



## Organic viticulture enhanced the activity of native entomopathogenic nematodes in DOCa Rioja soils (North of Spain)

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### ABSTRACT

Vineyards and their associated socio-economic activities are relevant sectors worldwide. Still, this agroecosystem is one of the most intensely managed crops and erosion-prone land areas. The conventional viticulture practices to control pests, diseases, and weeds, like tillage and agrochemical applications, accelerate the loss of soil biodiversity and compromise the presence of beneficial soil organisms such as the entomopathogenic nematodes (EPNs). Such human disturbances in the agroecosystems can strongly affect abiotic (e.g., soil texture and properties) and biotic factors (natural enemies and potential competitors) that modulate the EPN activity as biological control agents. For the first time in viticulture, this study aimed to investigate the impact of differentiating management on the EPN community and associated soil organisms and if their assemblage will provide indicators of better practices for sustainable farming. We hypothesized that organic pest management and alternative strategies to tillage might enhance the abundance and activity of the native EPN community in vineyard soils. In autumn 2019, we collected two composite soil samples from 80 vineyards distributed across the Guaranteed Designation of Origin (denominated DOCa) Rioja region. The sites belonged to one category of each of the two factors: pest management (integrated vs. organic, 40 plots each) and soil managing (tillage vs. cover cropping, 48 and 32 vineyards, respectively). Isolated through sucrose-gradient centrifugation and employing species-specific primers/probe qPCR sets, we investigated the presence of ten EPN species and associated soil organisms: four free-living nematodes (FLNs), six nematophagous fungi (NF), and two ectoparasitic bacteria (EcPB). Besides, we estimated the EPN activity using the traditional insect-bait method. We included in the analysis twenty soil variables to characterize the evaluated treatments and assess their impact on soil organism distribution. Our results provide evidence on the support of organic viticulture to beneficial soil organisms, notably the activity of native EPNs. We also reported a higher abundance of *S. feltiae* (the predominant steinernematid species in Europe) and FLNs for organic farming than IMP, while the presence of NF and EcPB resulted in unaffected. Contrarily, the soil management practices considered did not differ in their impact on EPNs or their natural enemies/competitors, even if contrasted for several soil properties. Future research may expand the screened soil-dwelling species using novel molecular techniques to unravel their complex interactions and determine the best farming practices to preserve soil health.

### 1. Introduction

Viticulture and enology are relevant socioeconomic and cultural sectors worldwide that provide a broader range of services and goods to people and nature than the simple production of grapes and wine (Santos et al., 2020). The current viticulture has to afford appropriate

yields while supporting ecosystem services such as regulating climate and hydrological services, preserving the habitat, or maintaining cultural heritage (Orgiazzi et al., 2016). Soils are possibly the most complex and biodiverse ecosystem on Earth (Orgiazzi et al., 2016). Soil biodiversity enables self-perpetuating of the ecosystem functions of carbon transformations, nutrient cycling, maintenance of soil structure, and the

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regulation of pests and diseases (Kibblewhite et al., 2008; Gunstone et al., 2021). Agricultural intensification accelerates the loss of soil biodiversity (Veresoglou et al., 2015), particularly in the last decade due to the overuse of chemical applications (FAO, 2020). Despite rising environmental awareness by farmers and society, which for instance drive in recent years the organic viticulture in the higher European wine-producing countries (FiBL, 2019), the vineyard is still one of the most intensely managed crops and erosion-prone land systems (Nicholls et al., 2008; Rodrigo-Comino et al., 2018; Winter et al., 2018; Karimi et al., 2020).

Located on both sides of the Ebro River, the Guaranteed Designation of Origin (denominated DOCa) Rioja (CR Rioja wine, 2021) is the oldest in Spain (1925), one of the principal vine-growing, wine-producing, and global exporter countries (OIV, 2020). Grapevine management in the region often involves conventional tillage and the widespread use of pesticides (herbicides, fungicides, insecticides, and acaricides) in substantial numbers, responsible for significant environmental impacts, including soil and water pollution (Pose-Juan et al., 2015; Herrero-Hernández et al., 2017). Integrated Pest Management (IPM) appeared as a viable solution to these problems. Strongly encouraged and regulated by the European Union (EC, 2009), IPM aims to keep the occurrence of potential pests, diseases, and weeds below the Economic Damage Threshold, following a series of principles ranging from preventive and monitoring strategies to intervention reducing as possible the use of pesticides (Barzman et al., 2015). Still, the wine sector and public opinion give today more attention to more sustainable practices (Aschemann-Witzel and Zielke, 2017). Organic viticulture, based in the European Union on the Regulation (EU) (2018)/848 containing the principles of organic production and labeling, is understood as an overall system that aims to reduce the use of approved pesticides by adopting alternative strategies that promote environmental care, biodiversity, preservation of natural resources, and high animal welfare standards (Provost and Pedneault, 2016; EU, 2018). Indeed, a recent meta-analysis estimated that organic viticulture promotes soil biodiversity over three times more than conventional practices (Karimi et al., 2020). Besides, soil management is also critical for soil and plant protection in crops. Traditional tillage negatively affects most soil microbes and their functioning and promotes the mineralization of organic matter and soil erosion, particularly in European vineyards, commonly placed on slopes (Santos et al., 2020). More sustainable solutions to reduce tilling, like cover cropping or mulching, decrease soil erosion and favor soil biodiversity by increasing organic matter content and porosity, structural stability, and water retention of soil crops (Santos et al., 2020).

Soil preservation brings several indirect benefits to farmer crops. Many soil invertebrates, like nematodes, mites, or different predators and parasitoids, play a role in controlling agricultural pests (Gunstone et al., 2021). The entomopathogenic nematodes (EPNs), well-known pathogens of soil inhabitant arthropods, are responsible for many ecological goods and services (Campos-Herrera et al., 2012; Lewis et al., 2015). Their non-feeding, infective juvenile (IJ) stages often occur in natural and agricultural soils where locate suitable hosts (Stock, 2015). Once penetrate within their hemocoel, IJs rapidly kill them (48–72 h after infection) with the aid of protein toxins and secondary metabolites produced by mutualistic enteric  $\gamma$ -Proteobacteria (Boemare, 2002; Bode, 2009; Dillman et al., 2012). Physical or chemical disturbance of soils resulting from human activities such as tillage and agrochemical applications can affect the occurrence and persistence of EPNs (Stuart et al., 2015). For example, a previous study conducted in La Rioja revealed a negative correlation between EPN activity and the intensity of agricultural management, with high activities in natural areas and perennial organic crops (vineyards included), low in conventional perennial crops, and rare in annual crops, regardless of pest control practices (Campos-Herrera et al., 2008). Similarly, recent investigations performed in an experimental DOCa Rioja vineyard concluded that turning conventional tillage to spontaneous cover cropping could favor

the occurrence of above and below beneficial organisms, including EPNs (Sáenz-Romo et al., 2019; Blanco-Pérez et al., 2020). In addition to soil chemical and physical alterations, soil biota such as bacteria, fungi, or nematodes of other nature, also interact with EPNs and modulate their service as biological control agents (Helmberger et al., 2017). Nowadays, advances in molecular tools allow determining their relative importance within the EPN food web in an appropriate ecological context (Campos-Herrera et al., 2012). Diverse free-living nematode (FLN) and EPN species co-emerge from larvae used in insect baits (Duncan et al., 2003; Campos-Herrera et al., 2012, 2015a, 2019a; Jaffuel et al., 2016, 2018; Blanco-Pérez et al., 2020), a competition that plausibly reduces the virulence of emerging IJs (Blanco-Pérez et al., 2019). Also, the nematophagous fungi (NF), found in all main fungal taxonomic groups, are widespread in natural and agricultural soils, remaining as saprophytes until switching to their parasitic stage, developing specialized structures in the presence of nematodes (Nordbring-Hertz et al., 2006). The mechanisms behind these interactions seem to be environmental and species-specific dependent, but still mostly unknown (Koppenhöfer et al., 1996; El-Borai et al., 2009; Bueno-Pallero et al., 2018). Regional and temporal studies showed that the presence of particular NF species can contribute to the persistence of specific EPNs, although still poorly understood which are the scenarios that favor species-specific NF predation of this kind (Pathak et al., 2017; Campos-Herrera et al., 2019b). Besides, microorganisms such as ectoparasitic bacteria (EcPB) in the genus *Paenibacillus* can also compromise the fitness of some EPN species by reducing the motility and virulence of IJs (El-Borai et al., 2005; Enright and Griffin, 2005).

Despite the enormous potential of native EPNs in agroecosystems to control large host ranges, little is known about plausible soil-dwelling target pests in vineyards yet (Campos-Herrera et al., 2021; Marín et al., 2021). However, we should not underestimate the importance of conserving natural enemies of arthropod pests such as EPNs, particularly in the context of a changing climate that may vary the spectrum of pests and diseases affecting vineyards in warmer regions since, for example, they increasingly survive during warmer winters (Santos et al., 2020). Recognizing the factors that drive the activity and abundance of EPNs in the vineyard should allow the establishment of best practices to favor suitable ecological scenarios to enhance their functionality and long-term persistence in the agroecosystems. Additionally, soil nematode community members, involved in primary roles in soil food webs (Eisenhauer and Guerra, 2019), are good bio-indicators of soil health (Ferris et al., 2001). Moreover, recent studies provided evidence that linked high occurrences and activities of EPN species with sustainable agricultural practices (Campos-Herrera et al., 2008, 2014; Blanco-Pérez et al., 2020). However, limited to a few vineyards, these results can not define a whole wine-production area. Comprising 80 vineyards distributed throughout the DOCa Rioja region, our study aimed to explore the impact of differentiating management on the EPN community and associated soil organisms and if their assemblage will provide indicators of better practices for sustainable viticulture. We speculated that organic viticulture and the implementation of alternative strategies to regular tillage might enhance the abundance and the activity of naturally occurring EPN species compared to conventional practices. The main objectives of this study were to (i) quantify EPN abundance and activity, (ii) quantify the abundance of target soil organisms associated with EPNs (NF, FLNs, and EcPB), and (iii) contribute to discriminate the abiotic factors that drive them.

## 2. Material and methods

### 2.1. Survey scheme, sampling methods, and soil properties analyses

During early autumn 2019, we surveyed 80 vineyards distributed crosswise the DOCa Rioja region (Fig. 1; Supplementary data 1, Table S1), located in a warm-summer Mediterranean climate (classified as Csb by the Köppen-Geiger system) with a continental influence. We

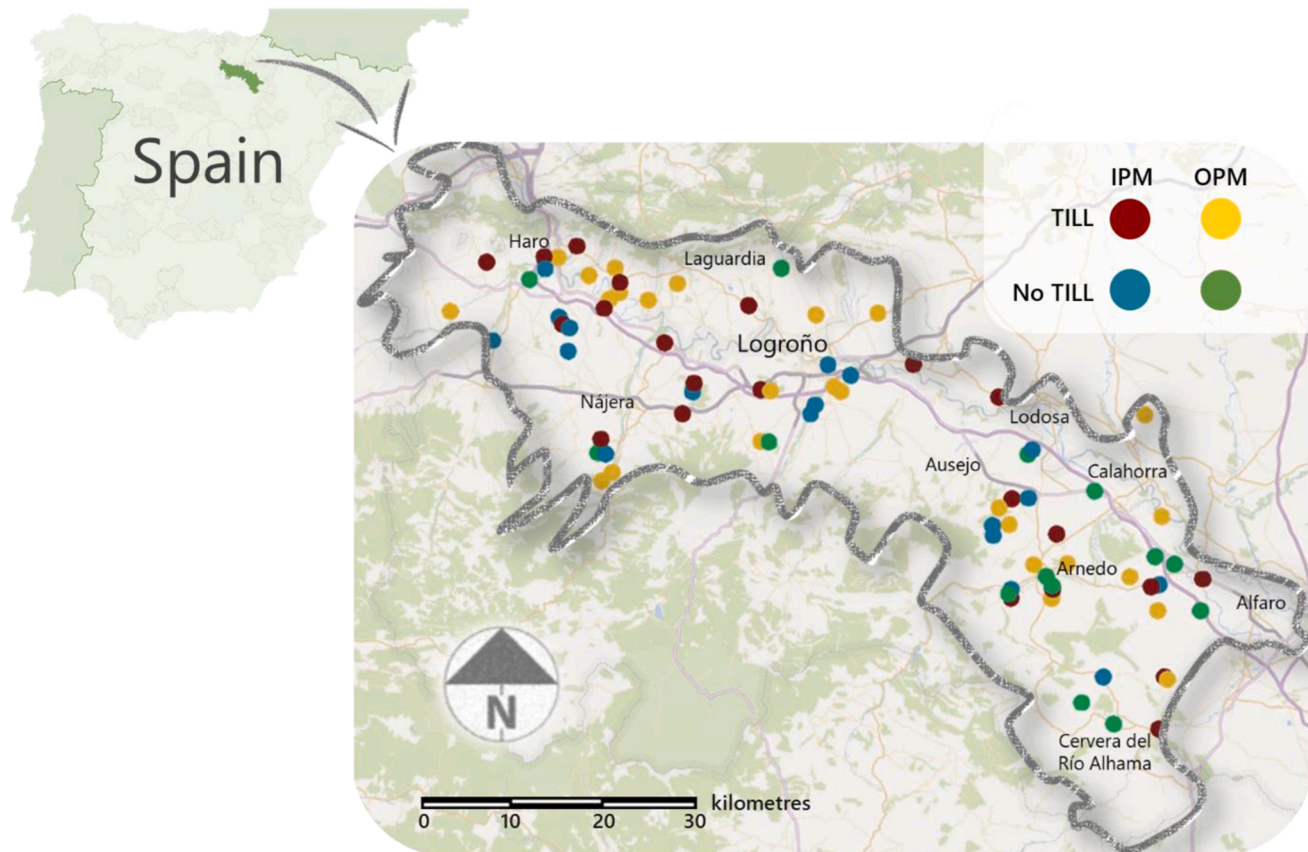


Fig. 1. Distribution of the sampled DOCa Rioja vineyards.

