

Short- and long-term responses of nematode communities to predicted rainfall reduction in Mediterranean forests

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ABSTRACT

Climate change is a fundamental process affecting terrestrial ecosystems. However, there is relatively little knowledge about its impacts on soil communities, with a large degree of uncertainty regarding their resistance to predicted alterations in temperature and, particularly, precipitation. Moreover, most studies exploring the response of soil biota to predicted rainfall reduction have focused on mesic environments and soil microbes, which limit our ability to find general patterns across ecosystems and soil organisms. In this study, we analysed the impact of predicted climate change scenarios of rainfall reduction on soil food webs of Mediterranean water-limited forests using nematodes as bioindicators. We took advantage of replicated rainfall exclusion infrastructures (30% exclusion) established in *Quercus* forests of southern Spain in 2016 (2-year exclusion) and of southern France in 2003 (15-year exclusion) to explore the sensitivity of the soil food web to predicted reductions at short- and long-term scales. Rainfall reduction had large negative short-term effects on nematode abundance, particularly of lower trophic groups (bacterivores and fungivores). Rainfall reduction had also consistent short- and long-term impacts on community composition (decrease of fungivores, marginal increase of omnivores) and nematode-based indicators of soil food web structure (higher maturity and structure index, lower prey:predator ratio). These results can be considered indicative of a low resistance of the soil food web to rainfall reductions predicted by climate change. Overall, our findings demonstrate the sensitivity of water-limited forests to further reductions in soil water availability, which might substantially alter their soil communities and likely affect the many ecosystem processes that they control.

1. Introduction

In the last two decades, multiple studies have shown the large capacity of recent and future changes in temperature and precipitation to modify terrestrial ecosystems (IPCC, 2014). As a result, we have today ample knowledge regarding the consequences of climate change for the performance of plant species, as well as the implications of such changes for the organization of plant communities and ecosystem functioning (Bjorkman et al., 2018; Peñuelas et al., 2018; Harrison et al., 2020). On the contrary, there is relatively little knowledge about the impacts of climate change on soil communities, with a large degree of uncertainty

regarding their resistance (i.e. ability to remain unchanged when subjected to a disturbance) to predicted alterations in temperature and precipitation (Blankinship et al., 2011, 2011de Vries and Griffiths, 2018). Studies on the effects of altered precipitation are particularly scarce since they have traditionally received lower attention than the effects of warming (García-Palacios et al., 2015; Nielsen and Ball, 2015, 2015de Vries and Griffiths, 2018). However, the proliferation in recent years of rainfall exclusion infrastructures in the field has strongly contributed to improve this knowledge, providing empirical support for the large capacity of climate change-related drought to alter the abundance and composition of soil communities, but also evidencing the

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strong variability of these impacts through spatio-temporal gradients and among taxonomic groups (Blankinship et al., 2011; Ren et al., 2018).

Climate change impacts on soil organisms can vary strongly in space and time. Across spatial climatic gradients, the response of soil communities to increasing aridity is likely non-linear (Xiong et al., 2020). In fact, available evidence suggests that a given percentage of rainfall reduction can cause larger impacts on soil communities in mesic systems than in arid systems (Liu et al., 2016; Ren et al., 2018; Franco et al., 2019). A mechanistic explanation for the high inherent resistance of communities in arid systems is that they are dominated by specialized species selected over time for their high drought tolerance (Vandeghehuchte et al., 2015; Curiel-Yuste et al., 2014). However, the number of studies that have explored the response of soil biota to rainfall reduction in water-limited ecosystems is still relatively low compared to mesic systems, limiting our ability to understand their sensitivity to a drier climate (Nielsen and Ball, 2015; Ren et al., 2018). Across time, it has been suggested that short-term impacts of climate change on soil organisms might be of larger magnitude than long-term impacts (Holmstrup et al., 2017; Ren et al., 2018), due to acclimation and evolutionary adaptation of soil populations to the new environmental conditions (Manzoni et al., 2012; Romero-Olivares et al., 2017; Yin et al., 2019). However, we lack comparative short-versus long-term studies investigating the response over time of soil biota to climate change. A better understanding of the variability in soil biota responses to climate change would strongly benefit from an increase in the number of standardized studies with similar methodology that allows direct comparison of results across space and time (Halbritter et al., 2020).

Current knowledge of climate change impacts on soil organisms, and of rainfall reduction effects in particular, is very much based on the study of the two main kingdoms of soil microorganisms (fungi and bacteria), whereas soil animals have been comparatively unexplored (García-Palacios et al., 2015; Ren et al., 2018; Nielsen, 2019). Within soil fauna, nematodes represent the most abundant group (Wilson and Kakouli-Duarte, 2009) and can serve as a unique model taxon for the study of rainfall reduction effects on the soil compartment. They are aquatic organisms that inhabit the water films on the surface of soil particles, and therefore their movement and activity might be particularly vulnerable to the direct effects of drought (Freckman et al., 1987; Coleman et al., 2004). Moreover, they cover a variety of life histories (ranging from colonizer to persister strategies) and feeding preferences, including bacterivores, fungivores, herbivores, omnivores and predators (Yeates et al., 1993). This variety of life histories and trophic habits have allowed the development of nematode-based indices that reflect the overall state of the soil food web, such as its maturity and complexity (maturity and structure index), the availability of resources (enrichment index) or the predominant decomposition pathway (fungivores:bacterivores ratio; Bongers and Ferris, 1999; Ferris et al., 2001; Neher, 2001; Du Preez et al., 2022). Despite their potential interest, experimental studies of the response of soil nematodes to changes in precipitation are relatively scarce, and largely restricted to temperate grasslands (Eisenhauer et al., 2012; Sylvain et al., 2014; Cesarz et al., 2015; Ankrom et al., 2020). These studies have revealed a variety of complex responses of the nematode community to rainfall reduction, largely dependent on the nematode trophic habit, but mostly indicative of negative drought impacts on the structure and complexity of soil food webs (Franco et al., 2019; Siebert et al., 2020). However, because the magnitude of the effect of altered precipitation on soil biota is highly dependent on ecosystem type (Blankinship et al., 2011), it is uncertain how these results might be extrapolated to systems dominated by woody species such as forests.

In this study, we analysed the impact of predicted climate change scenarios of rainfall reduction on soil food webs of Mediterranean forests using nematodes as bioindicators. We took advantage of replicated rainfall exclusion infrastructures established in *Quercus* forests of southern Spain in 2016 (2-year exclusion, short-term site hereafter) and of southern France in 2003 (15-year exclusion, long-term site hereafter)

to explore the sensitivity of the soil food web to predicted reductions in precipitation (30%) at both short- and long-term scales.

