, at present, *montados* are undergoing an unprecedented rate of change. The intensification of grazing and ploughing activities in the undercover hampers tree regeneration and at the same time it disrupts the physical properties of soils, putting at risk the maintenance of this ecosystem in the long-term (Plieninger and Wilbrand 2001; Plieninger 2007; Bugalho et al. 2011a, b). Therefore, the preservation of the *montado* depends on the consistent application of a management strategy that promotes tree regeneration and soil protection (Pulido and Díaz 2005; Bugalho et al. 2011a; Arosa et al. 2014 submitted).

Nematodes constitute the most abundant group within the soil mesofauna, with free-living nematodes playing an indispensable role in decomposing processes occurring in soil ecosystems (Yeates 1979). The cycling of nutrients above ground is controlled by herbivores, including nematodes, mainly by stimulating soil microbial activity, nutrient recycling and increased nutrient acquisition by plants from soil (McNaughton et al. 1997; Bardgett et al. 1998). Trophic interactions can be assigned to nematodes in the majority of soils (e.g., Moorhead et al. 2002) and these can be classified into five functional groups: bacterivores, fungivores, plant-feeders, predators and omnivores. Bacterivores and fungivores are indirectly involved in decomposition and nitrogen and carbon mineralization by feeding on bacteria and fungi, including mycorrhiza (Ferris et al. 2004; Yeates and Coleman 1982). Plant-parasitic nematodes (hereafter referred to using the abbreviation PPN) interact with flora by feeding on plant roots or shoots (Ingham et al. 1985). Predator nematodes feed upon soil invertebrates including other nematodes, enchytraeids, tardigrades and protozoa (Moore and de Ruiter 1991). Omnivores do not hold a separate position in the food chain but may add connectedness to the food web (Coleman et al. 1993) by feeding on more than one food source, including bacteria, fungi, algae, protozoa and rotifers. Nematode analysis is currently gaining interest in ecological studies in order to assess the functioning of soils (Wilson and Kakouli-Duarte

2009). Nematode community indices are a useful tool for monitoring environmental conditions and soil ecosystem function (Bongers and Ferris 1999; Ferris et al. 2001; Neher 2001; Berkelmans et al. 2003). The nematode community can be characterized by the maturity index for free-living taxa (MI) and the maturity index for plant-parasitic taxa (PPI) (Bongers 1990; Bongers and Ferris 1999). For the calculation of MI, soil nematodes are categorized into a 1–5 coloniser (r-strategists) and persister (k-strategists) ranking, termed as cp value, following the assumption of a shift toward opportunistic species under stress conditions (Odum 1985; Bongers 1990). The MI is the weighted mean cp value of the number of individuals in a representative soil sample; index values increase for low levels of disturbance. The index values vary according to the levels of disturbance, from less than 2.0 in nutrient-enriched disturbed systems to  $\pm 4.0$  in pristine environments. Nematodes that feed on higher plants are omitted from the calculation of the MI because their occurrence and abundance is largely determined by the community structure, host status and vigour of plants growing in the soil. Consequently, the equivalent of the MI for plant-feeding nematodes (the plant-parasite index, PPI) is calculated separately (Bongers 1990, Bongers and Ferris 1999). The ratio PPI/MI (Bongers et al. 1997) is a sensitive indicator of enrichment. Moreover, the nematode channel ratio (NCR) expresses the energy efficiency in soil decomposition processes, providing information on the relative dominance of the bacterial or fungal energy channel based on the abundance of bacterial and fungal feeding nematodes in the soil ecosystem (Yeates 2003). These energy channels represent two lines of dead biomass consumption: the fungal energy channels predominate when organic material is of high C/N ratio, when organic material is of low C/N ratio bacterial decomposition channels predominate (Moore and Hunt 1988; Ferris and Bongers 2006; Costa et al. 2011).