studied the effect of soil management (regular tillage *versus* cover cropping, 48 and 32 sites, respectively) and pest and diseases management (integrated *versus* organic, 40 locations each) on the EPN community and associated soil food web. Thus, each vineyard was characterized for one of the levels of the evaluated treatments, resulting in four possible combinations: tilled IMP, no-tilled IMP, tilled organic farming, and no-tilled organic farming (Table S1). Conventional soil management of DOCa Rioja vineyards consists of secondary tillage (not less than four times a year), mainly to provide weed control in inter-rows throughout the growing season during the maturation of the vine, generally using row crop cultivators (11 arms) with teeth that work at least 10–15 cm depth in the surface (Fernández Alcázar, 2011). We selected different types of cover cropping (a few of them combined with organic mulching) as alternative strategies to tillage that need no or minimum mechanization of soil crops (Table S1). The pest management of vineyards was adjusted to the current Spanish and regional regulations of sustainable use of agrochemical products (RD, 2012; Table S2) and organic farming (RD, 2014; DRioja, 2019), depending on the treatment.

We randomly collected two independent composited samples per vineyard, each consisting of 20 single soil cores (2.5 cm  $\varnothing$  x ~20 cm DP.), taken with auger soil samplers under the crop canopy of middle rows to avoid possible border effects. The 20 cores per sample were combined in individual plastic bags (~1800 cm<sup>3</sup> of soil) and stored in a chamber (4 °C in the dark) until processed (within 2–4 days). Following the protocol described by Campos-Herrera et al. (2019a), each sample was manually homogenized and divided into three subsamples of 200 g of fresh soil (Wiesel et al., 2015). Two of the subsamples were employed (i) to characterize the abundance of EPN community and associated organisms through sucrose-gradient centrifugation procedure and qPCR analysis, and (ii) to determine the soil suppressive capacity by using the traditional insect-bait method. The third soil set was dried at 40 °C for

one week to measure the water content to express the number of identified organisms per 100 g of dry soil. Finally, we combined 100 g of oven-dried soil of both replicates of each vineyard to analyze the following soil properties (performed by Laboratorio Regional del Gobierno de La Rioja, La Grajera, Logroño, Spain): texture (sand, silt, and clay percentages) (Bouyoucos, 1936), pH (Millennia and Markewitz, 2004), electric conductivity, organic matter (Walkley and Black, 1934), macro-nutrients (P, K, and nitrates), oligo-nutrients (Mg, Ca, and SO<sub>4</sub>), micro-nutrients (Fe, Mn, Zn, Cu, Al, and B), and other elements (Na and Pb) (Mehlich, 1978, 1984). Monthly precipitations in the DOCa Rioja region in 2019 were also recorded by the Agro-climatic Information Service in La Rioja (SIAR; Fig. S1).

## 2.2. Soil mesofauna isolation and estimation of soil activities against insect larvae

Following the procedure described by Blanco-Pérez et al. (2020), nematodes and other soil organisms were co-extracted through sucrose-gradient centrifugation (Jenkins, 1964) from sieved (aperture width of 25  $\mu$ m) 200 g of fresh soil of each sample (Wiesel et al., 2015). Besides, we employed the traditional insect-bait method to estimate the soil activity ratios following a protocol adapted from Bedding and Akhurst (1975). Specifically, we calculated differentiated mortality percentages of final instars of *Galleria mellonella* (Lepidoptera: Pyralidae) larvae (reared at ICVV): total mortality (total-act), mortality associated with nematode emergences (nem-act), and percentage of larvae for which Koch's postulates were confirmed (EPN-act). Additionally, we maintained some of the nematode emergences, from first and Koch's postulates test rounds (VO and VM aliquots, respectively), to establish laboratory cultures following the procedure described by Woodring and Kaya (1988). Both suspensions of soil organisms obtained through sucrose-gradient centrifugation and RO/RM aliquots were

stored at  $-20^{\circ}\text{C}$  in the dark until used for DNA extraction procedures (see Blanco-Pérez et al., 2020 for a more detailed protocol).

### 2.3. Identification and quantification of soil organisms by real-time qPCR

For the DNA extraction procedure, we first mechanically disaggregated all samples with sterile blue pestles (15 seg) assembled to a Kontes™ Pellet Pestle™ motor (DWK Life Sciences GmbH, Mainz, Germany). We used the DNA extraction kits DNeasy PowerSoil Kit (QIAGEN GmbH Co., Hilden, Germany) and Speedtools tissue (Biotools, B&M Labs S.A., Madrid, Spain) for the samples obtained by sucrose-gradient centrifugation and RO/RM aliquots, respectively. DNA extractions were stored at  $-20^{\circ}\text{C}$  until used. We screened for 22 soil organisms using species-specific primers and probes in qPCR tests (Table 1; Supplementary data 2, Table S3). Previous studies reported the presence in La Rioja of most of the evaluated species: the EPNs *Steinernema carpocapsae*, *S. feltiae*, *S. kraussei*, *S. riojaense*, *S. affine*, *Heterorhabditis bacteriophora*, and *H. indica*, the FLNs *Pristionchus maupasi* and *Acroboloides*-group, the NF *Arthrobotrys oligospora*, *A. dactyloides*, *Purpureocillium*

**Table 1**  
Soil organisms tested.

Type of organism / species	Population	GenBank ACNO ITS Region	Reference for primers and probes sequences
Entomopathogenic nematodes			
<i>Heterorhabditis bacteriophora</i>	(commercial)	KJ938576	Campos-Herrera et al. (2011a)
<i>Heterorhabditis indica</i>	Btw	KJ938571	Campos-Herrera et al. (2011b)
<i>Heterorhabditis megidis</i>	(commercial)	KJ938577	Campos-Herrera et al. (2015b)
<i>Steinernema affine</i>	CH	KJ938567	Torr et al. (2007)
<i>Steinernema carpocapsae</i>	DOK-83	KJ818295	Campos-Herrera et al. (2011a)
<i>Steinernema feltiae</i>	RS-5	KJ938569	Campos-Herrera et al. (2011a)
<i>Steinernema intermedium</i>	82	AF171290	Campos-Herrera et al. (2015b)
<i>Steinernema kraussei</i>	OS	KJ696686	Campos-Herrera et al. (2015b)
<i>Steinernema riojaense</i>	RM-30	MK503133	Blanco-Pérez et al. (2020)
<i>Steinernema</i> sp. <i>affine</i> -group	VO-53	MW480137	(current study)
Free-living nematodes			
<i>Acroboloides</i> -group	RT1-R15C	JQ237849	Campos-Herrera et al. (2012)
<i>Oscheius tipulae</i>	MG68 P29	KJ938579	Campos-Herrera et al. (2015a)
<i>Oscheius onirici</i>	MG67 P20	KJ938578	Campos-Herrera et al. (2015a)
<i>Pristionchus maupasi</i>	AM-3	MG551681	Campos-Herrera et al., 2019a
Nematophagous fungi			
<i>Catenaria</i> sp.	1D	JN585805	Pathak et al. (2012)
<i>Arthrobotrys dactyloides</i>	H55	KJ938574	Pathak et al. (2012)
<i>Arthrobotrys musiformis</i>	11	KJ938572	Pathak et al. (2012)
<i>Arthrobotrys oligospora</i>	8	KJ938573	Pathak et al. (2012)
<i>Hirsutella rhossiliensis</i>	2931	KM652168	Zhang et al. (2006)
<i>Purpureocillium lilacinum</i>	9357	KJ938575	Atkins et al. (2005)
Ectoparasitic bacteria			
<i>Paenibacillus nematophilus</i>	NEM2	AF480936	Campos-Herrera et al. (2011a)
<i>Paenibacillus</i> sp.	SdTc1FEE1	JF317562	Campos-Herrera et al. (2011a)

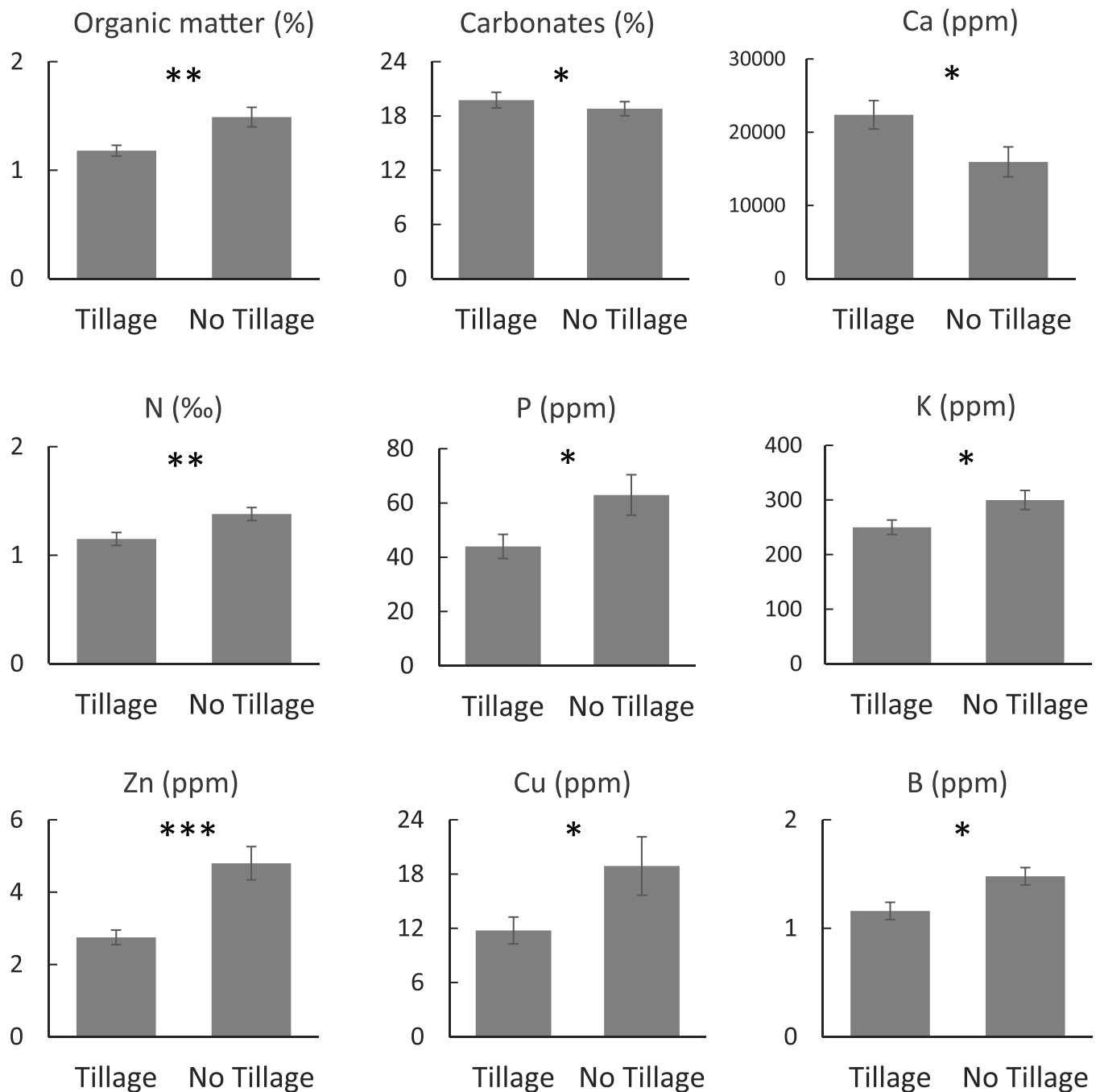
*lilacinum*, *Hirsutella rhossiliensis*, and *Catenaria* sp., and the EcPB *Paenibacillus* sp. (Campos-Herrera et al., 2007, 2008; Blanco-Pérez et al., 2020). Also, the EPN *S. intermedium*, the FLNs *Oscheius tipulae* and *O. onirici*, the NF *Arthrobotrys musiformis*, and the EcPB *Paenibacillus nematophilus* had been identified in the Iberian Peninsula (García del Pino, 2005; Campos-Herrera et al., 2011a, 2016, 2019a). On the other hand, there is still no record for *Heterorhabditis megidis* in Southwest Europe but Northern and Eastern European countries (Hominick, 2002). Besides, we designed a species-specific primers/probe set (Supplementary data 2) for the EPN species *Steinernema* sp. *affine*-group (recorded by Valadas et al., 2014 in continental Portugal, but misidentified as *S. intermedium* according to Dr. Vladimír Půža, Czech Academy of Sciences, Czech Republic), isolated from aliquots of insect-baits conducted in this study. Moreover, we included additional nematode species (13 EPNs and 5 FLNs) to validate the qPCR tools, avoiding cross-amplification and adapting previously published protocols to our experimental conditions (Table S4). Pure cultures of most evaluated soil organisms were preserved as described by Blanco-Pérez et al. (2020) (Supplementary data 2).