We aimed to answer four main questions: i) How does experimental rainfall reduction affect the abundance, diversity and composition of nematode communities in water-limited Mediterranean forests? Based on the ecology of nematodes, we hypothesized that rainfall reduction would reduce overall abundance of nematode species and their diversity. Moreover, we expected this negative impact to be particularly large at higher trophic levels (omnivores and predators), since their life-history traits (i.e. long generation time, large body size, low fecundity) make them more vulnerable to disturbances than nematodes at lower trophic levels; ii) What do changes in nematode indices tell us about the impact of rainfall reduction on soil food webs of Mediterranean forests? Based on previous knowledge, we expected rainfall reduction to translate into a simplified and less structured soil food web (i.e. lower values of the maturity and structure index), a change towards the slow fungal decomposition channel (i.e. higher fungivores:bacterivores ratio), and a lower top-down pressure on the soil food web (i.e. higher prey:predator ratio); iii) Are impacts of rainfall exclusion on soil food webs detectable at both short- and long-term temporal scales? We expected impacts to be restricted mainly to the short-term due to the potential for the evolutionary adaptation of soil nematodes over time to water stress. Finally, we conducted a detailed mechanistic study at the short-term site that allowed us to answer a fourth question: iv) Which abiotic and biotic factors are driving rainfall exclusion effects on nematode communities? We used structural equation models (SEMs) to explore the relative importance of changes in soil abiotic properties (soil moisture, soil organic matter), biotic microbial (soil microbial biomass C and N) and plant (litter biomass, root chemistry) properties, and trophic relationships as drivers of the impact of rainfall reduction on the abundance of main nematode trophic groups (Fig. S1, Table S1). By answering these questions, we aim to contribute to advance current knowledge about the short- and long-term implications of increasing drought on soil food webs of forest ecosystems.

2. Methods

2.1. Study sites

The study was conducted at two different locations in southern Europe: Los Alcornocales Natural Park in Southern Spain and the Puéchabon National Forest in Southern France (Fig. S2). Both areas are occupied by Mediterranean forests dominated by evergreen *Quercus* species (*Q. suber* in Spain, *Q. ilex* in Puéchabon). These naturally established forests have a long history of human use. In Los Alcornocales Natural Park, cork harvesting and manufacture has been a main economic activity since the end of the 19th century, whereas the Puéchabon National Forest was managed as a coppice for centuries until 1942, when the last clearcut was performed. In Los Alcornocales, *Q. suber* coexists with the deciduous *Q. canariensis* and a sparse understory dominated by *Erica arborea*, whereas in Puéchabon *Q. ilex* is accompanied by a more diverse and abundant (c. 25% cover) understory dominated by *Phyllirea latifolia*, *Buxus sempervirens*, *Pistacia terebinthus* and *Juniperus oxycedrus*. The climate at both sites is typical Mediterranean, with mild winters and long, dry summers. Mean annual precipitation is 890 mm at Los Alcornocales and 910 mm at Puéchabon (mean for the series 1984–2017), although most precipitation concentrates between October and April. Soils in Los Alcornocales derive from a bedrock dominated by Oligo-Miocene sandstones and have a sandy loam texture, whereas in Puéchabon soils derive from a bedrock of Jurassic limestone and have a silty clay loam texture (Table S2).

2.2. Rainfall exclusion general design

We took advantage of rainfall exclusion infrastructures installed at each of the two study sites in different years (2016 in Spain and 2003 in

France) but following a similar design. Six plots (20×15 m in Los Alcornocales, and 14×10 m in Puéchabon) were established at each site distributed in three pairs (hereafter, blocks), with a mean distance of 30 m among blocks. From each pair, one plot was assigned to a rainfall exclusion treatment, whereas the other plot served as control. The rainfall exclusion treatment was designed to reduce ca. 30% the amount of rainfall, reproducing future climate change scenarios for the Mediterranean region (model CMIP5 for the extreme scenario RCP 8.5 for the period 2081–2100; Stocker, 2013). Rainfall was excluded using PVC gutters that covered 30% of each plot area in order to exclude a similar amount of natural precipitation. Gutters were placed at least 1 m height over the forest floor supported by a metal structure. In control plots, the same gutters were placed upside down to simulate potential secondary effects of the infrastructure on the forest microenvironment while maintaining the natural precipitation regime (Fig. S3). Total basal area and tree density were similar for the two treatments at the two study sites prior to the construction of the infrastructure (Limousin et al., 2009; Homet et al., 2021, Table S2). A buffer area of 1 m was considered within each plot in order to avoid any possible edge effects. The effect of the rainfall exclusion treatment on soil moisture was continuously quantified at both sites: in Spain soil moisture was measured at four depths (10, 20, 30 and 40 cm) using a FDR sensor (PR2 Profile Probe, Delta-T Devices, Cambridge, UK), whereas in France it was measured at 0–15 and 0–30 cm depth using water content reflectometers (CS615, Campbell Scientific Ltd., Loughborough, UK). Rainfall reduction translated into significant reductions of soil moisture at both experimental sites (Limousin et al., 2008) (Tables S3 and S4; Figs. S4 and S5).

2.3. Nematode sampling, identification, and indices

In spring 2018, soil samples were collected at both study sites within 15 days of difference. Therefore, at the time of the sampling, rainfall exclusion had been applied for 2 years in Spain and for 15 years in France. Soil samples were collected under the canopy of 6 trees per plot with a hand auger (three replicates per tree, \varnothing 3 cm \times 20 cm depth, previous removal of the litter layer) and stored in plastic bags at 4 °C until processing. The three replicates per tree were combined and mixed by hand ($n = 72$ samples in total). Nematodes were extracted following well-established protocols from 100 ml per sample by the sugar centrifugation method (Jenkins, 1964). After we had heat killed and fixed the nematodes in 4% formalin, we counted all nematodes in the samples at $100 \times$ magnification. In addition, 100 individuals randomly chosen per sample were identified in temporary slides to the family and genus level (Andrássy, 2005, 2007, 2009; Bongers, 1994) at $400 \times$ and $1000 \times$ magnification. This taxonomic classification allowed calculating by extrapolation the abundance of each family/genus per sample. Estimated nematode abundance was expressed as number of individuals per g of soil dry weight (ind/g). Richness and the Shannon index (H' , Shannon and Weaver, 1949) of genera were calculated as measures of nematode diversity. The same sampling protocol was used at the Spanish site in spring 2016 to control for pre-treatment differences in the nematode community. Pre-treatment values of nematode communities were not available for the French site.