The main objective of this study is to provide a functional interpretation of the soil nematode community structure in *montados* in three sites due to land management practices and through time. We assess if differences in the nematode communities reflect differences in management of *montados* regarding agricultural, pastoral and forestry uses. Sites with high levels of grazing and soil ploughing for agriculture, are believed to be occupied by organisms that are less

able to colonize and become established presenting lower values of maturity indices. On the other hand, we consider the effect of time which, by modifying soil physical–chemical properties, can also affect nematode densities (Yeates et al. 1997; Bardgett and Cook 1998; Costa et al. 2011). Additionally, we will evaluate the potential burden of PPN in the different types of *montado* to estimate whether these can affect cork oak seedlings, therefore representing a limitation to regeneration.

## Methods

### Experimental sites

Our study area was set in Montemor-o-Novo, Portugal, most specifically in the Herdade do Freixo do Meio (38°42'N, 8°19'W), an organic farm that manages 1,140 ha of cork-holm oak *montado*. Within the study area, we selected three sites of approximately 30 ha each representing different management precedents concerning agricultural, pastoral and forestry uses. Cork harvesting and tree pruning were the main forestry activities undertaken in the three sites, despite the fact that they did not take place every year.

Site A was a grassland with scattered cork and holm oaks (25 trees/ha). This site was mechanically discoloured in autumn for the sowing of clover (*Trifolium* spp.) and intensive sheep grazing occurred during part of the year (winter, spring and autumn).

Site B was a dense cork-holm oak *montado* (40 trees/ha) with shrub undergrowth of *Cistus* spp. and *Asparagus acutifolius*. There were no agricultural uses in this site, although it was moderately used by livestock (pigs in winter and sheep in autumn).

Site C was a dense cork oak *montado* (45 trees/ha), including a minor number of holm oaks and stone pines (*Pinus pinea*), and a high density heterogeneous understory composed by shrub formations of *Cistus* spp., *A. acutifolius*, *Ulex australis* subsp. *welwitschianus*, *Pistacia lentiscus*, *Arbutus unedo*, *Phillyrea angustifolia*, *Crataegus monogyna*, *Quercus coccifera* and *Lavandula pedunculata*. This site management comprised only forestry uses.

To account for climatic differences among sites, temperature and relative humidity measures were obtained with a thermohygrometer (HOBO Pro v2 logger, Onset Computer Corporation, USA) at each

site. These thermohygrometers were placed 1.5 m above the ground under tree canopy and data were hourly recorded for 1 year starting in January 2012. Soil chemical properties and moisture content were also examined in the three sites twice, coinciding with spring and autumn.

### Nematode sampling, extraction and identification

Sampling was carried out in the three study sites four times during 2012. As each sampling coincided with a season, to simplify the text sampled time will be named as winter, autumn, spring and summer. Samples of rhizosphere soil (10 samples/site/season) were collected at a depth of 10–20 cm, placed in individual plastic bags (1,000 cm<sup>3</sup>) and kept in cold storage at 4 °C until processed. Soil samples were collected within sites at random but the presence of a cork oak seedling was mandatory in order to use its root for the extraction of endoparasitic nematodes. We used an adaptation of the generalist Tray Method for nematode extraction: living mobile nematodes were extracted from 100 cm<sup>3</sup> sieved soil samples for 72 h and endoparasitic nematodes were extracted from sieved root samples for 240 h (Whitehead and Hemming 1965). Preparations were observed under an inverted microscope at 100–400× magnification. We counted all extracted nematodes in the sample. For the determination of the feeding group we counted a subsample of 100 nematodes that were classified into functional groups (bacterivores, fungivores, PPN, predators and omnivores) according to their digestive system structure (Tarjan and Hopper 1974; Bongers and Bongers 1998). With these results both maturity indices (MI and PPI) and NCR were calculated. In order to describe PPN community and to determine if these should have an effect on cork oaks, we further identified PPN to genus level (Mai and Mullin 1996).