Final concentrations of species-specific primers and probes, synthesized by Biotools (Madrid, Spain), were 400 and 200 nM, respectively, except for *Steinernema* sp. *affine*-group, which was 100 and 40 nM. Probes were labeled at the 5' and 3' ends with the fluorogenic reporter dye FAM and the quencher BQH-1, respectively. The qPCR tests and quantifications were performed with the Quantimix Easy Probe mix (Biotools, Madrid, Spain) in Bio-Rad iCycler iQ® 96-well PCR plates, covered with Bio-Rad iCycler iQ® Optical tape, on the Bio-Rad CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). We run 38 cycles for nematodes species and 50 for NF and EcPB species. Before qPCR runs, all DNA samples were analyzed for quality and quantity in a Nanodrop system (Thermo Scientific 2000 C spectrophotometer) and adjusted to 1 ng/μl for all the organisms except NF, adjusted to 10 ng/μl. We optimized protocols and established positive controls described by Blanco-Pérez et al. (2020) (Supplementary data 2).

### 2.4. Statistical analyses

We ran generalized mixed models (GLMMs), executed with SPSS 25.0 (SPSS Statistics, SPSS Inc., Chicago, IL, USA), testing the effect of differentiated farming practices of DOCA Rioja vineyards, pest management (two levels: integrated *versus* organic), soil management (two levels: regular tillage *versus* cover cropping), and their interactions on DNA quantifications and abundance/frequencies of occurrence of EPNs, FLNs, NF, EcPB, and soil activity measures. The quantifications obtained by sucrose-gradient centrifugation were expressed per 100 g of dry soil: EPNs as number of IJs, EcPB as copies of plasmids, and FLNs and NF as standardized 0–1 range of the values recorded for each species (see Blanco-Pérez et al., 2020 for further details). The soil activities (total-act, nem-act, and EPN-act) recorded in the insect baits were represented as larval percentages and frequencies of occurrence. Before running the statistical analysis, quantitative variables were  $\log(x + 1)$  transformed. We ran GLMMs with a gamma distribution (log-link function) for quantifying soil organisms and binomial distribution (logit-link function) for soil activities and frequencies of occurrence. We initially considered covariates in exploratory GLMM tests of all the abiotic variables that showed significant differences between treatments for general linear model (GLM) tests (Fig. 2; Supplementary data 3, Table S5). Subsequently, we included soil organic matter to test for differences in soil activities and quantifications of DNA, total FLNs, and specific target organisms. We used the Least-Squares Means  $\pm$  standard error of the mean (SEM) as descriptive statistics. We employed Microsoft Excel 2016 (3D Maps) for visualizing the sample sites and species distributions.

For the multivariate analyses of selected soil organisms and abiotic factors, performed with CANOCO 5 (ter Braak and Šmilauer, 2002; Šmilauer and Lepš, 2014), we first chose some of the abiotic factors as



**Fig. 2.** General linear model testing for differences of soil properties between tilled and no-tilled soils. Asterisks indicate significant differences at  $*P < 0.05$ ,  $**P < 0.01$ , and  $***P < 0.001$ . Values are least-square means  $\pm$  SE (see Table S5 for complete statistics).

exploratory predictors after avoiding strong co-linearities (Table S6). Then, both biotic and abiotic variables were standardized by dividing by the highest values, ranking all values 0–1 (Šmilauer and Lepš, 2014). Values over 3.0 maximum length (SD units) for detrended canonical correspondence analysis (DCCA) indicate heterogeneous communities, and canonical correspondence analysis (CCA, constrained axes) is recommended (ter Braak and Šmilauer, 2002). We run CCA (interspecies correlations) with a Monte Carlo permutation ( $n = 499$ ) and automatic forward selection for the assignment of significant ( $P < 0.05$ ) abiotic factors (using the Bonferroni correction for  $P$  values). The final results were visualized with bi-plot scaling (CANOCO 5).

### 3. Results

#### 3.1. Soil properties and DNA quantification

We did not find differences for the evaluated abiotic factors between integrated and organic pest management but between regular tilling and cover cropping (Fig. 2; Supplementary data 3, Table S5). Overall, we recorded lower values for soil organic matter, N, P, K, Zn, Cu, and B in tilled soils but higher for carbonates (Fig. 2; Table S5). Among all these variables, only the soil organic matter was included in GLMMs testing the effect of our treatments on DNA quantifications and the abundances and occurrence of the target species *S. feltiae*, *O. tipulae*, *A. oligospora*, *H. rhossiliensis*, *Catenaria* sp., and *Peaenibacillus* sp. Contrary, soil

management did not affect the quantities of DNA obtained through sucrose-gradient centrifugation but pest management, higher in organic viticulture than IPM, although mediated by high values of soil organic matter (Fig. S2).

### 3.2. Abundance and frequency of occurrence of the soil organisms

We detected species of all the groups of soil organisms evaluated all across the DOCa Rioja region (Fig. 3), with no significant differences among treatments for the total EPN abundance and frequency of occurrence (Fig. 4A; Table 2). We detected four out of the ten EPN species screened: *S. feltiae*, *S. riojaense*, and *H. bacteriophora* recorded in all treatments, and *Steinernema* sp. *affine*-group only missed in tilled IMP vineyards (Fig. 4A; Table 2). Only for *S. feltiae* we reported statistical differences among treatments: higher abundance in organic viticulture and slightly higher frequency of occurrence ( $P < 0.1$ ) in tilled soils, both mediated by soil organic content (Table 2; Table S7). Regarding FLNs, we reported higher abundance in organic vineyards than IPM, specifically for *Acroboloides*-group and *O. tipulae*, both mediated by high values of soil organic matter (Table 2; Table S7), but not for soil management (Fig. 4B). In addition, we noticed the presence of the other two screened species, *O. onirici* and *P. maupasi*. The species *O. tipulae* was also detected in higher numbers in no-tilled soils, while *O. onirici* and *P. maupasi* were more abundant in bare soils (Table 2; Table S7). On the other hand, we detected five out of the six screened NF species: *A. dactyloides*, *A. oligospora*, *Catenaria* sp., *H. rhossiliensis*, and

*P. lilacinum* (Fig. 4C). The viticulture practices evaluated did not affect the NF identified (Fig. 4C), except *A. dactyloides*, recorded in higher numbers and frequencies in organic viticulture, again mediated by high values of soil organic matter (Table 2; Table S7). Finally, we found one out of the two screened EcPB species, *Paenibacillus* sp., in higher abundance in organic vineyards than IPM, also mediated by high values of soil organic, while not for soil management (Fig. 4D; Table S7).

### 3.3. Soil activities rates

For all the soil activities evaluated, we recorded significantly higher values in organic viticulture than IPM, also registered in a higher frequency of organic vineyards for total-act and nem-act, but not for soil management (Fig. 5, Fig S4 and S5; Table S8). The same nematode species identified through sucrose gradient centrifugation emerged from insect baits plus the steinernematid species *S. carpocapsae* and *S. kraussei*. The species *S. feltiae*, *O. tipulae*, *P. maupasi*, and *Acroboloides*-group occurred in all treatments except no-tilled IPM, for which no nematodes were found. The EPNs *Steinernema* sp. *affine*-group and *S. riojaense* happened in organic viticulture and *H. bacteriophora* in IPM. Finally, *S. kraussei* and *O. onirici* emerged from tilled IMP and *S. carpocapsae* from no-tilled organic vineyards only (Fig. 5A), none of them detected in the Koch's postulates tests (Fig. 5B). Nevertheless, we recorded no significant differences among treatments for any nematode species identified, neither from VO nor VM aliquots.

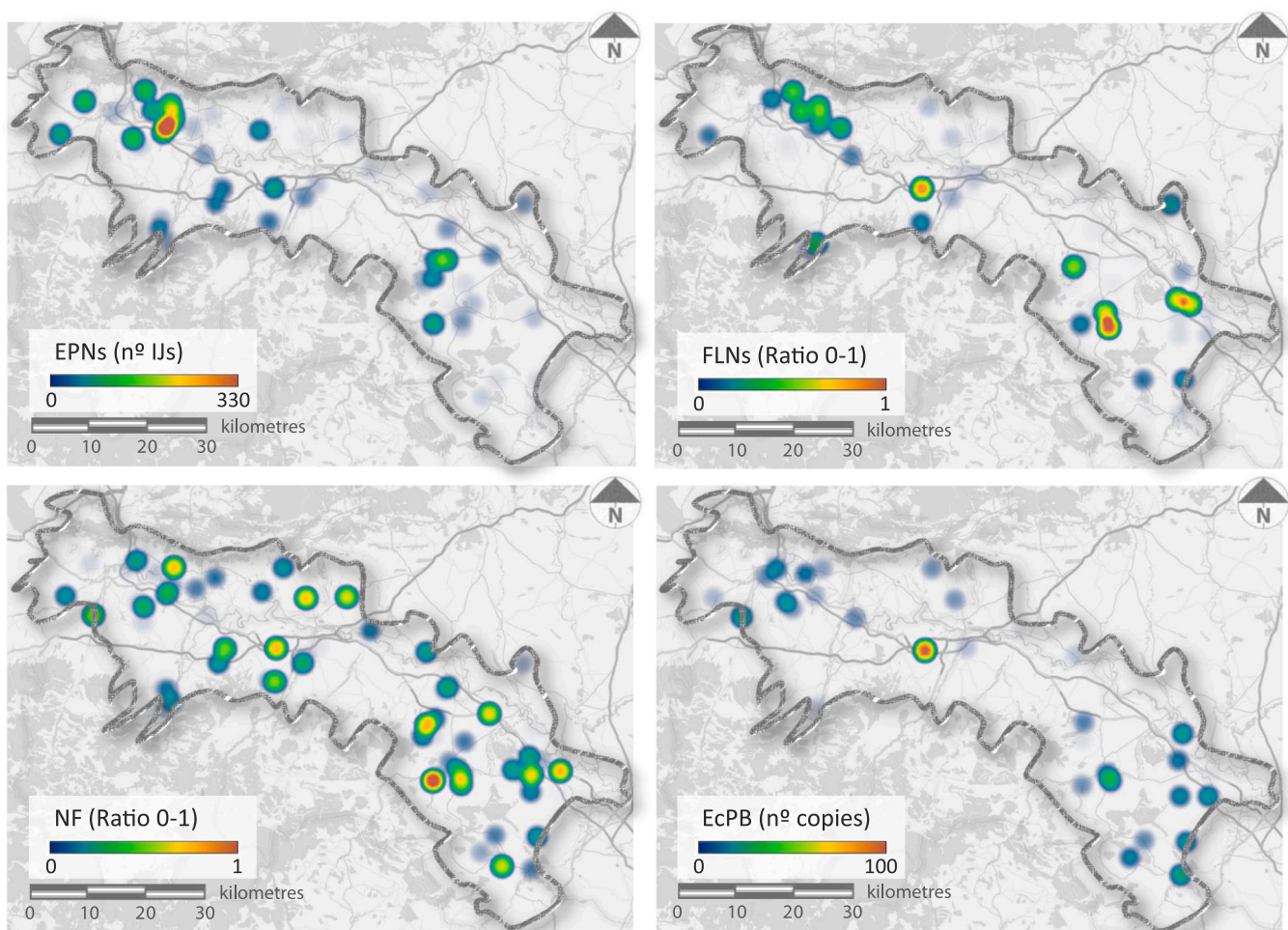
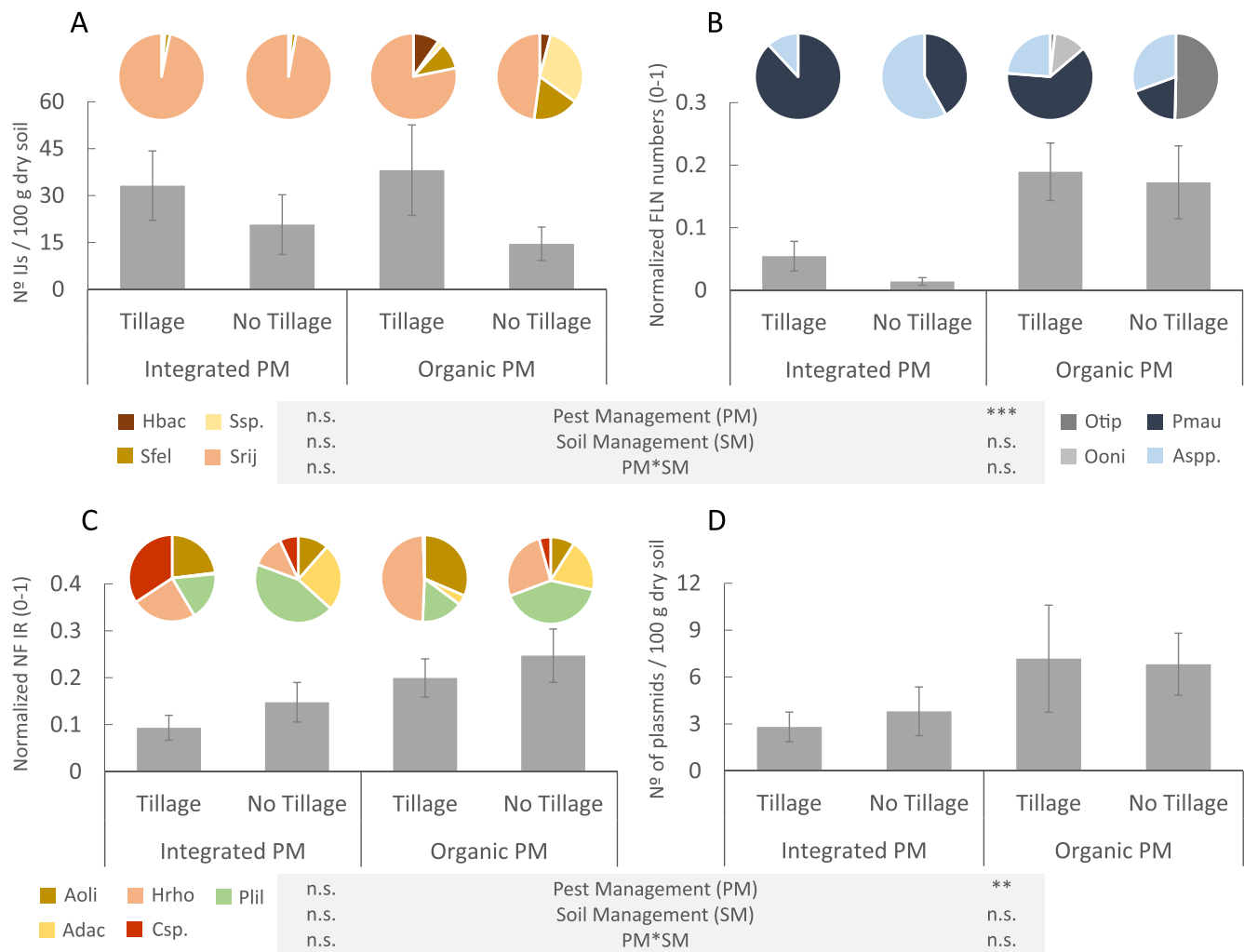


