Agriculture, Ecosystems and Environment 301 (2020) 107028

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Impact of vineyard ground cover management on the occurrence and activity of entomopathogenic nematodes and associated soil organisms

Rubén Blanco-Pérez[a], María Gloria Sáenz-Romo[b], Ignacio Vicente-Díez[a], Sergio Ibáñez-Pascual[a], Elena Martínez-Villar[b], Vicente Santiago Marco-Mancebón[b], Ignacio Pérez-Moreno[b], Raquel Campos-Herrera[a,*]

[a] Departamento de Viticultura, Instituto de Ciencias de la Vid y del Vino (CSIC, Universidad de La Rioja, Gobierno de La Rioja). Finca La Grajera, Ctra. de Burgos Km. 6 (LO-20 - salida 13), 26007 Logroño, Spain

[b] Departamento de Agricultura y Alimentación, Universidad de La Rioja, C/ Madre de Dios 51, 26006 Logroño, Spain

ARTICLE INFO

Keywords:
Agroecology
Beneficial soil organisms
Cover crop
Free-living nematodes
Nematophagous fungi
Sustainable viticulture
Tillage

ABSTRACT

Viticulture is a valuable sector worldwide with an extraordinary socio-economic impact in Spain. Numerous pests and diseases threaten vineyards, and their management primarily relies on the use of conventional agrochemicals. The current paradigm of sustainability pursues the implementation of ecologically sound strategies in vineyard ecosystems. The use of cover crops is arising as an alternative with numerous benefits, including favoring above-belowground biodiversity and the presence of beneficial soil organisms such as the entomopathogenic nematodes (EPNs). We hypothesized that the use of specific cover crops in vineyards might enhance the natural occurrence and activity of EPNs by modulating the assemblage with associated organisms. We performed the experiments in an ongoing experimental vineyard (*Vitis vinifera* var Tempranillo, clon RJ-26, rootstock '110-Richter') located in Logroño (Spain), drove with different soil management systems (three replicates each): conventional tillage practice and the cover crops (i) seeded with *Bromus catharticus* (Poaceae), (ii) flower-driven, and (iii) spontaneous. We took four soil composite samples per plot (n = 48 per sampling time) late spring and early autumn in two consecutive years (2017 and 2018). By using species-specific primers/probes qPCR sets, we screened for the presence and abundance of eight EPNs species and 12 related soil organisms: six nematophagous fungi, four free-living nematodes, and two ectoparasitic bacteria. Additionally, we assessed the EPN activity by the traditional insect-bait method. Overall, we recorded higher EPN numbers or activity rates on cover crops than on bare soils. However, some of the results were divergent among no-till treatments. We observed not only higher EPN abundance and activity on spontaneous covers but lower numbers of antagonistic organisms, particularly endoparasitic nematophagous fungi. Thus, according to our results, the use of spontaneous covers could be the most promising strategy to support the conservation biological control service provided by the naturally occurring EPN species in vineyards, plus with a low cost for the sector.

1. Introduction

The wine sector represents a relevant socioeconomic asset worldwide. Spain is among the top five grape-producing countries (FAOSTAT, 2018), has the largest area of vineyards in the world (almost one million hectares), and with France and Italy, is the world leader for wine exports (OIV, 2018). Since conventional viticulture production practices, such as the widespread use of agrochemicals and soil tillage, make the vineyard one of the most intensive management systems (Nicholls et al., 2008), defining sustainable strategies is critical for the sector within the current context of environmental protection. Indeed, areas devoted to organic grape production significantly increased during the last two decades, particularly in Spain, Italy (each with more than 100,000 ha) and France (with over 78,000 ha), the countries that cover the largest organic grape areas (FiBL, 2019; Provost and Pedneault, 2016).

Sustainable viticulture considers the vineyard as an ecosystem where every resource is optimized to maintain rich biodiversity and decrease pest and disease pressures (Thies and Tscharntke, 1999). Consequently, viticulture must face the current challenge of reducing agrochemicals by providing alternative management strategies that respect the environment. When sources like water are not limited, the

* Corresponding author.
E-mail address: raquel.campos@icvv.es (R. Campos-Herrera).

https://doi.org/10.1016/j.agee.2020.107028
Received 21 March 2020; Received in revised form 15 May 2020; Accepted 21 May 2020

use of cover crops in vineyards is arising as an alternative with numerous benefits, including reducing soil erosion, providing additional organic matter, improving soil structure, and favoring above-below-ground biodiversity (Coll et al., 2011; McGourty and Reganold, 2005; Sáenz-Romo et al., 2019a; Shapira et al., 2017; Wheaton et al., 2008). Continuous no-tillage practices stratify the soil, concentrating organic matter, nutrients, and microbial activity near the soil surface (Doran, 1980). Thus, crop residue decomposition occurs mostly through the activity of a diverse soil biota community in which complex interactions are established (House and Stinner, 1983). Conversely, organic matter, nutrients, and biodiversity decrease in conventionally tilled soils because decomposition and mineralization process occurs faster and over a deeper area of the soil, involving fewer types of organisms, primarily microbiota (House and Stinner, 1983).