### Statistical analysis

Analyses of variance (ANOVA), followed by post hoc Tukey tests ( $\alpha = 0.05$ ), were used to test for temporal differences among sites in the number of individuals of each functional group per preparation. Data were log-transformed for normality and homogeneity of variances. To test for differences in the number of bacterivores, PPN and total number of nematodes per preparation we used Kruskal–Wallis tests, followed by

multiple comparisons of mean ranks for all groups, because variance was not homogeneous, even after data transformation. ANOVAs were also performed to test for differences among sites in mean daily temperature and relative humidity. All analyses were conducted using Statistica version 8.0 (StatSoft Inc. 2007).

Principal components analyses (PCA) were performed using CANOCO for Windows version 4.5 (ter Braak and Smilauer 2002) to describe temporal patterns in PPN genera composition among sites. This analysis reduces the dimensionality of the PPN genera while retaining most of the variation in the data set. Samples can be plotted allowing us to visually assess similarities and differences between PPN genera. The number of individuals of each PPN genera per preparation (log-transformed) were used as dependent variables through time.

Generalized Linear Models (GLM) were used to test the temporal and site effects for the indices of ecosystem function (MI, PPI, PPI/MI and NCR). Significant differences among groups were further elucidated through the post hoc Tukey test at the 0.05 level. GLM were performed with Statistica version 8.0 (StatSoft Inc. 2007).

## Results

### Soil nematodes

A mean of  $751 \pm 660$  nematodes were counted per preparation extracted from the soil ( $100 \text{ cm}^3$ ). The most abundant nematodes were bacterivores followed by fungivores, especially in site A and B while in site C the abundance of fungivore was higher than the other functional groups (bacterivores, PPN, predators and omnivores). Predators and omnivores were considerably less abundant in all sites (Fig. 1). There were no significant differences in the total number of nematodes among sites ( $H_{2,117} = 0.84$ ,  $p = 0.656$ ), however, it was significantly highest in winter ( $H_{3,116} = 68.35$ ,  $p < 0.001$ ). Significant differences in nematodes abundance among sites were only found in fungivores ( $F_{2,117} = 4.15$ ,  $p = 0.018$ ), although these did not occur through time. Nevertheless, an increasing gradient of fungivore abundance was found every sampled time from site A to site C. All functional groups showed significant or nearly significant differences over time

(bacterivores:  $H_{3,116} = 59.37$ ,  $p < 0.001$ ; fungivores:  $F_{3,116} = 32.17$ ,  $p < 0.001$ ; PPN:  $H_{3,116} = 40.82$ ,  $p < 0.001$ ; predators:  $F_{3,116} = 43.92$ ,  $p < 0.001$ ; omnivores:  $F_{3,116} = 2.65$ ,  $p = 0.053$ ) and higher abundances were found in winter. Fungivore abundance was significantly lower in summer (sites A and C) and autumn (sites A, B and C), as was the abundance of predators in the three sites.

The GLM applied to the indices of ecosystem function showed the highest values of MI ( $F_{12,278.1} = 5.88$ ,  $p < 0.05$ ), PPI ( $F_{12,278.1} = 7.79$ ,  $p < 0.01$ ) and PPI/MI ( $F_{12,278.1} = 6.06$ ,  $p < 0.01$ ) in spring. Within sites, only the NCR was significantly lower in site C ( $F_{8,210} = 4.32$ ,  $p < 0.05$ ) (Table 1).