Fig. 3. Distribution in the DOCa Rioja region of the cumulative abundance of the soil organisms extracted through sucrose-gradient centrifugation: entomopathogenic nematodes (EPNs), free-living nematodes (FLNs), nematophagous fungi (NF), and ectoparasitic bacteria (EcPB).



**Fig. 4.** Effect of different management practices in DOCa Rioja vineyards on abundance of (A) infective juveniles (IJ) of the entomopathogenic nematode species *Heterorhabditis bacteriophora* (Hbac), *Steinernema sp. affine-group* (Ssp.), *S. feltiae* (Sfel), and *S. riojaense* (Srij), (B) the free-living nematodes (FLNs) *Oscheius tipulae* (Otip), *O. onirici* (Ooni), *Pristionchus maupasi* (Pmau), and *Acrobeloides-group* (Aspp.), (C) nematophagous fungi (NF) infection ratio (IR) of the species *Arthrobotrys oligospora* (Aoli), *A. dactyloides* (Adac), *Purpureocillium lilacinum* (Pliil), *Hirsutella rhossiliensis* (Hrhos), and *Catenaria sp.* (Csp.), and (D) the ectoparasitic bacterial species *Paenibacillus sp.* Asterisks indicate significant differences from generalized linear mixed model tests at  $***P < 0.001$  and  $**P < 0.01$  (n.s., not significant). Values are least-square means  $\pm$  SE. Soil species averages are represented in pies.

### 3.4. Multivariate analysis of abiotic factors and target soil organisms

Since the DCCA gradient was 3.1 SD units long, we performed CCA with the selected abiotic factors (Table S5) as exploratory variables, and the soil organisms isolated through sucrose-gradient centrifugation as response variables except for the NF species *Catenaria sp.* and the FLN species *O. tipulae* and *O. onirici*, present in less than 10% of the vineyards (Leš and Hadincová, 1992). Four soil properties (pH and clay, organic matter, and Cu content) significantly ( $P < 0.1$ ) contributed to explaining the soil composition and target species assemblage. The explained fitted variation of the two first axes reached 65%, Axis 1 primarily influenced by Cu and clay content, and Axis 2 by pH and soil organic matter (Fig. 6). Regarding the EPN species, *S. feltiae* was linked to high soil organic matter content and low pH and *S. riojaense* to high Cu and clay content, an opposite trend that observed for *Steinernema sp. affine-group*. The heterorhabditid *H. bacteriophora*, located near the origin axes, was unrelated to the predictor factors. The FLNs in the genus *Acrobeloides* correlated with low clay and organic matter contents and high pH, while the NF species *H. rhossiliensis* and *P. lilacinum* were located in the opposite quadrant. The NF species in the genus *Arthrobotrys* were associated with low organic matter content and high pH, particularly *A. dactyloides*. The FLN *P. maupasi* and the ECPB *Paenibacillus sp.* were

widespread with no apparent association with the independent variables.

## 4. Discussion

### 4.1. Impact of pest management

The literature provides not many examples of the effect of different agronomic management systems on the EPN community. For instance, Eilers-Kirk et al. (2000) observed that the inundative release of *S. riobravisi* IJs successfully controls *Acalymma vittatum* (Coleoptera: Chrysomelidae) populations in organic cucumber crops, in relative terms better than IMP. Contrary, no differences were found for the abundance and activity of EPNs and associated organisms in an ambitious study comprising four farming systems applied on three different crops (Jaffuel et al., 2016). However, and in agreement with our hypothesis, we observed higher soil activity rates for organic farming than IPM, also for those associated with nematode emergences, including EPNs. Conversely, we did not find significant differences for the total abundance of EPN species between the evaluated pest management treatments, probably due to the high numbers detected for *S. riojaense* also in IPM vineyards. Up to now, *S. riojaense*, the prevalent EPN species

**Table 2**

Descriptive ( $\bar{x} \pm \text{SEM}$ ) by treatments of abundances and frequencies of occurrence of the evaluated soil organisms. Asterisks indicate significant differences from generalized linear mixed models tests at \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , and \*  $P < 0.05$  ( $+P < 0.1$ ; n.s., not significant). Abundances (per 100 g of dry soil) measured as infective juveniles for entomopathogenic nematodes (EPNs), and ng/ $\mu\text{l}$  of pure culture for free-living nematodes (FLNs), and nematophagous fungi (NF). Codes: PM, pest management; SM, soil management; IPM, integrated pest management; OPM, organic pest management; Till, tillage; OM, soil organic matter.

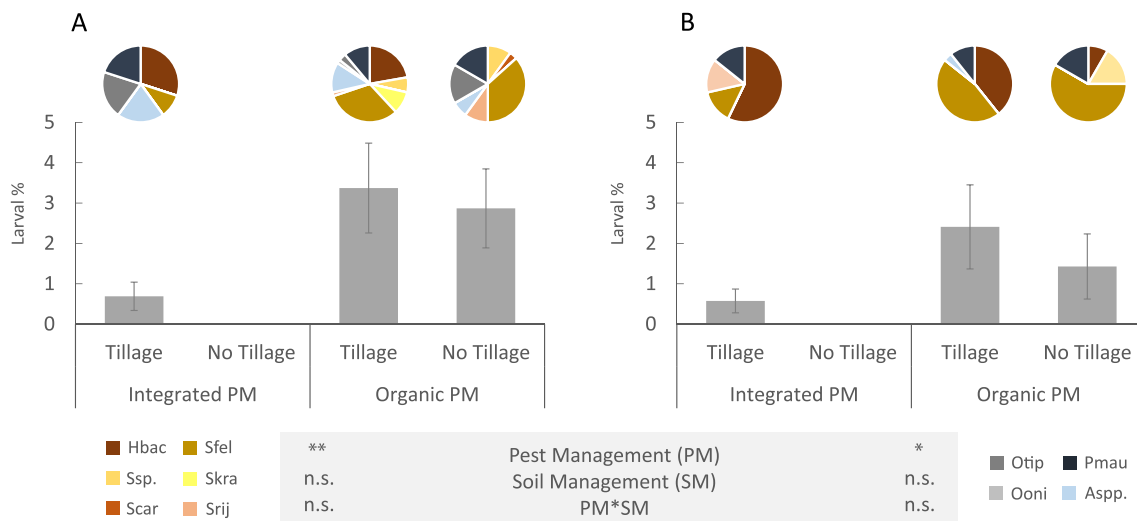
	Abundances				Factors and covariables				Frequencies of occurrence				Factors and covariables			
	Treatments				PM	SM	PM*SM	OM (%)	Treatments				PM	SM	PM*SM	OM (%)
	IPM-Till	IPM-NoTill	OPM-Till	OPM-NoTill					IPM-Till	IPM-NoTill	OPM-Till	OPM-NoTill				
EPN species																
<i>Heterorhabditis bacteriophora</i>	0.11	0.23	3.73	0.60	n.	n.	n.s.	-	0.02	0.03	0.15	0.04	n.	n.	n.s.	-
<i>Steinernema feltiae</i>	$\pm 0.11$	$\pm 0.23$	$\pm 1.79$	$\pm 0.60$	s.	s.			$\pm 0.02$	$\pm 0.03$	$\pm 0.05$	$\pm 0.04$	s.	s.		
<i>Steinernema riojaense</i>	0.62	0.37	3.82	2.51	**	n.	n.s.	***	0.23	0.14	0.44	0.32	n.	+	n.s.	*
<i>Steinernema sp. affine-group</i>	$\pm 0.24$	$\pm 0.24$	$\pm 2.34$	$\pm 0.94$		s.			$\pm 0.06$	$\pm 0.06$	$\pm 0.07$	$\pm 0.09$	s.			
<i>Oscheius tipulae</i>	32.13	20.11	29.77	6.98	n.	n.	n.s.	-	0.30	0.39	0.40	0.32	n.	n.	n.s.	-
<i>Oscheius onirici</i>	$\pm 11.10$	$\pm 9.57$	$\pm 13.92$	$\pm 2.90$	s.	s.			$\pm 0.07$	$\pm 0.08$	$\pm 0.07$	$\pm 0.09$	s.	s.		
<i>Pristionchus maupasi</i>	0.33	0	0.77	4.49	n.	n.	n.s.	-	0.07	0	0.12	0.07	n.	n.	n.s.	-
<i>Purpureocillium lilacinum</i>	$\pm 0.24$		$\pm 0.39$	$\pm 4.25$	s.	s.			$\pm 0.04$		$\pm 0.04$	$\pm 0.05$	s.	s.		
FLN species																
<i>Acrobeloides-group</i>	6,5E-03	8,2E-03	4,5E-02	5,3E-02	*	n.	n.s.	**	0.82	0.83	0.98	0.96	n.	n.	n.s.	-
<i>Oscheius tipulae</i>	$\pm 1,8E-03$	$\pm 2,6E-03$	$\pm 1,7E-02$	$\pm 3,6E-02$		s.			$\pm 0.06$	$\pm 0.06$	$\pm 0.02$	$\pm 0.04$	s.	s.		
<i>Oscheius onirici</i>	0	0	3,4E-03	8,7E-02	***	***	**	***	0	0	0.04	0.25	+	n.	n.s.	*
<i>Pristionchus maupasi</i>	0	0	$\pm 2,4E-03$	$\pm 4,3E-02$	n.	**	*	-	0	0	0.04	0	n.	n.	n.s.	-
<i>Arthrobotrys dactyloides</i>	4,8E-02	5,9E-03	1,2E-01	3,3E-02	n.	*	n.s.	-	0.16	0.03	0.35	0.18	+	n.	n.s.	-
<i>Arthrobotrys oligospora</i>	$\pm 2,4E-02$	$\pm 5,9E-03$	$\pm 3,4E-02$	$\pm 2,3E-02$	s.				$\pm 0.06$	$\pm 0.03$	$\pm 0.07$	$\pm 0.07$	s.			
<i>Catenaria sp.</i>	4,1E-04	3,7E-02	7,4E-03	4,8E-02	n.	**	n.s.	**	0.05	0.17	0.10	0.29	+	**	n.s.	*
<i>Hirsutiella rhossiliensis</i>	$\pm 4,0E-04$	$\pm 2,8E-02$	$\pm 3,7E-03$	$\pm 1,9E-02$	s.				$\pm 0.03$	$\pm 0.06$	$\pm 0.04$	$\pm 0.09$				
<i>Purpureocillium lilacinum</i>	2,1E-02	1,7E-02	6,3E-02	2,2E-02	n.	n.	n.s.	-	0.11	0.08	0.15	0.07	n.	n.	n.s.	-
<i>Purpureocillium lilacinum</i>	$\pm 1,1E-02$	$\pm 1,0E-02$	$\pm 2,7E-02$	$\pm 1,6E-02$	s.	s.			$\pm 0.05$	$\pm 0.05$	$\pm 0.05$	$\pm 0.05$	s.	s.		
<i>Hirsutiella rhossiliensis</i>	3,2E-02	1,0E-02	1,1E-03	1,1E-02	n.	n.	n.s.	***	0.07	0.03	0.02	0.07	n.	n.	n.s.	*
<i>Purpureocillium lilacinum</i>	$\pm 2,4E-02$	$\pm 1,0E-02$	$\pm 1,1E-03$	$\pm 1,0E-02$	s.	s.			$\pm 0.04$	$\pm 0.03$	$\pm 0.02$	$\pm 0.05$	s.	s.		
<i>Purpureocillium lilacinum</i>	2,3E-02	1,8E-02	9,7E-02	6,5E-02	n.	n.	n.s.	-	0.20	0.14	0.23	0.14	n.	n.	n.s.	-
<i>Purpureocillium lilacinum</i>	$\pm 8,3E-03$	$\pm 1,7E-02$	$\pm 3,5E-02$	$\pm 4,1E-02$	s.	s.			$\pm 0.06$	$\pm 0.06$	$\pm 0.06$	$\pm 0.07$	s.	s.		
<i>Purpureocillium lilacinum</i>	1,7E-02	6,5E-02	3,1E-02	1,0E-01	n.	***	***	-	0.70	0.67	0.79	0.71	n.	n.	n.s.	-
<i>Purpureocillium lilacinum</i>	$\pm 4,3E-03$	$\pm 2,3E-02$	$\pm 6,7E-03$	$\pm 4,0E-02$	s.				$\pm 0.07$	$\pm 0.08$	$\pm 0.06$	$\pm 0.09$	s.	s.		