Each nematode was assigned to a trophic group (bacterivore, fungivore, herbivore, omnivore or predator, according to Yeates et al., 1993) and a c-p group (colonizer-persister scale with five levels) indicative of their life-history strategy (Bongers and Ferris, 1999). Trophic and c-p groups can be combined forming functional guilds (Bongers and Bongers, 1998). Feeding types and c-p groups were assigned using the NINJA automated calculation system (Sieriebriennikov et al., 2014). Six indices indicative of food web characteristics were also calculated using NINJA (Sieriebriennikov et al., 2014): the maturity index for free-living taxa (MI), the plant parasitic index (PPI), the structure index (SI), the enrichment index (EI), the fungivores:bacterivores ratio (F:B) and the prey:predator ratio. The maturity index is an indicator of the ecological successional status of a soil community, considering the relative

abundance of nematodes with different life-history strategy: those in the higher c-p scale (longer life cycles and K-strategists) vs. those in the lower c-p scale (opportunistic, fast-growing, and r-strategists) (Bongers, 1990; Bongers and Ferris, 1999). The maturity index is calculated as the weighted mean of the % of free-living nematodes in each c-p group: $MI = \sum v_i f_i$, where v_i is the c-p value in the i_{th} taxon, and f_i is the frequency of the i_{th} taxon in a sample (Bongers, 1990). The plant parasitic index is the equivalent of the maturity index for plant-feeding nematodes. The interpretation of the plant parasitic index is the inverse to the maturity index, with undisturbed systems showing a high maturity index but a low plant parasitic index (Bongers et al., 1997). The structure index measures the complexity of the soil food web, with high scores indicating a structured and complex food web (Ferris et al., 2001). It is calculated as $100 \times [s/(s + b)]$, where s is the structure component (calculated as the weighted frequencies of the functional guilds Ba3–Ba5, Fu3–Fu5, Pr3–Pr5 and Om3–Om5), and b is the basal component (calculated as the weighted frequencies of Ba2 and Fu2 nematodes, Ferris et al., 2001). The enrichment index is a measure of nutrient enrichment, generally associated with nutrient addition or with the mortality of other organisms reflecting a nutrient flush, describing therefore the resource availability in the soil (Ferris and Bongers, 2006). It is calculated as $100 \times [e/(e + b)]$, where e is the enrichment component (calculated as the weighted frequencies of Ba1 and Fu2 nematodes), and b is the basal component (Ferris et al., 2001). The fungivores:bacterivores ratio (F:B) was calculated as an indicator of the dominant decomposition channel in the soil, with low values suggesting a dominance of the fast bacterial decomposition channel and high values of the slower fungal channel. Finally, the prey:predator ratio was calculated as a proxy for top-down pressure within the nematode community, considering the sum of the abundances of fungivore, bacterivore and herbivore nematodes as prey and the sum of omnivore and predator nematodes as predators. We considered omnivores as predators due to the predatory feeding habit of Dorylaimida (Yeates et al., 1993), which was the dominant order of omnivore nematodes in our study (see Results).

2.4. Characterization of soil abiotic properties and microbial and plant properties

At the short-term site, we measured soil organic matter, microbial biomass and tree root chemistry and litter production to explore possible mechanisms behind the effect of the rainfall exclusion treatment on the nematode community. Data were obtained from samples collected in spring 2018 under the same trees where nematodes were quantified (6 trees per plot, 36 samples in total). Soils (0–10 cm depth) were sampled following the same protocol as for nematodes. Tree roots were sampled in the first 0–20 cm of the soil profile by following large roots from the base of each tree trunk in at least two different cardinal directions. A representative sample of the fine roots (<0.2 mm \varnothing) attached to the larger roots were collected for each individual tree. Soil and root samples were kept moist in sealed plastic bags and transported inside an ice box to the laboratory, where they were stored at 4 °C until processing. Soil samples were sieved through a 2 mm mesh sieve, and used for quantification of organic matter content using the calcination method (Howard and Howard 1990) and of soil microbial biomass (in terms of C and N) following the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). Roots were gently washed to remove soil particles, dried at 45 °C during 48 h and finely ground with liquid nitrogen. Secondary compounds (total phenols) and non-structural carbohydrates (soluble sugars and starch) were extracted from 300 to 20 mg (respectively) of dry tissue of each root sample. Total phenols were quantified colorimetrically by the Folin-Ciocalteu assay (Moreira et al., 2009) and non-structural carbohydrates (NSC) using the anthrone method (Hansen and Møller, 1975). Litter production was sampled using litterbags (0.5 m \varnothing) located under the canopy of each tree (2–3 bags per tree, depending on the tree size). The content of each litterbag was

emptied at the beginning of each spring and taken to the lab, where it was dried (70 °C during 48 h) and weighed to calculate the litter biomass (g/m²) produced annually per tree. Litter production during the period 2017–2018 was used in the statistical analyses.

2.5. Data analysis

We first used Generalized Linear Models (GLMs) to test the effect of rainfall exclusion on the richness, diversity (H'), total abundance, abundance of different trophic groups, abundance of the dominant families (>0.1 indv/g), and indices (maturity index, plant parasitic index, structure index, enrichment index, F:B and prey:predator) of the soil nematode community. Richness was modelled using a Poisson error distribution, whereas the remaining variables were modelled using a normal distribution. Site, rainfall exclusion and their interaction were included as fixed factors. To control for the nested structure of the experimental design, block was included as an additional fixed factor nested in site. Block was included as a fixed factor because it did not have enough levels (i.e. > 4) to be considered as a random factor (Ieno and Zuur, 2015). For the Spanish experimental site, pre-treatment differences in the nematode community between control and rainfall exclusion plots were explored also using GLM. Since some pre-treatment differences appeared (Table S5, Fig. S6), models testing the effect of the rainfall exclusion treatment were also run for this site including pre-treatment values as covariables.

To test for statistical differences on the composition of the nematode community across sites and rainfall exclusion treatments, we used principal coordinate analysis (PCoA) and PERMANOVA test with 999 permutations. Prior to the analysis, the matrix containing abundances for the different taxonomic groups identified across all sites at the genus level was squared-root transformed to reduce the weight of the most common taxa in the analysis (Santonja et al., 2017). Then, distances between groups were calculated using the Bray-Curtis distance index, which ranges between 0 and 1 (Bray and Curtis, 1957).

Finally, we used structural equation models (SEMs) to explore the mechanisms underlying the effects of short-term rainfall exclusion on the nematode community at the Spanish site. SEMs were not conducted for the French site due to unavailability of enough data about the abiotic and biotic determinants of nematode abundance. Before the analysis, pairwise correlations were conducted to check for the existence of strong correlations (Pearson $r > 0.8$) among variables included in the SEM (Fig. S7). We also transformed (log, square root) several variables to fulfil normality and homoscedasticity assumptions. We hypothesized that rainfall exclusion would cause alterations of soil abiotic properties, microbial biomass, and tree litter production and root chemistry. Changes in litter production would affect soil abiotic properties, which in turn would affect abundance of all nematode trophic groups. On the contrary, changes in root chemistry would directly affect only herbivore nematodes and microbial biomass, whereas changes in microbial biomass would affect mostly bacterivores and fungivores. Regarding relationships between the different nematode trophic groups, we tested three alternatives: directional relationships that implied a top-down regulation in the trophic food web, directional relationships that implied a bottom-up regulation in the trophic food web, and bi-directional relationships that might reflect similar preferences for microsite conditions. Hypothesized mechanisms are summarized in Table S1. According to this causal structure, the initial path diagram was tested (Fig. S1), selecting or discarding the different relationships until we reached consensus of a model providing biological realism and a good statistical fit following several indices (root mean square error of approximation (RMSEA), comparative fit index (CFI) and standardized root mean square residual (SRMR)) (Kline, 2016). The rainfall exclusion treatment was introduced in the model as a dummy variable (0, 1). Plant properties (litter biomass, root phenols and root NSC) were introduced as three independent observed variables, whereas soil abiotic properties and microbial biomass were introduced as two composite variables. Soil

abiotic properties was composed by soil water content and organic matter, whereas microbial biomass was composed by microbial biomass measured in terms of carbon and nitrogen. As an integrative measure of soil water content, we used the cumulative value during the fall and winter months previous to the sampling date (October–March) in the first 20 cm of the soil profile (see Table S6 for a summary of the mean values of all variables included in the SEM).