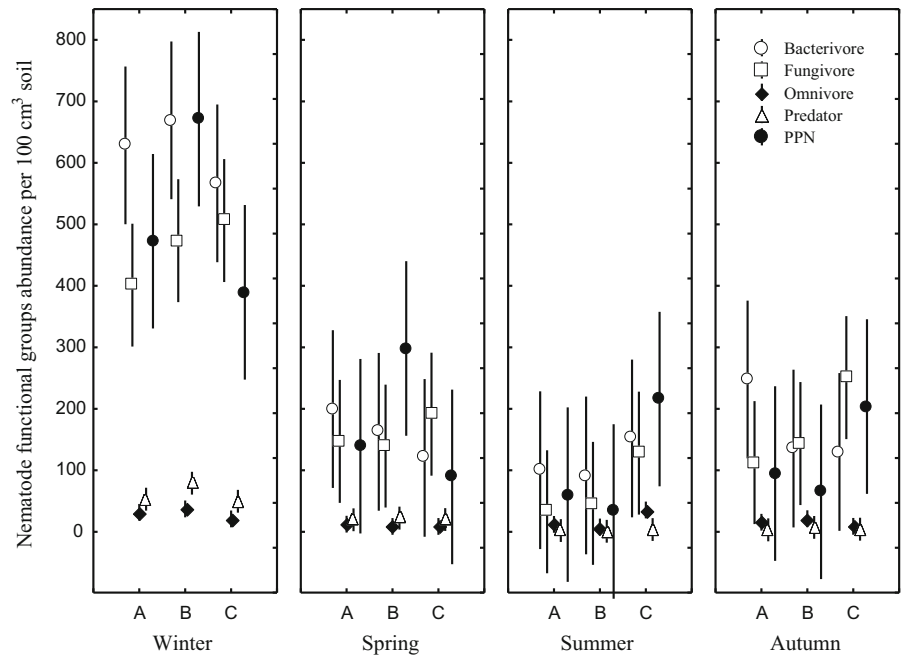
### Endoparasitic and living mobile PPN

Endoparasitic nematodes were not found in any of the preparations extracted from sieved roots of cork oak seedlings ( $n = 120$ ). On the contrary, PPN extracted from soil samples consisted of 11 genera (number of samples where present): *Criconemella* (31), *Gracilacus* (60), *Helicotylenchus* (98), *Hemicycliophora* (2), *Heterodera* (16), *Longidorus* (42), *Pratylenchus* (92), *Rotylenchus* (74), *Trichodorus* (5), *Tylenchorhynchus* (70) and *Xiphinema* (41).

The PCA (Fig. 2) reduced the original  $30 \times 11$  matrices, one for each sampling, to two independent principal components that explained 24.2 % and 21.8 % (winter), 30.2 % and 18.7 % (spring), 24.5 % and 18.1 % (summer), and 24.8 % and 18.3 % (autumn) of the variation, respectively.

Every sampling showed a regular discrimination between site A and samples from the two other sites, according to PPN variability. The genera that most contributed for this segregation were *Gracilacus*, *Helicotylenchus*, and to a lesser extent *Criconemella* and *Rotylenchus*. In spring, the variability of the genus *Rotylenchus* did not stand for that distinction, supporting instead the separation of samples from site B. Samples from site B were generally discriminated by the variability of the genus *Xiphinema*, although most noticeably in spring. Moreover, in summer, samples from site B showed an inverse relationship with the variability of *Longidorus* and *Tylenchorhynchus*. Overall, no particular PPN genus fomented the segregation of samples from site C, except in autumn when it was determined by the variability of *Criconemella*.

**Fig. 1** Temporal abundance of soil nematodes, classified into functional groups, detected in the three sites (see “Methods” for information on experimental sites). Data correspond to the mean  $\pm$  SD of ten replicates per site