in this study, has been only identified by Blanco-Pérez et al. (2020) in an experimental DOCa Rioja vineyard but in lower numbers. However, the high abundance recorded through sucrose-gradient centrifugation contrasted with the poor presence of *S. riojaense* obtained in insect baits and only from organic vineyards. Although the biology and ecology of this newly described species are still mostly unknown (Pūža et al., 2020), this discrepancy might be due to the possible underperformance of *S. riojaense* for the host chosen in our bio-test (*G. mellonella* larvae). Indeed, Adams and Nguyen (2002) illustrated similar issues for EPNs of a narrow host range as *S. scarabaei* or *S. scapterisci*. Considering the rest of the identified EPN species, we only reported significant differences for higher abundance in organic viticulture of *S. feltiae*, the prevalent steinernematid species in Europe (Hominick, 2002; Bhat et al., 2020), and surveys completed in the Iberian Peninsula in the last decades (García del Pino and Palomo, 1996; Campos-Herrera et al., 2007, 2019a, 2019b; Valadas et al., 2014; Blanco-Pérez et al., 2020). Although these findings over mainland Spain and Portugal reported evidence of *S. feltiae* in a wide variety of habitats, studies performed in La Rioja found higher activity rates and abundance for this particular EPN species in less disturbed ecosystems like natural habitats, organic crops, and no-tilled vineyards (Campos-Herrera et al., 2007, 2010; Blanco-Pérez et al., 2020). The common practice of organic fertilizer applications in organic farming often enhances soil organic matter content (Leifeld and Fuhrer,

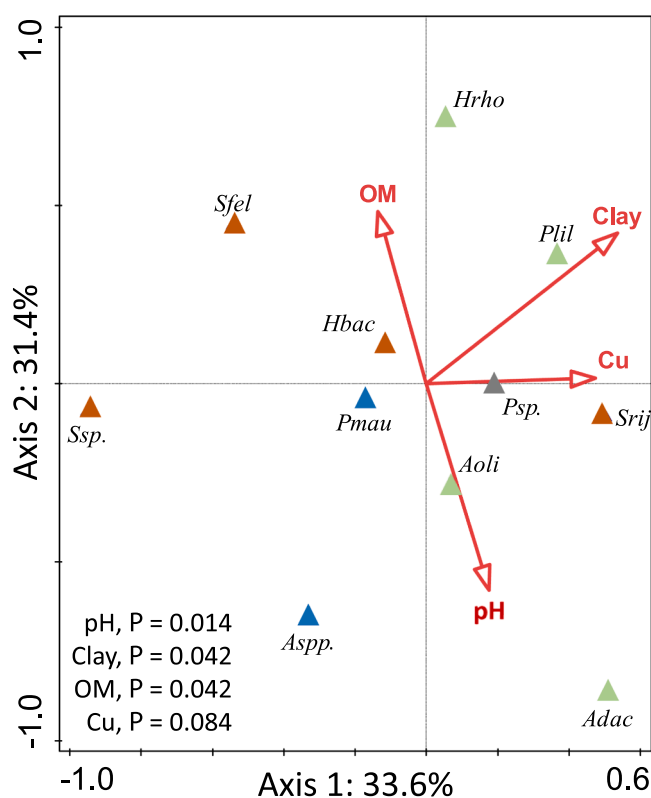
2010). Even if this assertion was not fully satisfied in our study, we established a correlation between organic matter and *S. feltiae* abundance in the overall soil community assemblage analysis (CCA). Linford's hypothesis proposed that organic matter inputs to soil generate rapid FLN population growth followed by their natural enemies (Linford, 1937; Cooke, 1962). Possibly, the higher soil organic matter contents that characterize organic vineyards favor the development of sustainable hosts for nematodes as *S. feltiae* to allow their long-term persistence. Other EPN species (*H. bacteriophora*, *Steinernema sp. affine-group*, *S. carpocapsae*, and *S. kraussei*) occurred at lower numbers, with no significant differences between the evaluated pest management practices. It is important to note that these three steinernematid species occurred only in organic viticulture, perhaps indicating the importance of this type of management to support the biodiversity within EPN communities.

The total abundance of our target FLN species was significantly higher in organic viticulture than IPM, particularly for bacterivores species in the genus *Oscheius* and *Acrobeloides* and, in agreement with Linford's hypothesis, enhanced by high soil organic matter contents. Bacterial feeder nematodes are good predictors of soil health in terms of soil structure, pH, and organic matter content (van den Hoogen et al., 2019). However, the few existing reports approaching the effects of organic farming on the nematode community suggested no clear





**Fig. 5.** Effect of different management practices in DOCa Rioja vineyards on soil activities measured as percentages of *Galleria mellonella* larvae (A) that showed nematode emergences and (B) positive for the Koch's postulates. Asterisks indicate significant differences from generalized linear mixed model tests at  $^{**}P < 0.01$  and  $^{*}P < 0.05$  (n.s., not significant). Values are least-square means  $\pm$  SE (see Table S8 for complete statistics). Averages of the nematodes identified are represented in pies: the entomopathogenic nematode species *Heterorhabditis bacteriophora* (Hbac), *Steinernema* sp. affine-group (Ssp.), *S. carpocapsae* (Scar), *S. feltiae* (Sfel), *S. kraussei* (Skra), and *S. riojaense* (Srij); and the free-living nematode species *Oscheius tipulae* (Otip), *O. onirici* (Ooni), *Pristionchus maupasi* (Pmau), and *Acrobeloides*-group (Aspp.).



**Fig. 6.** Canonical correspondence analysis among significant ( $P < 0.1$ ) abiotic factors (arrows) and soil organisms (triangles) isolated through sucrose gradient centrifugation from DOCa Rioja vineyards. Codes: entomopathogenic nematode species (orange triangles) *Heterorhabditis bacteriophora* (Hbac), *Steinernema* sp. affine-group (Ssp.), *S. feltiae* (Sfel), and *S. riojaense* (Srij); free-living nematode species (blue triangles) *Pristionchus maupasi* (Pmau) and *Acrobeloides*-group (Aspp.); nematophagous fungi species (green triangles) *Arthrobotrys oligospora* (Aoli), *A. dactyloides* (Adac), *Purpureocillium lilacinum* (Plil), and *Hirsutiella rhossiliensis* (Hrhos), and the ectoparasitic bacterium (grey triangle) *Paenibacillus* sp. (Psp.); OM, soil organic matter content. The P values were adjusted with the Bonferroni correction.

benefits. For example, the study of soil fertility and soil food web structure in semiarid vineyards and olive orchards in South-Central Spain did not distinguish between conventional and organic management but between crop systems (Sánchez-Moreno et al., 2018). On the other hand, other studies reported higher nematode abundance in organic viticulture compared to conventional practices but only concerning specific trophic groups: fungal-feeding nematodes in Southern French vineyards (Coll et al., 2012), and omnivore-predator species occurring in inter-rows in Northern Israel (Schlüter et al., 2022). Anyhow, our study did not intend to characterize the free-living community but fit its co-occurrence within the EPN soil food web. Various studies have observed declines in the fitness of some EPN species in the presence of FLNs in the genus *Pellioditis* (Duncan et al., 2003), *Acrobeloides* (Campos-Herrera et al., 2012), and *Oscheius* (Blanco-Pérez et al., 2019). As highlighted by Blanco-Pérez et al. (2020), the relative abundance of diverse soil organisms associated with the EPN soil food web could partially explain the numbers we reported for EPN species. Our results showed that organic viticulture did not favor the proliferation of the NF species evaluated but the EcPB *Paenibacillus* sp. This bacterium, initially linked with *Steinernema diaprepesi* (Nguyen et al., 2007), has been detected in the Iberian Peninsula, putatively associated with other steinernematids close related to *S. diaprepesi* in the Clade V (Campos-Herrera et al., 2019a; Blanco-Pérez et al., 2020), especially *S. riojaense* (Púza et al., 2020). Whether a possible association *S. riojaense*-*Paenibacillus* sp. limit the nematode movement is a matter of speculation that deserves further attention. In any case, it could explain, partially at least, the high abundance in the soil but the low activity of *S. riojaense* in organic vineyards. Overall, organic farming could enhance the occurrence and biodiversity in vine rows of certain soil mesofauna groups, nematofauna included, so probably EPNs have to deal with more potential competitors.

#### 4.2. Impact of soil management

Although several studies revealed that inter-row tillage favors soil biota degradation in vineyards (Karimi et al., 2020), its impact on the nematofauna is still mostly unknown. Shapiro-Ilan et al. (1999) observed significantly higher persistence for the EPN *S. carpocapsae* in corn-soybean rotation ecosystems treated with crop residues based on soybean stubble than in bare soils. In the same direction, Susurluk and

Ehlers (2008) noted that no-tilled managements of different crops increase the persistence of *H. bacteriophora*, probably by maintaining host occurrence, improving soil health conditions, and reducing temperature fluctuations. Moreover, Blanco-Pérez et al. (2020) remarked a correlation between EPN abundance and activity and physical disturbance of inter-rows, recording higher EPN values for cover cropping than tilling, especially in the absence of mechanization in spontaneous cover crops. In disagreement with these results and our hypothesis, we observed that neither abundance nor activity of EPNs significantly varied among vineyards that differed in the inter-row soil management. Our observations also contrast with the results of several studies based on baiting methods and conducted in other crop systems, although with reservations. For example, no-tillage or strip-till significantly increased the infection of *G. mellonella* larvae by EPNs compared with traditional tillage in North Carolina, while other factors, such as soil properties, pesticide inputs, or type and quality of cover cropping, could similarly affect EPNs or the availability of target hosts (Brust, 1991; Hummel et al., 2002). Besides, Millar and Barbercheck (2002) suggested opposite sensitivities for different EPN species to the conditions created in tilled soils. Even if conventional tillage in viticulture rarely is intensive and does not directly affect rows (the sampling area in this study), we found several differences in the abiotic factors between till and no-till treatments that could modulate the occurrence of EPNs in a species-specific manner. For instance, metal ions like Zn and Cu, recorded at higher rates in our no-tilled vineyards, can exhibit very high toxicity on steinernematids but much less on heterorhabditids (Jaworska et al., 1996, 1997). This presumption seems to agree with our observations for *Steinernema* sp. *affine*-group and *S. feltiae*, both negatively affected by high Cu contents, while an opposite trend was noted for *S. riojaense*, closely associated with Cu in our CCA analysis. This example gives us an idea of the difficulty of determining the relative importance of different abiotic factors when evaluating their impact on the EPN community in complex soil environments.