All statistical analyses were conducted using the R software (R version 4.2.1). Linear Models were performed using package “stats” and “lm” and “glm” function (R Core Team, 2022), PERMANOVA analyses were performed using package “vegan” and “adonis2” function (Oksanen et al., 2013), and structural equation models (SEMs) were conducted using package “lavaan” and “sem” function (Rosseel, 2012).

3. Results

3.1. Impact of rainfall exclusion on nematode diversity, abundance and composition

The rainfall exclusion treatment did not affect the richness of the nematode community at any of the two study sites, and had only marginal positive effects on diversity (H') (Table 1). However, rainfall exclusion had an effect on the total abundance of nematodes, but only at the short-term site (significant Site × Rainfall exclusion interaction, Table 1). At this site, nematode abundance was 39% lower in rainfall exclusion plots than in control plots (Fig. 1). Results for nematode richness, diversity, abundance and indices at the short-term site were not significantly affected by the introduction of pre-treatment values as covariables (Table S7), suggesting that initial differences did not influence the effect of the rainfall exclusion treatment.

Bacterivores and fungivores were the most abundant groups at both the short-term (2.41 ± 0.31 and 1.78 ± 0.15 ind/g, respectively) and long-term (6.08 ± 0.64 and 4.65 ± 0.38 , respectively) sites, and the most affected by the rainfall exclusion treatment (Fig. 1). The effect of rainfall exclusion on bacterivore abundance differed among sites (significant Site × Rainfall exclusion interaction, Table 1), being largely

Table 1

Results of the Generalized Linear Models analysing the effect of rainfall exclusion on nematode diversity, abundance, and indices at the short- and long-term sites. Values represent Z-values for richness and F-values for the rest of variables. Block was introduced as a fixed factor to control for the nested structure of the experimental design. Asterisks denote significant effects: ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05, †p < 0.1.

Variable	Site	Block [Site]	Rainfall exclusion	Site × Rainfall exclusion	R ²
Diversity					
Richness	0.08	3.82	0.99	0.74	0.07
H'	0.33	1.27	2.97†	1.33	0.12
Abundance					
Total	106.47****	2.67*	3.46†	4.02*	0.64
Bacterivore	62.69****	3.47*	1.47	5.55*	0.54
Fungivore	94.49****	7.51***	10.34**	1.76	0.66
Herbivore	47.79***	1.18	1.78	1.21	0.44
Predator	2.19	1.76	1.31	0.14	0.13
Omnivore	84.06***	3.22*	3.21†	2.62	0.59
Indices					
Maturity index	0.32	1.99	10.37**	0.38	0.21
Plant Parasitic index	1.17	0.78	1.03	0.02	0.07
Structure index	4.75**	1.66	9.73**	0.42	0.23
Enrichment index	45.71****	4.14**	0.01	0.79	0.47
Fungivores/Bacterivores	0.09	2.98*	3.39†	3.31†	0.21
Prey/predator	15.81**	5.28**	10.59**	0.51	0.40

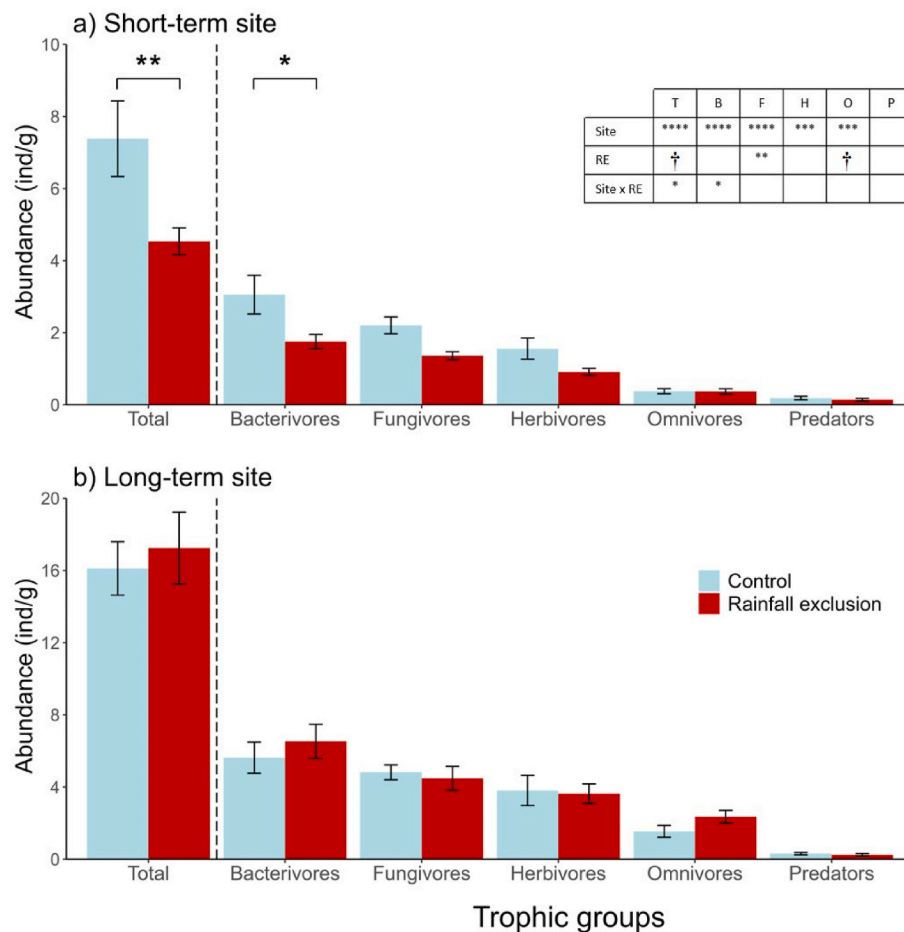


Fig. 1. Effects of rainfall exclusion on the abundance (mean \pm SE) of soil nematodes, both total and separated by trophic groups, at the short-term (a) and long-term (b) experimental sites. Abundances are expressed as the number of individuals per gram of soil. The inset table shows the significance of the effects of site, rainfall exclusion and its interaction on each of the variables analysed (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, † $p < 0.1$). When the interaction was significant, site-specific effects are indicated with asterisks in the figure.