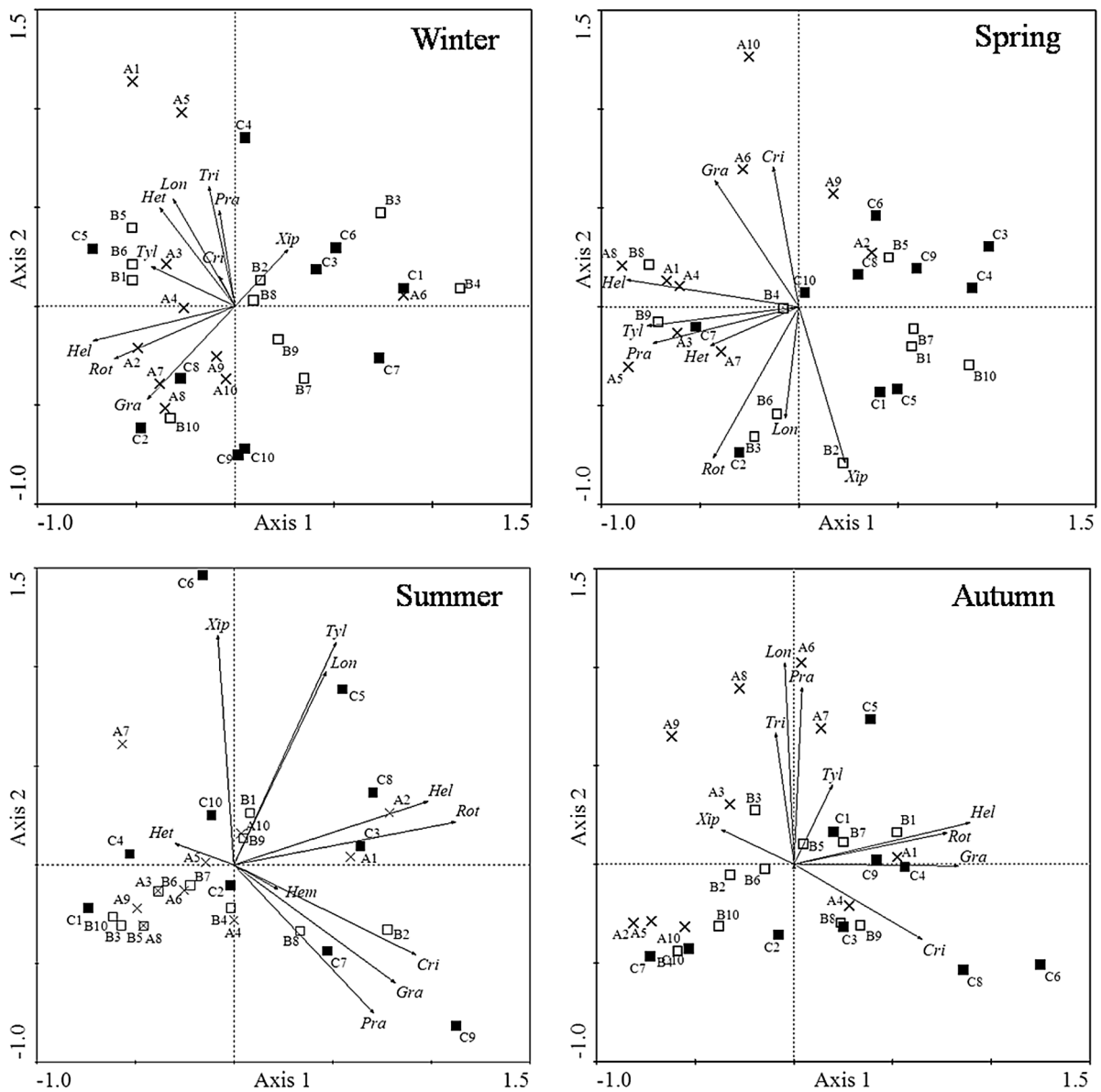
**Table 1** Indices of ecosystem function (*MI* maturity index for free-living nematodes, *PPI* maturity index for plant-parasitic nematodes, *NCR* nematode channel ratio) calculated per season in the three sites (see “Methods” for information on experimental sites)