In addition to soil properties, biotic factors can also affect the survival and reproduction of EPNs (Stuart et al., 2015). We found no differences between tilled and no-tilled vineyards for the total quantification of any other group of the soil organisms evaluated but for specific species. Alternative strategies to tillage and herbicide applications like cover cropping may enhance the nematode community by affecting trophic links associated with their soil food web (Sánchez-Moreno et al., 2015). However, in our study, no-tilled vineyards only appeared to favor the occurrence of the FLN *O. tipulae* and the NF *A. dactyloides* and *P. lilacinum*, while the nematode species *P. maupasi* and *O. onirici* were more abundant in tilled soils. The complex interactions that these soil organisms could establish with EPNs are not easy to interpret. For instance, the versatility of roles attributed to nematodes in the genus *Pristionchus* in natural conditions can serve as an illustration. The wide-spread hermaphroditic species *P. maupasi* is commonly present in rotting vegetal substrates (Félix et al., 2018), closely associated with scarabaeoid beetles in the genus *Melolontha* (Herrmann et al., 2006). The type of relationship with its hosts is controversial. According to Félix et al. (2018), *P. maupasi* displays a phoretic association with cockchafer to disperse the dauer juveniles (the stress-resistant, alternative third juvenile stage) and colonize new food sources. Certainly, Hong et al. (2008) concluded that *P. maupasi* is attracted to the most dispersive forms of cockchafer. But Herrmann et al. (2006) considered that the nematodes in the genus *Pristionchus* follow a necromeny behavior, an association much more specific with their hosts that is suggested, in evolutionary terms, as an intermediate step preceding true parasitism (Dillman et al., 2012). Although there is no evidence for considering *P. maupasi* a parasite of insects or a competitor of EPNs for hosts (Herrmann et al., 2006; Blanco-Pérez et al., 2019), the living and feeding habits of soil nematodes under natural conditions is still mostly unknown. Indeed, the specialized buccal cavity of *Pristionchus* and other diplogastrid nematodes allow them to feed on bacteria, fungi, and other nematodes (von Lieven, 2003), so they could

also behave as predators if conditions require it. On the other hand, *in vitro* experiments showed that *O. onirici* could compromise *S. feltiae* virulence when acting as scavengers, a plausible strategy for EPNs to survive (Blanco-Pérez et al., 2019).

As mentioned, some FLN species can compete with EPNs for resources, but the nature of their interaction is still uncertain (Blanco-Pérez et al., 2017). We expected to notice a more straightforward relationship between NF and EPNs. Based on their observations, Blanco-Pérez et al. (2020) suggested that endoparasitic NF (*H. rhossiliensis* and *Catenaria* sp.) could be more efficient in killing EPNs than trapping NF (*Arthrobotrys* spp.). Since we obtained higher quantifications for *A. dactyloides* in no-tilled vineyards and no differences for *H. rhossiliensis* and *Catenaria* sp. between soil management treatments, our results did not ratify this premise. On the opposite, as also highlighted by Blanco-Pérez et al. (2020), the higher numbers reported for the eggs-trapping fungal species *P. lilacinum* on no-tilled vineyards could be due to an underestimation of the FLN abundance.

## 5. Conclusions

Our study supported the hypothesis that organic viticulture can favor the EPN activity and the abundance, at least, of the EPN *S. feltiae*, the predominant steinernematid species in Europe (Hominick, 2002; Bhat et al., 2020). In addition, we also observed evidence of higher quantities of FLNs and identified more nematode species (EPNs and FLNs) in organic agriculture. Nonetheless, we found no differences for the total EPN abundance between organic and integrated pest management, perhaps due to complex interactions among soil members assemblage to their soil food web. Conjointly, these results could indicate a higher resilience against pests and the healthiness of organic vineyard soils. Contrary, alternative strategies to tillage affected only a few of the evaluated soil species, without general distinctions on the EPN community or associated soil organisms. The vineyards selected for this study comprised, actually, multi-faceted management practices that could disturb soil biotic and abiotic characteristics differently. Since the nematode community could react to these singular disturbances even in a species-specific manner, our objective of establishing the causal effects of differentiated viticulture practices on the activity and abundance of native EPN species is considerably complex (Fiscus and Neher, 2002; Stuart et al., 2015). Proper viticulture practices will maximize profits and reduce potential problems (Provost and Pedneault, 2016), for instance, promoting optimal conditions for the occurrence of natural enemies of possible pests and pathogens in soil vineyards. Here we provide evidence on the support of organic viticulture to the activity of beneficial soil organisms, the EPNs. Future implementation of advanced molecular tools based on Next-Generation Sequencing (NGS) analysis (Geisen et al., 2018; Dritsoula et al., 2020) will allow expanding the number of soil species investigated and hence, will contribute to a better understanding of the assemblages of EPNs and other soil organisms in natural conditions, and how abiotic factors affect them in the agroecosystems. These baseline data will provide objective assessments to add value to organic management strategies in sustainable viticulture.

## 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.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2022.107931](https://doi.org/10.1016/j.agee.2022.107931).

## References

- Adams, B.J., Nguyen, K.B., 2002. Taxonomy and systematic. In: Gaugler, R. (Ed.), *Entomopathogenic Nematology*. CABI Publishing, Wallingford, UK, pp. 1–33. <https://doi.org/10.1079/9780851995670.0001>.
- Aschmann-Witzel, J., Zielke, S., 2017. Can't buy me green? A review of consumer perceptions of and behavior toward the price of organic food. *J. Consum. Aff.* 51, 211–251. <https://doi.org/10.1111/joca.12092>.
- Atkins, S.D., Clark, I.M., Pande, S., Hirsch, P.R., Kerry, B.R., 2005. The use of real-time PCR and species-specific primers for the identification and monitoring of *Paecilomyces lilacinus*. *Microb. Ecol.* 51, 257–264. <https://doi.org/10.1016/j.femsec.2004.09.002>.
- Barzman, M., Bärberi, P., Birch, A.N.E., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Hommel, B., Jensen, J.E., Kiss, J., Kudsk, P., Lamichhane, J.R., Messéan, A., Moonen, A.C., Ratnadass, A., Ricci, P., Sarah, J.L., Sattin, M., 2015. Eight principles of integrated pest management. *Agron. Sustain. Dev.* 35, 1199–1215. <https://doi.org/10.1007/s13593-015-0327-9>.
- Bedding, R.A., Akhurst, R.J., 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* 21, 109–110. <https://doi.org/10.1163/187529275x00419>.
- Bhat, A.H., Chaubey, A.K., Askary, T.H., 2020. Global distribution of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*. *Egypt. J. Biol.* <https://doi.org/10.1186/s41938-020-0212-y>.
- Blanco-Pérez, R., Bueno-Pallero, F.Á., Neto, L., Campos-Herrera, R., 2017. Reproductive efficiency of entomopathogenic nematodes as scavengers. Are they able to fight for insect's cadavers? *J. Invertebr. Pathol.* 148, 1–9. <https://doi.org/10.1016/j.jip.2017.05.003>.
- Blanco-Pérez, R., Bueno-Pallero, F.Á., Vicente-Díez, I., Marco-Mancebón, V.S., Pérez-Moreno, L., Campos-Herrera, R., 2019. Scavenging behavior and interspecific competition decrease offspring fitness of the entomopathogenic nematode *Steinernema feltiae*. *J. Invertebr. Pathol.* 164, 5–15. <https://doi.org/10.1016/j.jip.2019.04.002>.
- Blanco-Pérez, R., Sáenz-Romo, M.G., Vicente-Díez, I., Ibáñez-Pascual, S., Martínez-Villar, E., Marco-Mancebón, V.S., Pérez-Moreno, L., Campos-Herrera, R., 2020. Impact of vineyard ground cover management on the occurrence and activity of entomopathogenic nematodes and associated soil organisms. *Agric. Ecosyst. Environ.* 301, 107028. <https://doi.org/10.1016/j.agee.2020.107028>.
- Bode, H.B., 2009. Entomopathogenic bacteria as a source of secondary metabolites. *Curr. Opin. Chem. Biol.* 13, 224–230. <https://doi.org/10.1016/j.cbpa.2009.02.037>.
- Boemare, N., 2002. Biology, taxonomy and systematics of *Photorhabdus* and *Xenorhabdus*. In: Gaugler, R. (Ed.), *Entomopathogenic Nematology*. CABI Publishing, Wallingford, UK, pp. 35–56. <https://doi.org/10.1079/9780851995670.0035>.
- Bouyoucos, G.J., 1936. Directions for making mechanical analyses of soils by the hydrometer method. *Soil Sci.* 42, 225–230. <https://doi.org/10.1097/00010694-193609000-00007>.
- Brust, G.E., 1991. Augmentation of an endemic entomogenous nematode by agroecosystem manipulation for the control of a soil pest. *Agric. Ecosyst. Environ.* 36, 175–184. [https://doi.org/10.1016/0167-8809\(91\)90014-0](https://doi.org/10.1016/0167-8809(91)90014-0).
- Bueno-Pallero, F.Á., Blanco-Pérez, R., Dionísio, L., Campos-Herrera, R., 2018. Simultaneous exposure of nematophagous fungi, entomopathogenic nematodes and entomopathogenic fungi can modulate belowground insect pest control. *J. Invertebr. Pathol.* 154, 85–94. <https://doi.org/10.1016/j.jip.2018.04.004>.
- Campos-Herrera, R., El-Borai, F.E., Duncan, L.W., 2012. Wide interguild relationships among entomopathogenic and free-living nematodes in soil as measured by real time qPCR. *J. Invertebr. Pathol.* 111, 126–135. <https://doi.org/10.1016/j.jip.2012.07.006>.
- Campos-Herrera, R., Piedra-Buena, A., Escuer, M., Montalbán, B., Gutiérrez, C., 2010. Effect of seasonality and agricultural practices on occurrence of entomopathogenic nematodes and soil characteristics in La Rioja (Northern Spain). *Pedobiologia* 53, 253–258. <https://doi.org/10.1016/j.pedobi.2009.11.004>.
- Campos-Herrera, R., El-Borai, F.E., Stuart, R.J., Graham, J.H., Duncan, L.W., 2011a. Entomopathogenic nematodes, phoretic *Paenibacillus* spp., and the use of real time quantitative PCR to explore soil food webs in Florida citrus groves. *J. Invertebr. Pathol.* 108, 30–39. <https://doi.org/10.1016/j.jip.2011.06.005>.
- Campos-Herrera, R., Johnson, E.G., Stuart, R.J., Graham, J.H., Duncan, L.W., 2011b. Long-term stability of entomopathogenic nematode spatial patterns in soil as measured by sentinel insects and real-time PCR assays. *Ann. Appl. Biol.* 158, 55–68. <https://doi.org/10.1111/j.1744-7348.2010.00433.x>.
- Campos-Herrera, R., El-Borai, F.E., Ebert, T.E., Schumann, A., Duncan, L.W., 2014. Management to control citrus greening alters the soil food web and severity of a pest-disease complex. *Biol. Control* 76, 41–51. <https://doi.org/10.1016/j.biocontrol.2014.04.012>.
- Campos-Herrera, R., Stuart, R.J., Pathak, E., El-Borai, F.E., Duncan, L.W., 2019b. Temporal patterns of entomopathogenic nematodes in Florida citrus orchards: evidence of natural regulation by microorganisms and nematode competitors. *Soil Biol. Biochem.* 128, 193–204. <https://doi.org/10.1016/j.soilbio.2018.10.012>.
- Campos-Herrera, R., Escuer, M., Labrador, S., Robertson, L., Barrios, L., Gutiérrez, C., 2007. Distribution of the entomopathogenic nematodes from La Rioja (Northern Spain). *J. Invertebr. Pathol.* 95, 125–139. <https://doi.org/10.1016/j.jip.2007.02.003>.
- Campos-Herrera, R., Gómez-Ros, J.M., Escuer, M., Cuadra, L., Barrios, L., Gutiérrez, C., 2008. Diversity, occurrence, and life characteristics of natural entomopathogenic nematode populations from La Rioja (Northern Spain) under different agricultural management and their relationships with soil factors. *Soil Biol. Biochem.* 40, 1474–1484. <https://doi.org/10.1016/j.soilbio.2008.01.002>.
- Campos-Herrera, R., Púza, V., Jaffuel, G., Blanco-Pérez, R., Čepulyte-Rakauskiene, R., Turlings, T.C.J., 2015a. Unraveling the intraguild competition between *Oscheius* spp. nematodes and entomopathogenic nematodes: Implications for their natural distribution in Swiss agricultural soils. *J. Invertebr. Pathol.* 132, 216–227. <https://doi.org/10.1016/j.jip.2015.10.007>.
- Campos-Herrera, R., Rodríguez Martín, J.A., Escuer, M., García-González, M.T., Duncan, L.W., Gutiérrez, C., 2016. Entomopathogenic nematode food webs in an ancient, mining pollution gradient in Spain. *Sci. Total Environ.* 572, 312–323. <https://doi.org/10.1016/j.scitotenv.2016.07.187>.
- Campos-Herrera, R., Blanco-Pérez, R., Bueno-Pallero, F.Á., Duarte, A., Nolasco, G., Sommer, R.J., Rodríguez Martín, J.A., 2019a. Vegetation drives assemblages of entomopathogenic nematodes and other soil organisms: evidence from the Algarve, Portugal. *Soil Biol. Biochem.* 128, 150–163. <https://doi.org/10.1016/j.soilbio.2018.10.019>.
- Campos-Herrera, R., Jaffuel, G., Chiriboga, X., Blanco-Pérez, R., Fesselet, M., Púza, V., Mascher, F., Turlings, T.C.J., 2015b. Traditional and molecular detection methods reveal intense interguild competition and other multitrophic interactions associated with native entomopathogenic nematodes in Swiss tillage soils. *Plant Soil* 389, 237–255. <https://doi.org/10.1007/s11104-014-2358-4>.
- Campos-Herrera, R., Vicente-Díez, I., Blanco-Pérez, R., Chelkha, M., González-Trujillo, M., del M., Puelles, M., Čepulyte-Rakauskiene, R., Pou, A., 2021. Positioning entomopathogenic nematodes for the future viticulture: exploring their use against biotic threats and as bioindicators of soil health. *Turk. J. Zool.* 45, 335–346. <https://doi.org/10.3906/zoo-2106-40>.
- Coll, P., Le Cadre, E., Villenave, C., 2012. How are nematode communities affected during a conversion from conventional to organic farming in southern French vineyards? *Nematology* 16, 665–676.
- Cooke, R.C., 1962. Ecology of nematode-trapping fungi in soil. *Ann. Appl. Biol.* 50, 507–513. <https://doi.org/10.1111/j.1744-7348.1962.tb06045.x>.
- CR Rioja wine [WWW Document], 2021. Cons. Regul. la Denominación Orig. Calif. La Rioja. URL (<https://www.riojawine.com/en/home-en/>).
- van den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D.A., de Goede, R.G.M., Adams, B.J., Ahmad, W., Andriuzzi, W.S., Bardgett, R.D., Bonkowski, M., Campos-Herrera, R., Cares, J.E., Caruso, T., de Brito Caixeta, L., Chen, X., Costa, S.R., Creamer, R., Mauro da Cunha Castro, J., Dam, M., Djigal, D., Escuer, M., Griffiths, B.S., Gutiérrez, C., Høhberg, K., Kalinkina, D., Kardol, P., Kerguntuil, A., Korthals, G., Krashevska, V., Kudrin, A.A., Li, Q., Liang, W., Magilton, M., Marais, M., Martín, J.A.R., Matveeva, E., Mayad, E.H., Mulder, C., Mullin, P., Neilson, R., Nguyen, T.A.D., Nielsen, U.N., Okada, H., Rius, J.E.P., Pan, K., Peneva, V., Pellissier, L., Carlos Pereira da Silva, J., Pitteloud, C., Powers, T. O., Powers, K., Quist, C.W., Rasmann, S., Moreno, S.S., Scheu, S., Setälä, H., Sushchuk, A., Tiunov, A.V., Trap, J., van der Putten, W., Vestergård, M., Villenave, C., Waeyenberge, L., Wall, D.H., Wilschut, R., Wright, D.G., Yang, J. in, Crowther, T.W., 2019. Soil nematode abundance and functional group composition at a global scale. *Nature* 572, 194–198. <https://doi.org/10.1038/s41586-019-1418-6>.
- Dillman, A.R., Chaston, J.M., Adams, B.J., Ciche, T.A., Goodrich-Blair, H., Stock, S.P., Sternberg, P.W., 2012. An entomopathogenic nematode by any other name. *PLoS Pathog.* 8, 8–11. <https://doi.org/10.1371/journal.ppat.1002527>.
- DRioja, 2019. Decreto 21/2019 de la CC La Rioja, de 31 de mayo, por el que se regula el ejercicio de funciones en materia certificación y control de la producción ecológica en La Rioja y se aprueba el Reglamento de Funcionamiento y Régimen Interno del Consejo de la Pro. BOR 68, 8507–8522.
- Dritsoula, A., Campos-Herrera, R., Blanco-Pérez, R., Duncan, L.W., 2020. Comparing high throughput sequencing and real time qPCR for characterizing entomopathogenic nematode biogeography. *Soil Biol. Biochem.* 145, 107793. <https://doi.org/10.1016/j.soilbio.2020.107793>.
- Duncan, L.W., Dunn, D.C., Bague, G., Nguyen, K., 2003. Competition between entomopathogenic and free-living bacterivorous nematodes in larvae of the weevil *Diaprepes abbreviatus*. *J. Nematol.* 35, 187–193.
- EC, 2009. Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. *Off. J. Eur. Union* 52, 1–50.
- Eisenhauer, N., Guerra, C.A., 2019. Global maps of soil-dwelling nematode worms. *Nature* 572, 187–188. <https://doi.org/10.1038/d41586-019-02197-0>.