negative (43% reduction) at the short-term site but not significant at the long-term site (Fig. 1). Regarding fungivores, the rainfall exclusion treatment had a consistent negative effect on its abundance at both sites (Table 1), but of larger magnitude at the short-term site (38% vs. 7% reduction, Fig. 1). The abundance of herbivore and predator nematodes was not affected by rainfall exclusion at any site, whereas omnivores abundance showed a marginal, positive response to the experimental treatment that was particularly evident at the long-term site (Table 1, Fig. 1). When explored at the family level, rainfall exclusion had consistent negative effects across sites on some dominant families of bacterivores (Cephalobidae) and fungivores (Aphelenchoididae, Filenchus). However, other dominant families were only affected at one of the sites (e.g. the bacterivorous Rhabditidae or the omnivore Nordiidae at the long-term site) or showed contrasting responses among sites (bacterivores Plectidae and Monhysteridae) (Table S8, Fig. 2). Among herbivores, treatment effects were detected on the abundance of just one family (Cricematidae), which decreased under rainfall exclusion but only significantly at the short-term site (Table S8, Fig. 2). The effect of rainfall exclusion on omnivores was restricted to the dominant family Qudsianematidae, which showed a marginally significant increase under rainfall exclusion at both sites, and the family Nordiidae which also increased under rainfall exclusion but only at the long-term site (Table S8, Fig. 2). These differential impacts across trophic groups and families translated into nematode communities of contrasting composition at control versus rainfall exclusion plots at both sites, as supported by the results of the PERMANOVA analysis (Table S9, Fig. S8).

3.2. Impact of rainfall exclusion on nematode indices

The rainfall exclusion treatment had a significant effect on the

maturity index, the structure index, the prey:predator ratio, and (only marginally) the F:B ratio (Table 1). Moreover, these effects were always consistent across sites (i.e. non-significant Site \times Rainfall exclusion interactions, Table 1), except for the F:B ratio (marginally significant Site \times Rainfall exclusion interaction). Rainfall exclusion caused an increase in both the maturity index and the structure index, as well as a decrease in the prey:predator ratio (Fig. 3). The effect on the F:B ratio was only detected at the long-term site, where rainfall exclusion caused a 26% decrease in the ratio (Fig. 3). Rainfall exclusion had no effects on the plant parasitic index or the enrichment index at any site (Table 1).

3.3. Drivers of the short-term impact of rainfall exclusion on nematode communities

The final SEM model adequately fit the data on the soil food web at the short-term site (RMSEA = 0.065, SRMR = 0.133, p -value = 0.241). Rainfall exclusion did not affect litter biomass or root phenols, but had a positive effect on root NSC (Fig. 4). This change in NSC did not translate into changes in the abundance of herbivore nematodes, which were only negatively affected by root phenols. Rainfall exclusion caused a direct significant negative effect on soil abiotic properties (soil water content and organic matter), which in turn had a positive effect on the abundance of bacterivores, fungivores and herbivores (Fig. 4). Rainfall exclusion had no direct effects on soil microbial biomass, although microbial biomass had direct positive effects on the abundance of bacterivore and fungivore nematodes. Among the several path diagrams tested, the final SEM did not include directional relationships among nematode trophic groups, but positive correlations. Particularly strong were correlations between fungivores and bacterivores, as well as between predators (omnivores and predators) and lower trophic levels