|               | MI              | PPI             | PPI/MI          | NCR             |
|---------------|-----------------|-----------------|-----------------|-----------------|
| <b>Winter</b> |                 |                 |                 |                 |
| A             | 1.68 $\pm$ 0.25 | 2.99 $\pm$ 0.22 | 1.83 $\pm$ 0.34 | 0.58 $\pm$ 0.19 |
| B             | 1.60 $\pm$ 0.14 | 3.13 $\pm$ 0.31 | 1.96 $\pm$ 0.21 | 0.60 $\pm$ 0.14 |
| C             | 1.61 $\pm$ 0.17 | 3.00 $\pm$ 0.34 | 1.87 $\pm$ 0.18 | 0.53 $\pm$ 0.16 |
| <b>Spring</b> |                 |                 |                 |                 |
| A             | 1.71 $\pm$ 0.29 | 3.37 $\pm$ 0.21 | 2.03 $\pm$ 0.43 | 0.53 $\pm$ 0.17 |
| B             | 1.74 $\pm$ 0.26 | 3.54 $\pm$ 0.25 | 2.07 $\pm$ 0.28 | 0.52 $\pm$ 0.17 |
| C             | 1.83 $\pm$ 0.26 | 3.63 $\pm$ 0.36 | 2.00 $\pm$ 0.21 | 0.39 $\pm$ 0.17 |
| <b>Summer</b> |                 |                 |                 |                 |
| A             | 1.52 $\pm$ 0.15 | 3.01 $\pm$ 0.29 | 2.00 $\pm$ 0.25 | 0.66 $\pm$ 0.14 |
| B             | 1.57 $\pm$ 0.18 | 2.92 $\pm$ 0.09 | 1.87 $\pm$ 0.18 | 0.73 $\pm$ 0.11 |
| C             | 1.80 $\pm$ 0.26 | 3.07 $\pm$ 0.51 | 1.71 $\pm$ 0.21 | 0.55 $\pm$ 0.19 |
| <b>Autumn</b> |                 |                 |                 |                 |
| A             | 1.79 $\pm$ 0.26 | 3.10 $\pm$ 0.49 | 1.78 $\pm$ 0.46 | 0.51 $\pm$ 0.13 |
| B             | 1.44 $\pm$ 0.25 | 3.11 $\pm$ 0.40 | 2.23 $\pm$ 0.58 | 0.68 $\pm$ 0.19 |
| C             | 1.85 $\pm$ 0.26 | 2.79 $\pm$ 1.04 | 1.55 $\pm$ 0.61 | 0.32 $\pm$ 0.20 |

Data correspond to the mean  $\pm$  SD of ten replicates

Soil and climatic data

Temperature and relative humidity data recorded during this study agree with the Mediterranean climate of long, hot, dry summer and a mild, humid winter (mean annual temperature = 15.5 °C, mean annual relative humidity = 73.6 %). Among sites mean daily temperature was lowest in B and relative humidity was highest in B (temperature:  $F_{2,1,095} = 2.96$ ,  $p > 0.05$ ; relative humidity:  $F_{2,1,095} = 5.06$ ,  $p < 0.01$ ). Concerning the two samplings, the three sites showed no differences with respect to soil chemical properties and soil moisture content (Table 2). All tested soils were dry and acidic, which compromises the uptake of macronutrients, and had low levels of stable organic matter. These types of soils are prone to erosion and plant cover and soil biota may play crucial roles in the maintenance of a functioning soil layer. The organic matter content increased in the autumn, possibly due to the accumulation of dead plant material in the previous summer, without a correspondent decrease in soil pH. The increased levels of stable organic matter in the autumn may improve soil structure and provide nutrients, after the water and temperature stresses of the previous summer.



**Fig. 2** Biplots with PPN genera temporal variability and sample scores, according to site (A X-mark, B empty square, C filled square). Codes for genera are Cri Cricionemella, Gra Gracilacus, Hel Helicotylenchus, Hem Hemicylophora, Het

*Heterodera*, Lon Longidorus, Pra Pratylenchus, Rot Rotylenchus, Tri Trichodorus, Tyl Tylenchorhynchus, and Xip Xiphinema

## Discussion

Temporal variations in nematode communities were most evident during winter when there was a rise in the abundance of the f4 functional groups in every experimental site. The high availability of food sources, e.g., fungi, plant roots or shoots and microbial

communities, during that period may explain these differences (Ferris et al. 2004). Concordantly, there is a simultaneous increase of soil moisture and organic matter, due to litter decomposition, in winter (Sohlenius et al. 2011). Although temperatures are more favourable to decomposition processes in the spring for *montado* ecosystems, microbial activity peaks in