- El-Borai, F.E., Duncan, L.W., Preston, J.F., 2005. Bionomics of a phoretic association between *Paenibacillus* sp. and the entomopathogenic nematode *Steinernema diaepresi*. *J. Nematol.* 37, 18–25.
- El-Borai, F.E., Bright, D.B., Graham, J.H., Stuart, R.J., Cubero, J., Duncan, L.W., 2009. Differential susceptibility of entomopathogenic nematodes to nematophagous fungi from Florida citrus orchards. *Nematology* 11, 231–241. <https://doi.org/10.1163/156854109x429565>.
- Ellers-Kirk, C.D., Fleischer, S.J., Snyder, R.H., Lynch, J.P., 2000. Potential of entomopathogenic nematodes for biological control of *Acalymma vitatum* (Coleoptera: Chrysomelidae) in cucumbers grown in conventional and organic soil management systems. *J. Econ. Entomol.* 93, 605–612. <https://doi.org/10.1603/0022-0493-93.3.605>.
- Enright, M.R., Griffin, C.T., 2005. *Paenibacillus nematophilus* on the entomopathogenic nematode *Heterorhabditis megidis*. *J. Invertebr. Pathol.* 88, 40–48. <https://doi.org/10.1016/j.jip.2004.10.002>.
- EU, 2018. Regulation (EU) 2018/848 of The European Parliament and of The Council of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007. *Off. J. Eur. Union*.
- F. García del Pino Natural occurrence of EPN in Spain COST 850 WGM on Natural Occurrence and Evolution of EPNs 2005 České Budejovice 14 17.
- FAO, 2020. State of Knowledge of Soil Biodiversity - Status, Challenges and Potentialities: Report 2020. Rome. <https://doi.org/10.4060/cb1928en>.
- Félix, M.A., Allion, M., Hsu, J.C., Richaud, A., Wang, J., 2018. *Pristionchus* nematodes occur frequently in diverse rotting vegetal substrates and are not exclusively necromenic, while *Panagrellus redivivoides* is found specifically in rotting fruits. *PLoS One* 13, 1–21. <https://doi.org/10.1371/journal.pone.0200851>.
- Fernández Alcázar, J.I., 2011. Costes de cultivo en viñedo. *Cuad. Campo* 46, 4–13.
- Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Appl. Soil Ecol.* 18, 13–29. [https://doi.org/10.1016/S0929-1393\(01\)00152-4](https://doi.org/10.1016/S0929-1393(01)00152-4).
- FiBL, 2019. The World of Organic Agriculture, The World of Organic Agriculture Statistic and emerging trends 2019. Research Institute of Organic Agriculture (FiBL) and IFOAM – Organics International, Frick and Bonn. <https://doi.org/10.4324/9781849775991>.
- Fiscus, D.A., Neher, D.A., 2002. Distinguishing sensitivity of free-living soil nematode genera to physical and chemical disturbances. *Ecol. Appl.* 12, 565–575. [https://doi.org/10.1890/1051-0761\(2002\)012\[0565:DSOFLS\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2002)012[0565:DSOFLS]2.0.CO;2).
- García del Pino, F., Palomo, A., 1996. Natural occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Spanish soils. *J. Invertebr. Pathol.* 68, 84–90. <https://doi.org/10.1006/jip.1996.0062>.
- Geisen, S., Snoek, L.B., ten Hooven, F.C., Duyts, H., Kostenko, O., Bloem, J., Martens, H., Quist, C.W., Helder, J.A., van der Putten, W.H., 2018. Integrating quantitative morphological and qualitative molecular methods to analyse soil nematode community responses to plant range expansion. *Methods Ecol. Evol.* 9, 1366–1378. <https://doi.org/10.1111/2041-210X.12999>.
- Gunstone, T., Cornelisse, T., Klein, K., Dubey, A., Donley, N., 2021. Pesticides and soil invertebrates: a hazard assessment. *Front. Environ. Sci.* 9. <https://doi.org/10.3389/fenvs.2021.643847>.
- Helmberger, M.S., Shields, E.J., Wickings, K.G., 2017. Ecology of belowground biological control: entomopathogenic nematode interactions with soil biota. *Appl. Soil Ecol.* 121, 201–213. <https://doi.org/10.1016/j.apsoil.2017.10.013>.
- Herrero-Hernández, E., Rodríguez-Cruz, M.S., Pose-Juan, E., Sánchez-González, S., Andrades, M.S., Sánchez-Martín, M.J., 2017. Seasonal distribution of herbicide and insecticide residues in the water resources of the vineyard region of La Rioja (Spain). *Sci. Total Environ.* 609, 161–171. <https://doi.org/10.1016/j.scitotenv.2017.07.113>.
- Herrmann, M., Mayer, W.E., Sommer, R.J., 2006. Nematodes of the genus *Pristionchus* are closely associated with scarab beetles and the Colorado potato beetle in Western Europe. *Zoology* 109, 96–108. <https://doi.org/10.1016/j.zool.2006.03.001>.
- Hominick, W.M., 2002. Biogeography. In: *Entomopathogenic Nematology*. CAB, Wallingford, UK. <https://doi.org/10.1079/9780851995670.0035>.
- Hong, R.L., Svatoš, A., Herrmann, M., Sommer, R.J., 2008. Species-specific recognition of beetle cues by the nematode *Pristionchus maupasi*. *Evol. Dev.* 10, 273–279. <https://doi.org/10.1111/j.1525-142X.2008.00236.x>.
- Hummel, R.L., Walgenbach, J.F., Barbercheck, M.E., Kennedy, G.G., Hoyt, G.D., Arellano, C., 2002. Effects of production practices on soil-borne entomopathogens in Western North Carolina vegetable systems. *Environ. Entomol.* 31, 84–91. <https://doi.org/10.1603/0046-225X-31.1.84>.
- Jaffuel, G., Mäder, P., Blanco-Pérez, R., Chiriboga, X., Fliessbach, A., Turlings, T.C.J., Campos-Herrera, R., 2016. Prevalence and activity of entomopathogenic nematodes and their antagonists in soils that are subject to different agricultural practices. *Agric. Ecosyst. Environ.* 230, 329–340. <https://doi.org/10.1016/j.agee.2016.06.009>.
- Jaffuel, G., Blanco-Pérez, R., Hug, A.S., Chiriboga, X., Meuli, R.G., Mascher, F., Turlings, T.C.J., Campos-Herrera, R., 2018. The evaluation of entomopathogenic nematode soil food web assemblages across Switzerland reveals major differences among agricultural, grassland and forest ecosystems. *Agric. Ecosyst. Environ.* 262, 48–57. <https://doi.org/10.1016/j.agee.2018.04.008>.
- Jaworska, M., Sepiol, J., Tomasiak, P., 1996. Effect of metal ions under laboratory conditions on the entomopathogenic *Steinernema carpocapsae* (Rhabditida: Steinernematidae). *Water Air Soil Pollut.* 88, 331–341. <https://doi.org/10.1007/BF00294109>.
- Jaworska, M., Gorczyca, A., Sepiol, J., Tomasiak, P., 1997. Effect of metal ions on the *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae) under laboratory conditions. *Water Air Soil Pollut.* 93, 157–166. <https://doi.org/10.1023/A:1022140110106>.
- Jenkins, W.R., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Dis. Rep.* 48, 692. <https://doi.org/10.1007/s10333>.
- Karimi, B., Cahurel, J.Y., Gontier, L., Charlier, L., Chovelon, M., Mahé, H., Ranjard, L., 2020. A meta-analysis of the ecotoxicological impact of viticultural practices on soil biodiversity. *Environ. Chem. Lett.* 18, 1947–1966. <https://doi.org/10.1007/s10311-020-01050-5>.
- Kibblewhite, M.G., Ritz, K., Swift, M.J., 2008. Soil health in agricultural systems. *Philos. Trans. R. Soc. B* 363, 685–701. <https://doi.org/10.1098/rstb.2007.2178>.
- Koppenhöfer, A.M., Jaffee, B.A., Muldoon, A.E., Strong, D.R., Kaya, H.K., 1996. Effect of nematode-trapping fungi on an entomopathogenic nematode originating from the same field site in California. *J. Invertebr. Pathol.* 68, 246–252. <https://doi.org/10.1006/jip.1996.0092>.
- Leifeld, J., Fuhrer, J., 2010. Organic farming and soil carbon sequestration: what do we really know about the benefits? *Ambio* 39, 585–599. <https://doi.org/10.1007/s13280-010-0082-8>.
- Lepš, J., Hádincová, V., 1992. How reliable are our vegetation analyses? *J. Veg. Sci.* 3, 119–124. <https://doi.org/10.2307/3236006>.
- Lewis, E.E., Hazir, S., Hodson, A., Gulcu, B., 2015. Trophic relationships of entomopathogenic nematodes in agricultural habitats. In: Campos-Herrera, R. (Ed.), *Nematode Pathogenesis of Insects and Other Pests: Ecology and Applied Technologies for Sustainable Plant and Crop Protection*. Springer International Publishing, AG Switzerland, pp. 139–163. [https://doi.org/10.1007/978-3-319-18266-7\\_5](https://doi.org/10.1007/978-3-319-18266-7_5).
- Linford, M.B., 1937. Stimulated activity of natural enemies of nematodes. *Science* 85, 123–124. <https://doi.org/10.1126/science.85.2196.123>.
- Marín, D., Armengol, J., Carbonell-Bejerano, D.S., Escalona, J.M., Gramaje, D., Hernández-Montes, E., Intrigliolo, D.S., Martínez-Zapater, J.M., Medrano, H., Mirás-Avalos, J.M., Palomares-Rius, J.E., Romero-Azorin, P., Savé, R., Santesteban, L.G., De Herralde, F., 2021. Challenges of viticulture adaptation to global change: tackling the issue from the roots. *Aust. J. Grape Wine Res.* 27, 8–25. <https://doi.org/10.1111/ajgw.12463>.
- Mehlich, A., 1978. New extractant for soil test evaluation of phosphorus, potassium, magnesium, calcium, sodium, manganese and Zinc. *Commun. Soil Sci. Plant Anal.* 9, 477–492. <https://doi.org/10.1080/00103627809366824>.
- Mehlich, A., 1984. Mehlich 3 soil test extractant: a modification of mehlich 2 extractant. *Commun. Soil Sci. Plant Anal.* 15, 1409–1416. <https://doi.org/10.1080/00103628409367568>.
- Millar, L.C., Barbercheck, M.E., 2002. Effects of tillage practices on entomopathogenic nematodes in a corn agroecosystem. *Biol. Control* 25, 1–11. [https://doi.org/10.1016/S1049-9644\(02\)00042-7](https://doi.org/10.1016/S1049-9644(02)00042-7).
- Millennia, O., Markewitz, D., 2004. Book reviews. *Soil Sci. Soc. Am.* 104, 681–682. <https://doi.org/10.1016/j.agee.2004.07.007>.
- Nguyen, K., Hunt, D., Mráček, Z., 2007. Steinernematidae: species descriptions. In: Nguyen, K., Hunt, D. (Eds.), *Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbionts*, Brill, Leiden (The Netherlands), pp. 121–609. <https://doi.org/10.1163/ej.97890004152939.i-816.29>.
- Nicholls, C.I., Altieri, M.A., Ponti, L., 2008. Enhancing plant diversity for improved insect pest management in Northern California organic vineyards. *Acta Hort.* 785, 263–278. <https://doi.org/10.17660/ActaHortic.2008.785.32>.
- Nordbring-Hertz, B., Jansson, H.-B., Tunlid, A., 2006. Nematophagous Fungi. In: *ELN*. John Wiley & Sons, Ltd. <https://doi.org/10.1038/npg.els.0004293>.
- OIV, 2020. State of the world vitivinicultural sector in 2020, International Organisation of Vine and Wine. Paris, France.
- Orgiazzi, A., Bardgett, R.D., Barrios, E., Behan-Pelletier, V., Briones, M.J.I., Chotte, J.-L., De Deyn, G.B., Eggleton, P., Fierer, N., Fraser, T., Hedlund, K., Jeffery, S., Johnson, N.C., Jones, A., Kandeler, E., Kaneko, N., Lavelle, P., Lemanceau, P., Miko, L., Montanarella, L., Moreira, F.M.S., Ramirez, K.S., Scheu, S., Singh, B.K., Six, J., van der Putten, W.H., Wall, D., 2016. Global soil biodiversity atlas. In: *European Commission (Ed.). Publication Office of the European Union, Luxembourg*, p. 176.
- Pathak, E., Campos-Herrera, R., El-Borai, F.E., Duncan, L.W., 2017. Spatial relationships between entomopathogenic nematodes and nematophagous fungi in Florida citrus orchards. *J. Invertebr. Pathol.* 144, 37–46. <https://doi.org/10.1016/j.jip.2017.01.005>.
- Pathak, E., El-Borai, F.E., Campos-Herrera, R., Johnson, E.G., Stuart, R.J., Graham, J.H., Duncan, L.W., 2012. Use of real-time PCR to discriminate parasitic and saprophagous behaviour by nematophagous fungi. *Fungal Biol.* 116, 563–573. <https://doi.org/10.1016/j.funbio.2012.02.005>.
- Pose-Juan, E., Sánchez-Martín, M.J., Andrades, M.S., Rodríguez-Cruz, M.S., Herrero-Hernández, E., 2015. Pesticide residues in vineyard soils from Spain: spatial and temporal distributions. *Sci. Total Environ.* 514, 351–358. <https://doi.org/10.1016/j.scitotenv.2015.01.076>.
- Provost, C., Pedneault, K., 2016. The organic vineyard as a balanced ecosystem: improved organic grape management and impacts on wine quality. *Sci. Hortic.* 208, 43–56. <https://doi.org/10.1016/j.scienta.2016.04.024>.
- Půža, V., Campos-Herrera, R., Blanco-Pérez, R., Jakubíková, H., Vicente-Díez, I., Nermut, J., 2020. *Steinernema riojaense* n. sp., a new entomopathogenic nematode (Nematoda: Steinernematidae) from Spain. *Nematology* 22, 825–841. <https://doi.org/10.1163/15685411-00003343>.
- RD, 2012. Real Decreto 1311/2012 del Gobierno de España, de 14 de septiembre, por el que se establece el marco de actuación para conseguir un uso sostenible de los productos fitosanitarios. *BOE* 223, 65127–65171.
- RD, 2014. Real Decreto 833/2014 del Gobierno de España, de 3 de octubre, por el que se establece y regula el Registro General de Operadores Ecológicos y se crea la Mesa de coordinación de la producción ecológica. *BOE* 252, 84104–84109.