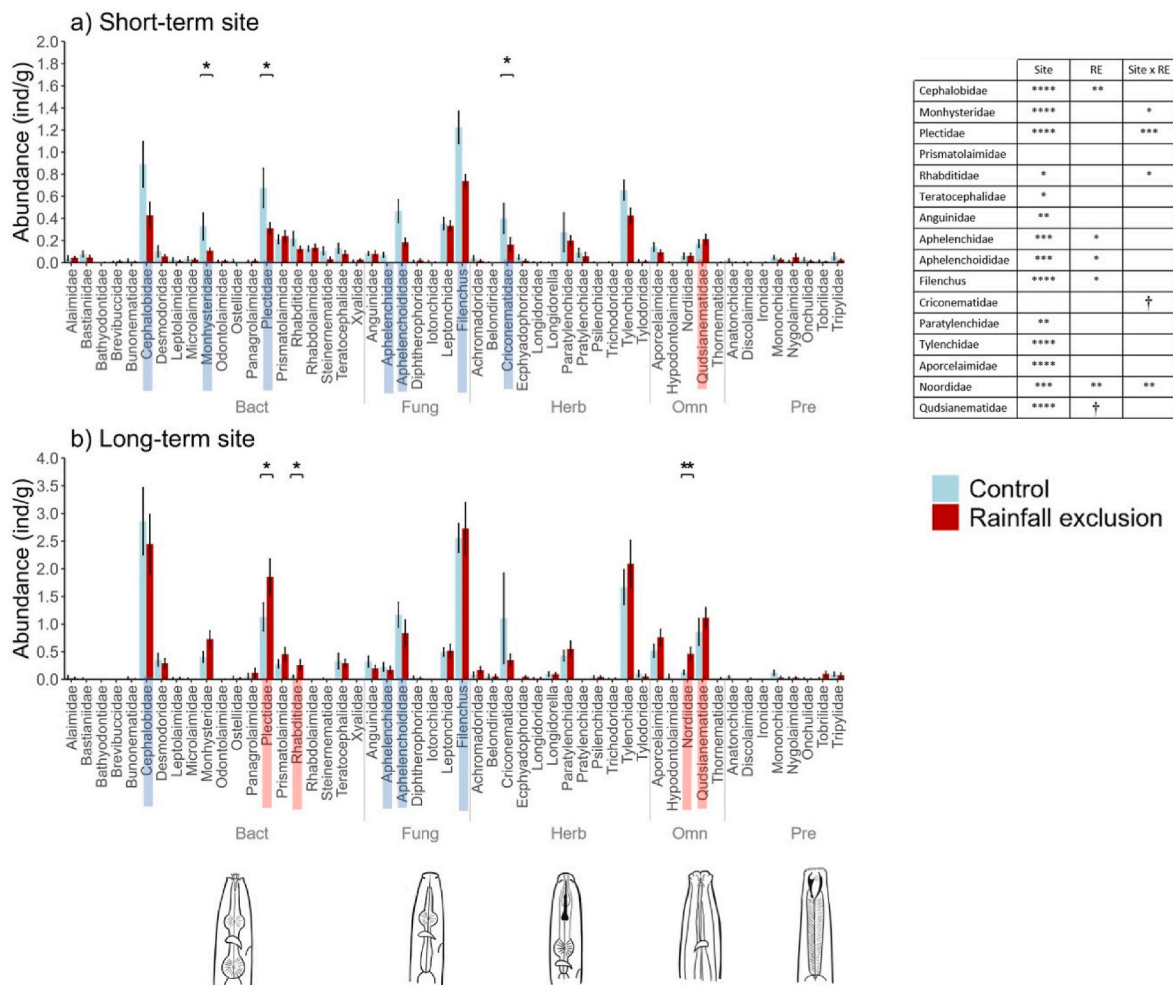


Fig. 2. Effects of rainfall exclusion on the total abundance (mean \pm SE) of nematode families, grouped by functional groups (Bact: bacterivores; Fung: fungivores; Herb: herbivores; Omn: omnivores; Pre: predators) at the short-term (a) and long-term (b) experimental sites. The *Filenchus* genus is represented as fungivore, separately from the Tylenchidae family, due to their different trophic habits. The inset table shows the significance of the effects of site, rainfall exclusion and its interaction on each family (****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05, †p < 0.1). When the interaction was significant, site-specific effects are indicated with asterisks in the figure. Families for which a significant effect of rainfall exclusion was found are indicated in the colour of the treatment where they reached the largest abundance. Silhouettes below the graphs represent the different trophic groups based on the structure of their mouthparts and are based on Ed Zaborsky illustrations, University of Illinois.

(fungivores and bacterivores) (Fig. 4).

4. Discussion

This study provides novel experimental evidence showing that rainfall reductions predicted by climate change models for the Mediterranean region (i.e. 30% reduction in annual precipitation) have both short- and long-term impacts on soil food webs of forest ecosystems. Our results therefore demonstrate the sensibility of water-limited forests to further reductions in soil water availability, which might substantially alter their soil communities and likely affect the many ecosystem processes that they control.

4.1. Impact of rainfall exclusion on the diversity, abundance and composition of nematode communities

Experimental evidence regarding the impacts of rainfall reduction on the abundance and diversity of soil nematodes is conflicting, with available studies showing a variety of complex responses including negative, neutral and positive impacts (Stevnbak et al., 2012; Sylvain et al., 2014; Cesarz et al., 2015; Torode et al., 2016; Franco et al., 2019; Ankrom et al., 2020; Siebert et al., 2020). Because nematodes are

aquatic organisms physiologically active only when water is available, we expected rainfall reduction to have a negative effect on their abundance and diversity in Mediterranean forests, where soil water content is extremely low during a large part of the year (June-October) (Figs. S4 and S5). Our results supported this prediction for nematode abundance, with a 30% rainfall reduction translating into a 39% reduction in the abundance of the nematodes, but only at the short-term site (Spain). This finding is consistent with previous studies that explored the impact of rainfall reduction on soil nematode abundance in mesic temperate forests (Landesman et al., 2011; Wang et al., 2021). Overall, our results therefore provide new evidence supporting that nematode abundance in water-limited Mediterranean forests show low resistance to rainfall reductions predicted by climate change models.

We also hypothesized that rainfall exclusion effects would vary among trophic groups, following previous literature showing higher trophic levels to be particularly sensitive to environmental changes (Preisser and Strong, 2004; Franco et al., 2019). Our results supported the prediction of differential responses among trophic groups consistently across sites, but in the opposite direction than expected. Thus, rainfall reduction had in general larger negative effects on lower trophic groups (bacterivores and fungivores) than on higher trophic groups (omnivores and predators), which even showed a marginal positive

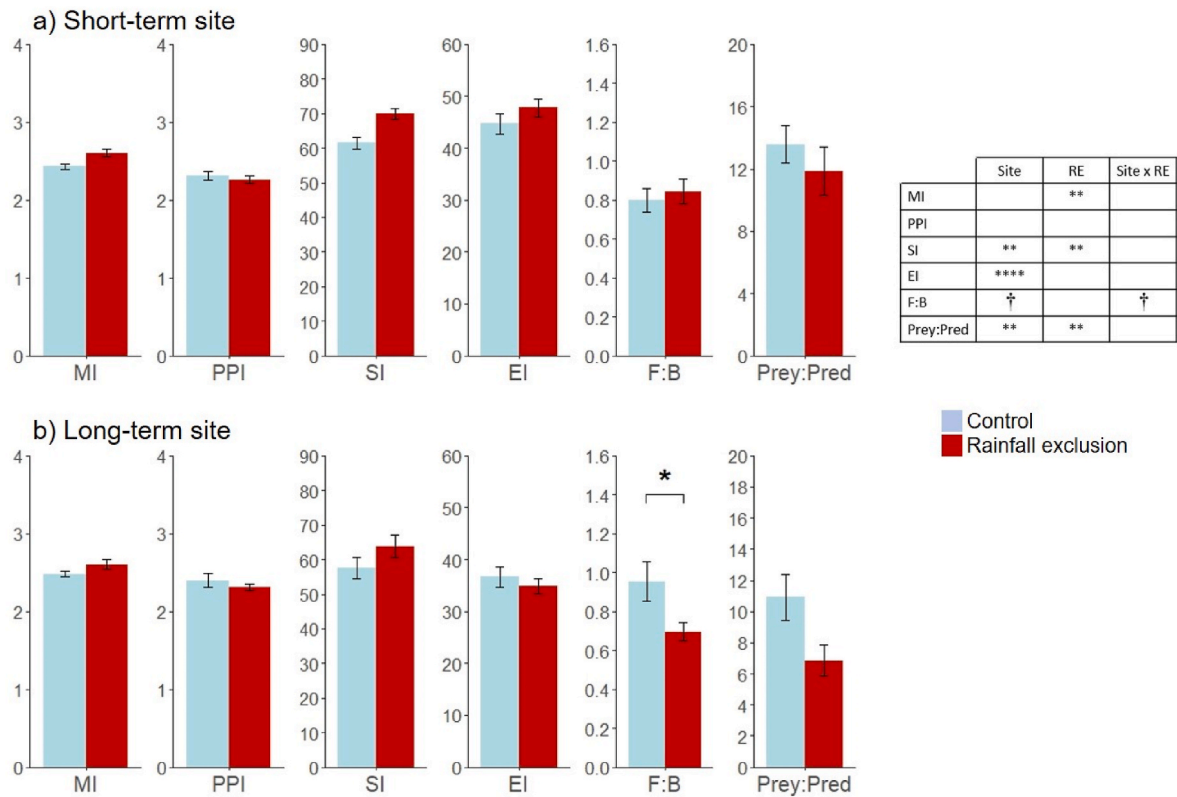


Fig. 3. Effects of rainfall exclusion on the nematode-based indices (mean \pm SE) at the short-term (a) and long-term (b) experimental sites. MI = maturity index, PPI = plant parasitic index, SI = structure index, EI = enrichment index, F:B = fungivores:bacterivores ratio; Prey:Pred = prey:predator ratio. The inset table shows the significance of the effects of site, rainfall exclusion and its interaction on each index (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, † $p < 0.1$). When the interaction was significant, site-specific effects are indicated with asterisks in the figure.