**Table 2** Soil chemical properties and soil moisture content in the three study sites in spring and autumn

|        | pH<br>(H <sub>2</sub> O) | Organic<br>matter (%) | C<br>(%) | N<br>(%) | C/N   | Soil moisture<br>(%) <sup>a</sup> |
|--------|--------------------------|-----------------------|----------|----------|-------|-----------------------------------|
| Spring |                          |                       |          |          |       |                                   |
| A      | 4.6                      | 1.32                  | 0.77     | 0.08     | 9.57  | 28.91 ± 5.86                      |
| B      | 4.7                      | 1.27                  | 0.74     | 0.07     | 10.52 | 22.94 ± 5.78                      |
| C      | 5.1                      | 1.88                  | 1.09     | 0.09     | 12.12 | 26.70 ± 4.35                      |
| Autumn |                          |                       |          |          |       |                                   |
| A      | 5.2                      | 2.33                  | 1.35     | 0.11     | 12.29 | 22.39 ± 3.06                      |
| B      | 5.6                      | 2.43                  | 1.41     | 0.12     | 11.75 | 17.30 ± 2.79                      |
| C      | 5.0                      | 1.52                  | 0.88     | 0.08     | 11.02 | 18.30 ± 2.79                      |

C carbon, N nitrogen

<sup>a</sup> Data are mean ± SD of ten replicates, measured in situ

the rainy season (autumn and winter), after the litter inputs resulting from the summer drought (Costa et al. 2013). The increased organic matter content of soil between the spring and the autumn may result from litter inputs from the understory shrub vegetation (Correia et al. 2014) however in site C, which had the most dense and diverse shrub understory, an increase in organic matter and macronutrients was not observed. In fact, the herbaceous vegetation of site A may have a larger contribution to the soil organic matter pool after the summer senescence than the dense shrub understory of site C (Otieno et al. 2011). Litter origin (shrub vs. herbaceous) and hence litter quality is also known to affect decomposition rates, with the former being decomposed at slower rates than the latter (Castro et al. 2010). The soil C/N ratio and soil pH increased with site disturbance (from A to C) in the spring, which agrees with results obtained elsewhere for areas under increasing cattle grazing regimes (Bardgett et al. 2001), but not in the autumn. None of the measured soil parameters could be related to populations of any of the nematode trophic groups or genera. Although it is widely assumed that soil properties influence nematode distribution and damage to crops in agricultural systems, a direct relationship cannot often be found between presence and/or abundance of given genera and soil physico-chemical parameters (Chen et al. 2012). However, the cycling of nutrients in the soil is controlled mainly by nematodes, by stimulating microbial growth when feeding on them, based on the comparative estimates of other studies (e.g., Ingham et al. 1985; Hunt et al. 1987;

Ferris et al. 1998). Bacterivorous and fungivorous nematodes appear to be the major contributors to N mineralisation. Increases in mineral N may follow the irrigation of dry soil (Sparling and Ross 1988; Lundquist et al. 1999) and consequently N mineralisation is probably offset by the considerable leaching of N that occurs during winter months (Poudel et al. 2001). However, grazing by aboveground herbivores can interfere over rates of vegetation succession, altering the quantity and quality of litter inputs to soil, which in turn affects soil biota and rates of soil nutrient cycling (Bardgett and Wardle 2003; Wardle et al. 2004).

Contrary to our expectations, distinct land uses had a small effect on nematode communities in *montados* while concerning functional assemblages. Nevertheless, there were noteworthy differences concerning fungivore abundance. This result is in agreement with the prominent role of fungi in ecosystem processes under low grazing, or completely unmanaged systems (Bardgett et al. 2001) and could be related to the input of lignin-rich, fungal-decomposition based litter originating from the understory shrubs (Castro et al. 2010). The temporal occurrence and abundance of many PPN genera was largely determined by land use. The genera *Gracilacus* and *Helicotylenchus* were associated to agriculture and livestock grazing (site A). Management was responsible for differences in plant community structure and composition among land uses, thus plant diversity increased from A to C. Given that PPN genera may have different levels of specificity to their hosts, it would be predictable that C site was the one with higher PPN diversity (Wardle et al. 2003; De Deyn et al. 2004; Vikeftoft et al. 2005). In our study, the number of PPN genera was identical in the three land uses; nonetheless, their variability was more homogeneous in site C.