- Rodrigo-Comino, J., Keesstra, S., Cerdà, A., 2018. Soil erosion as an environmental concern in vineyards: the case study of Celler del Roure, Eastern Spain, by means of rainfall simulation experiments. *Beverages* 4, 31. <https://doi.org/10.3390/beverages4020031>.
- Sáenz-Romo, M.G., Veas-Bernal, A., Martínez-García, H., Campos-Herrera, R., Ibáñez-Pascual, S., Martínez-Villar, E., Pérez-Moreno, I., Marco-Mancebón, V.S., 2019. Ground cover management in a Mediterranean vineyard: impact on insect abundance and diversity. *Agric. Ecosyst. Environ.* 283, 106571 <https://doi.org/10.1016/j.agee.2019.106571>.
- Sánchez-Moreno, S., Cano, M., López-Pérez, A., Rey Benayas, J.M., 2018. Microfaunal soil food webs in Mediterranean semi-arid agroecosystems. Does organic management improve soil health? *Appl. Soil Ecol.* 125, 138–147. <https://doi.org/10.1016/j.apsoil.2017.12.020>.
- Sánchez-Moreno, S., Castro, J., Alonso-Prados, E., Alonso-Prados, J.L., García-Baudín, J. M., Talavera, M., Durán-Zuazo, V.H., 2015. Tillage and herbicide decrease soil biodiversity in olive orchards. *Agron. Sustain. Dev.* 35, 691–700. <https://doi.org/10.1007/s13593-014-0266-x>.
- Santos, J.A., Fraga, H., Malheiro, A.C., Moutinho-Pereira, J., Dinis, L.T., Correia, C., Moriondo, M., Leolini, L., Dibari, C., Costafreda-Aumedes, S., Kartschall, T., Menz, C., Molitor, D., Junk, J., Beyer, M., Schultz, H.R., 2020. A review of the potential climate change impacts and adaptation options for European viticulture. *Appl. Sci.* 10, 1–28. <https://doi.org/10.3390/app10093092>.
- Schlüter, S., Gil, E., Doniger, T., Applebaum, I., Steinberger, Y., 2022. Abundance and community composition of free-living nematodes as a function of soil structure under different vineyard managements. *Appl. Soil Ecol.* 170, 104291 <https://doi.org/10.1016/j.apsoil.2021.104291>.
- Shapiro-Ilan, D.I., Obrycki, J.J., Lewis, L.C., Jackson, J.J., 1999. Effects of crop residue on the persistence of *Steinernema carpocapsae*. *J. Nematol.* 31, 517–519.
- Šmilauer, P., Lepš, J., 2014. *Multivariate Analysis of Ecological Data Using CANOCO 5*. Cambridge University Press, Cambridge, UK.
- Stock, S.P., 2015. Diversity, biology and evolutionary relationships. In: Campos-Herrera, R. (Ed.), *Nematode Pathogenesis of Insects and Other Pests: Ecology and Applied Technologies for Sustainable Plant and Crop Protection*. Springer International Publishing, AG Switzerland, pp. 3–27. [https://doi.org/10.1007/978-3-319-18266-7\\_1](https://doi.org/10.1007/978-3-319-18266-7_1).
- Stuart, R.J., Barbercheck, M.E., Grewal, P.S., 2015. Entomopathogenic nematodes in the soil environment: distributions, interactions and the influence of biotic and abiotic factors. In: Campos-Herrera, R. (Ed.), *Nematode Pathogenesis of Insects and Other Pests: Ecology and Applied Technologies for Sustainable Plant and Crop Protection*. Springer International Publishing, AG Switzerland, pp. 97–137. [https://doi.org/10.1007/978-3-319-18266-7\\_4](https://doi.org/10.1007/978-3-319-18266-7_4).
- Susurluk, A., Ehlers, R.U., 2008. Field persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora* in different crops. *BioControl* 53, 627–641. <https://doi.org/10.1007/s10526-007-9104-2>.
- ter Braak, C.J.F., Šmilauer, P., 2002. *CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5)*. (Microcomputer Power). www.canoco.com, Ithaca NY, USA.
- Valadas, V., Laranjo, M., Mota, M., Oliveira, S., 2014. A survey of entomopathogenic nematode species in continental Portugal. *J. Helminthol.* 88, 327–341. <https://doi.org/10.1017/S0022149x13000217>.
- Veresoglou, S.D., Halley, J.M., Rillig, M.C., 2015. Extinction risk of soil biota. *Nat. Commun.* 6, 8862. <https://doi.org/10.1038/ncomms9862>.
- von Lieven, A.F., 2003. Functional morphology and evolutionary origin of the three-part pharynx in nematodes. *Zoology* 106, 183–201. <https://doi.org/10.1078/0944-2006-00115>.
- Walkley, A., Black, I.A., 1934. An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* 37, 29–38. <https://doi.org/10.1097/00010694-193401000-00003>.
- Wiesel, L., Daniell, T.J., King, D., Neilson, R., 2015. Determination of the optimal soil sample size to accurately characterise nematode communities in soil. *Soil Biol. Biochem.* 80, 89–91. <https://doi.org/10.1016/j.soilbio.2014.09.026>.
- Winter, S., Bauer, T., Strauss, P., Kratschmer, S., Paredes, D., Popescu, D., Landa, B., Guzmán, G., Gómez, J.A., Guernion, M., Zaller, J.G., Batáry, P., 2018. Effects of vegetation management intensity on biodiversity and ecosystem services in vineyards: a meta-analysis. *J. Appl. Ecol.* 55, 2484–2495. <https://doi.org/10.1111/1365-2664.13124>.
- Woodring, J.L., Kaya, H.K., 1988. Steinernematid and heterorhabditid nematodes: A handbook of techniques, Southern Cooperative Series Bulletin. Arkansas Agricultural Experiment Station.
- Zhang, L.M., Liu, X.Z., Zhu, S.F., Chen, S.Y., 2006. Detection of the nematophagous fungus *Hirsutella rossiliensis* in soil by real-time PCR and parasitism bioassay. *Biol. Control* 36, 316–332. <https://doi.org/10.1016/j.biocontrol.2005.08.002>.