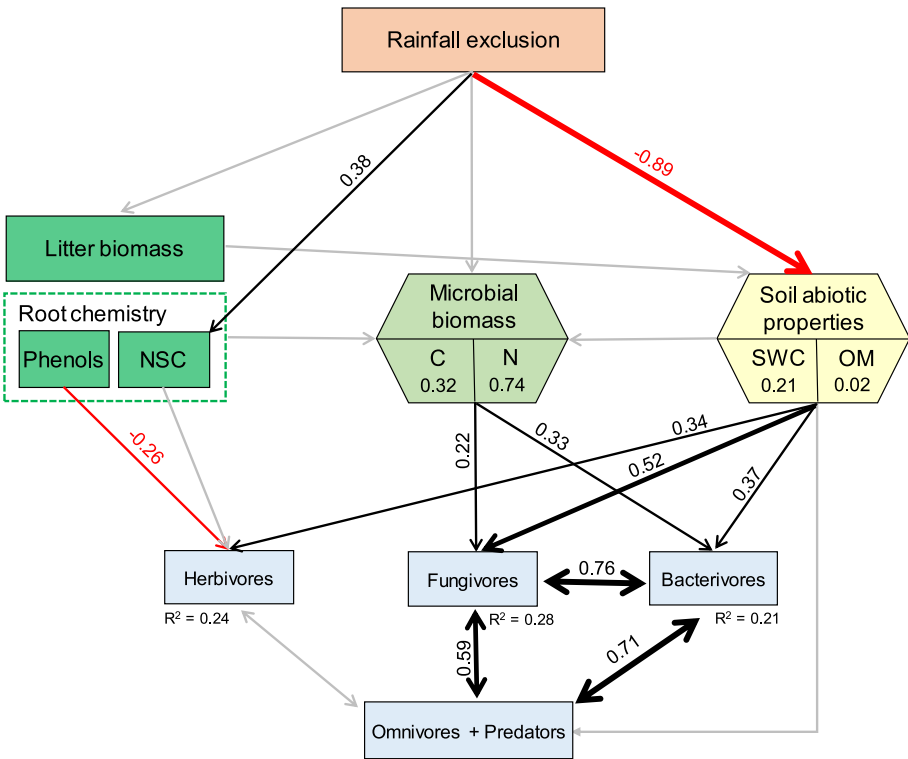


Fig. 4. Results of the structural equation model (SEM) linking rainfall exclusion with abiotic, microbial and plant drivers of the abundance of different nematode trophic groups. Hexagons represent composite variables and rectangles observed variables. Numbers adjacent to arrows are standardized path coefficients. Width of arrows is proportional to the strength of path coefficients. Red arrows indicate negative relationships, black arrows positive relationships, and grey arrows relationships not included in the final model. Numbers inside hexagons are the coefficients of the different indicator variables used to construct the composite variables (SWC, soil water content; OM, organic matter; C, microbial biomass carbon; N, microbial biomass nitrogen). R^2 represent the variance explained for each endogenous observed variable.

response to rainfall exclusion. A plausible explanation for the absence of negative effects on higher trophic groups might rely on their composition, since they were largely dominated by the family Qudsianematidae. This family is considered to have a high drought tolerance (Landesman et al., 2011; Bakonyi et al., 2007) and adaptability to extreme climates (Hoschitz, 2003), probably due to a remarkable anhydrobiosis ability (i.e. ability to survive the loss of most water and enter a state of suspended metabolism; Treonis et al., 2000). Moreover, long life cycles coupled with a broad feeding strategy are not likely to represent a disadvantage to cope with increasing drought, as has been reported for other stress sources. Rather, these characteristics could allow the exploitation of a wide variety of resources during favourable seasons, entering a state of diapause when conditions are adverse (Landesman et al., 2011).

A closer look at the family level revealed taxon specific responses of nematodes to predicted rainfall reduction, likely reflecting a large variation within the nematode community in their drought tolerance. In fact, a recent study along an aridity gradient found that, although most genera were more abundant in the wet part of the gradient, some reached their higher abundance in the drier part (Xiong et al., 2020). In our study, the negative effects of rainfall reduction on fungivores across sites were largely driven by the response of the Aphelenchoididae family, which seems to have a great susceptibility to drought (Landesman et al., 2011), whereas the positive effect on omnivores were driven by the drought-tolerant Qudsianematidae. The response of bacterivores was, however, more complex, since the two most abundant families, Cephalobidae and Plectidae, showed contrasting responses to rainfall exclusion both within and across sites, even though they belong to the same functional guild (i.e. c-p 2, Cesarz et al., 2015). Previous studies have also reached contrasting conclusions about the drought tolerance of these bacterivore families (Bakonyi et al., 2007; Landesman et al., 2011; Xiong et al., 2020). Clearly, further studies are needed to improve our understanding of the large variability present within nematode trophic and functional groups in their response to a drier climate. Such variability might be a key determinant of contrasting responses of nematode communities with different composition to a given climatic stressor.

4.2. Impact of rainfall exclusion on soil food webs based on nematode indices

A main advantage of using nematode communities as target group to understand climate change impacts on soils is that they allow the calculation of multiple indices that may reflect the overall state of soil food webs and the ecosystem (Du Preez et al., 2022). Based on previous studies, we hypothesized that rainfall reduction would translate into a simplified and less structured soil food web (i.e. lower values of the maturity and structure index) where top-down regulation of lower trophic levels (microbivores and plant feeders) might be impaired (i.e. higher prey:predator ratio). However, we found just the opposite and in a consistent manner across both experimental sites. Thus, the lower vulnerability to rainfall exclusion of omnivores and predators in contrast to lower trophic levels translated into an increase in the maturity and structure index, as well as in a reduction of the prey:predator ratio. These changes might be interpreted as a shift towards structured soil food webs with many trophic links and a large top-down control at both short and long temporal scales (Bongers, 1990; Ferris et al., 2001; Domínguez-Begines et al., 2019). Interestingly, a similar increase in the top-down control of soil food webs under rainfall reduction was found in a parallel study conducted at the short-term site with litter mesofauna (Homet et al., 2021). Soil networks with a high connectivity are considered to be more resistant against disturbances than simpler networks (Ferris et al., 2001; Xiong et al., 2017). Our findings therefore suggest that the predicted rainfall reduction for Mediterranean areas might cause long-lasting changes in the structure of the soil food web that might increase their capacity to withstand further disturbances.