Despite PPN being one of the most representative groups in the soil, endoparasitic nematodes were not found in roots of cork oak seedlings. As a significant number of root samples were sampled in the three land uses, we assume that PPN are not likely a limitation to cork oak regeneration since they did not affect seedling establishment.

Whilst data of nematode abundance was useful, the indices of maturity and trophic diversity are less variable than populations of individual trophic groups and thus may be more useful to detect trends in the ecological condition of soils (Neher and Darby 2009),

providing a more structured ordination. Our results of MI, PPI and PPI/MI ratio explained the significant changes in the nutritional status over time following modifications in the dynamics of vegetation (Bongers et al. 1997), because the *montado* shows great variability in vegetation structure through time. The highest values of MI, PPI and PPI/MI appeared in spring. According to Bongers (1999), the MI varies from 1 under enriched conditions to 3 or 4 under undisturbed conditions. An increasing population of bacterial feeders, with high reproduction rates, results in a decreasing MI. The persister nematodes (with higher cp values) are more resistant to disturbances and with higher values MI represents a less disturbed site. The PPI is comparable to MI but based on plant parasitic nematodes and may increase with increasing soil fertility, whereas the MI decreases (Bongers 1990; Bongers and Ferris 1999). This inverse relationship is not shown by our data, which supports the data suggesting organic farming systems are more sustainable through the lack of synthetic pesticide and fertilizer use, and better closed nutrient cycles (Mulder et al. 2005). As the MI and the PPI may show diverging trends, a merging of the information provided by the life strategy (MI) and the functional information given by the PPI is the and PPI/MI ratio (Bongers et al. 1997). We used this ratio as a useful additional parameter to detect differences through time. This ratio significantly varied with the highest values reached in spring. These ratios were higher than 1.6 in several of our observations, indicating that resource utilization by the plants may be far from optimal, according to Bongers et al. (1997) that suggests such high ratios are indicative of severely nutrient enriched habitats. In relation to site only the NCR showed significant differences. NCR is a good indicator to express the energy efficiency in soil decomposition process (Moore et al. 1988; Yeates 2003), providing information on the dominant way in which the breakdown of organic matter proceeds with the participation of bacteria or fungi. Values for this ratio are constrained between 0 (totally fungi mediated) and 1 (totally bacteria mediated). The highest values were attained in A and B, suggesting a higher activity of the bacterial energy channel in these sites with fast-growing plant species, which dominate in early succession and produce high quality litter (i.e., N-rich) promoting bacterial-dominated food webs. NCR values were lowest in autumn and in C ( $0.32 \pm 0.20$ ) evidencing a prevalence of the fungal-based

decomposition energy channel, where slow-growing plants dominate in late succession and produce low-quality, phenolic-rich litter that favours fungal dominated food webs (Wardle 2002; Bardgett 2005). In addition, sites A and B are areas where the soil is regularly mechanized and grazed, and the detected differences among sites could indicate that soil conditions in C are more stable (Viketoft et al. 2011).

This work is the first to use the soil nematodes as indicators of disturbance in the *montado* ecosystem. Nematode composition provided information on plant succession and changes in decomposition pathways in the soil food web. Our results stressed temporal variations in the structure of soil nematodes communities despite the effect of disturbance caused by land use. Future studies should be carried out in other areas of *montado* in order to confirm these patterns on a broad scale. Also, routine analysis of nematode fauna in different agroforestry systems affords rapid assessment of response to soil management practices and levels of stressors, so that sustainable optimal yields can be attained.

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