We also predicted that rainfall exclusion would cause a change

towards the slow fungal decomposition channel, as indicated by higher fungivore:bacterivore ratios (F:B ratio). This prediction was based on accumulated evidence showing that bacteria are more sensitive to a drier climate than fungi due to a higher dependence on moisture availability to access resources for growth (Ren et al., 2018). However, our results did not support this prediction at any site. At the short-term site, both bacterivores and fungivores showed large decreases (30–40%) induced by drought causing no alterations of the decomposition channel. On the other hand, in the long-term site, only fungivores were negatively affected by drought whereas bacterivores were stimulated, causing a decrease in the F:B ratio. Thus, the decomposition channel was affected only after long-term exclusion towards a dominance of the fast bacterial pathway. A plausible explanation that needs to be tested is that this impact was driven by changes in the structure and diversity of the plant community after more than a decade of rainfall exclusion. In fact, the long-term site had a diverse understorey that might have benefited from the negative effects of long-term drought on tree performance (Limousin et al., 2009; Gavinet et al., 2019). Previous studies have shown that soil bacterial communities in Mediterranean forests can benefit from a decline in tree health and the subsequent increase in the diversity and cover of understorey species with higher litter quality (Curiel-Yuste et al., 2012; Lloret et al., 2015). Our results therefore support limited direct effects of rainfall reduction on decomposition pathways in Mediterranean forests, but point towards possible indirect long-term impacts mediated by changes in the plant community. These changes would imply increased flows through the bacterial-decomposition channel, and therefore less efficient carbon cycling, since bacteria produce less biomass C per unit of C metabolized than fungi leading to a lower proportion of C stored in the soil (van der Heijden et al., 2008; Strickland and Rousk 2010; Zhang et al., 2022).

4.3. Impact of rainfall reduction on soil nematodes at short and long temporal scales

We expected impacts of rainfall reduction to be restricted mainly to the short-term. However, we found consistent changes at both short (2 years) and long temporal scales (15 years) in the composition of the nematode community (decrease of fungivores, marginal increase of omnivores) and the structure of the soil food web (higher maturity and structure index, lower prey:predator ratio). The fact that rainfall exclusion had impacts on the nematode community even after 15 years of treatment might be indicative of a low adaptive capacity of the soil food web of Mediterranean forests to a drier climate. Unfortunately, the scarcity of experimental studies that compare both short- and long-term effects on soil nematodes precludes the comparison of our results with those from other systems. To our knowledge, only the study by Holmstrup et al. (2017) reported data after 2 and 8 years of rainfall exclusion in a heathland ecosystem of Northern Europe, finding a decrease of drought effects on nematode diversity through time that might be indicative of certain adaptive capacity of the nematode community to the new climatic conditions. In view of these contrasting results, further long-term studies are urgently needed to allow reaching sound conclusions on the adaptation of soil nematodes to a drier climate.

4.4. Drivers of the short-term impact of rainfall exclusion on nematode communities

Our SEM provided relevant insights into the pathways through which rainfall exclusion affected the different nematode trophic groups at the short-term site. Rainfall exclusion had a strong negative direct effect on the abundance of nematodes in lower trophic levels (microbes and herbivores) mediated by a reduction in soil water content and organic matter, supporting the importance of abiotic conditions for these organisms (Ferris and Bongers, 2006; Wang et al., 2018; Xiong et al., 2020). Contrary to our expectations, rainfall exclusion did not indirectly affect the abundance of bacterivore and fungivore nematodes

through changes in microbial biomass. This is because, although microbial biomass had a positive effect on the abundance of microbivores, it was not affected by the rainfall exclusion treatment. The lack of rainfall exclusion effects on microbial biomass was unexpected, since soil microbes are strongly sensitive to alterations in rainfall (Blankinship et al., 2011; Nielsen and Ball, 2015). However, because microbial abundance in forest soils is strongly heterogeneous at the microscale and variable in time (Baldrian, 2017), we consider that further measurements are needed to validate this result. Our SEM did not detect either indirect effects of rainfall exclusion on nematode abundance mediated by changes in plant properties as litter biomass or root chemistry. Herbivore nematodes were negatively affected by the root content of phenols, which are a class of secondary compounds known to provide resistance against herbivores in *Quercus* species (Moreira et al., 2018a, 2018b). However, these compounds were not altered by the rainfall exclusion treatment. Overall, our results suggest that negative effects of rainfall exclusion on nematode abundance were largely direct and mediated by reductions in soil water content, which might restrict movement and access to prey limiting survival and population growth (Landesman et al., 2011).

Food webs are frequently structured by top-down and bottom-up forces, where higher trophic levels (e.g. omnivores, predators) are limited by resource availability and lower trophic levels (e.g. fungivores, bacterivores, herbivores) by predation pressure (Power 1992; Yeates et al., 1997). However, our final model of nematode abundance did not include directional relationships between lower and higher trophic groups, but strong correlations. Because these correlations were all positive, they could reflect shared environmental requirements that promoted overall higher nematode abundance at some microsites in our study forests. Therefore, our results suggest that the soil food web in the studied Mediterranean forest was more intensively influenced by environmental conditions than by trophic relationships.

5. Concluding remarks

Our study took advantage of standardized rainfall exclusion protocols to explore the short- (2 years) and long-term (15 years) effects of a drier climate (30% rainfall reduction) in Mediterranean forests of southern Europe. We recognize that a better replication in both space (more than one exclusion per country) and time (soil sampling in different seasons or years) would have increased the robustness of our conclusions. Moreover, we also recognize that the two study forests showed differences in soil properties of relevance for the nematode community (e.g. texture, soil organic matter; van den Hoogen et al., 2019) that limited our ability to directly compare the results from both sites. For example, the detection of negative rainfall exclusion effects on nematode abundance only at the short-term site might be indicative of some adaptation process of the population abundance to a drier climate over time. Alternatively, it could be just the result of a larger protection against drought offered by soil aggregates in the fine texture soils of the long-term site, where nematode abundance was generally much higher than in the short-term site (Görres et al., 1999; Neher et al., 1999; Yeates et al., 2002).

Despite these limitations, results were largely consistent among sites, suggesting that soil food webs of Mediterranean forests (as indicated by soil nematodes) are highly sensitive to reductions in precipitation at both short and long temporal scales. Whether these patterns can be generalizable to other forests or water-limited systems needs to be tested. We firmly believe that the increasing number of manipulative climate change experiments throughout the world, often in coordinated networks (i.e. DroughtNet, Knapp et al., 2017), offer a unique opportunity to improve this knowledge gap. Given the pivotal role of soil nematodes in the regulation of carbon and nutrient dynamics (Ingham et al., 1985; Ferris, 2010; Wagg et al., 2014) and their value as bio-indicators of soil health (Neher, 2001), a better understanding of their response to climatic alterations will offer very valuable insights into the

short- and long-term implications of climate change for the functioning of terrestrial ecosystems.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data and associated code to reproduce the results are stored with Figshare: 10.6084/m9.figshare.22093292

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2023.108974>.

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