Favored by high biodiversity rates, conservation biological control plays a key role in integrated and organic pest management programs (Altieri, 1999; Altieri and Nicholls, 2004). Among the soil organisms responsible for ecological goods and services, the entomopathogenic nematodes (EPNs) are well recognized as biological control agents and excellent models in ecological studies (Campos-Herrera et al., 2012; Lewis et al., 2015; Stuart et al., 2015). Nematodes in the families Steinernematidae and Heterorhabditidae show pathogenicity against arthropods in symbiosis with enteric γ-Proteobacteria species in the genera *Xenorhabdus* and *Photorhabdus*, respectively (Boemare, 2002). EPNs occur in soils of natural and agricultural areas worldwide in their non-feeding stage, called infective juvenile (IJ), capable of locating suitable hosts (Stock, 2015). Once inside, the symbiotic nematode-bacterium complex overcomes the host's immune response resulting in death by septicemia within 48–72 h of infection (Dillman et al., 2012). But first, IJs must cope with soil and environmental conditions (i.e. limited water content, extreme temperatures, etc.), overcome competitors for hosts, and avoid predators and other soil organisms that could compromise their reproductive success (Lewis et al., 2015; Stuart et al., 2015). Regarding specific biotic interactions, several surveys have revealed, through traditional and molecular methods, the co-emergence of IJs and free-living nematodes (FLNs) naturally occurring in soils (Liang et al., 2005) from insect larvae colonized by EPNs (Campos-Herrera et al., 2012, 2015, 2019; Duncan et al., 2003; Jaffuel et al., 2016, 2018). Aside from competitive interactions that could occur between these two groups of nematodes, recent *in vitro* experiments suggest that IJ pathogenicity could decrease when competing with FLNs for nutrient sources inside the insect cadaver (Blanco-Pérez et al., 2019). On the other hand, nematophagous fungi (NF), found in all major fungal taxonomic groups, are also widespread in natural and agricultural soils, where mainly survive as saprophytes until they switch to the parasitic stage in the presence of nematodes, developing traps or mature spores (Nordbring-Hertz et al., 2006). Thus, NF are natural enemies of EPNs, but the mechanisms behind their interactions are not well known except that EPN susceptibility to NF seems to be environmental and species-specific dependent (Bueno-Pallero et al., 2018; El-Borai et al., 2009; Koppenhöfer et al., 1996). Finally, other soil organisms such as ectoparasitic bacteria (EcPB), can alter the fitness of some EPN species by reducing their motility and pathogenicity (El-Borai et al., 2005; Enright and Griffin, 2005).

Due to their notable value as biological control agents against a wide range of soil-borne insect pests, there is increasing interest in identifying the factors that define EPN population dynamics in agroecosystems (Griffin, 2015; Lewis et al., 2015). Numerous studies link the use of cover crops and the nematode community with beneficial actions, for example, to evaluate soil health by using soil nematodes as bio-indicators (Bongers and Ferris, 1999; DuPont et al., 2009; Ferris et al., 2012; Ito et al., 2015), or to enhance the control of plant-parasitic nematodes (Hiltpold, 2015). In this regard, there is evidence of high EPN occurrence and activity in perennial crops, including vineyards, would be linked to soil health (Campos-Herrera et al., 2008, 2014). However, few studies address the relationship of cover crops presence with EPN occurrence. Susurluk and Ehlers (2008) suggested that no-till ground cover managements increase the persistence of the EPN species *Heterorhabditis bacteriophora* by supporting alternative hosts, improving soil health conditions, and reducing temperature fluctuations. However, recent studies advise that case–by–case investigations might be necessary to confirm a positive impact of cover crops on EPN populations. For example, black oat covers favored EPN activity in corn crops compared to conventional tillage practice, while the implementation of oil radish covers only implied higher records of heterorhabditid IJs without affecting the global EPN activity (Marquez, 2017). Similarly, for EPN augmentation experiments in winter wheat crops, *Steinernema feltiae* activity remained significantly higher in pea and mustard covers than in bare soils five months after IJ application but not its abundance. Conversely, no differences were observed when *H. bacteriophora* was applied, nor for the abundance of EPN natural enemies and antagonists such as NF and FLN species (Jaffuel et al., 2017).

Previous studies performed in the same experimental Denominación de Origen Calificada (DOCa) Rioja (https://www.riojawine.com/es-en/) vineyard, where we carried the current experiments, concluded that cover crops could promote beneficial above-ground entomofauna, effective in conservation biological control programs (Sáenz-Romo et al., 2019a, 2019b). Our study aimed to explore the impact of the implementation of different cover crops (grass-seeded, flower-driven, and spontaneous) on the presence and activity of EPN populations, and to identify the biotic and abiotic factors that could modulate them under those different ecological scenarios. We speculated that the use of cover crops might enhance the abundance and the activity of naturally occurring EPN species compared to conventional tillage practice. Thus, the main objectives of this study were (i) to quantify EPN abundance and activity, and (ii) to quantify the abundance of target soil organisms associated with EPNs, like natural enemies and potential competitors or antagonisms (NF, FLNs, and EcPB), on soils managed with different practices.

2. Material and methods

2.1. Field experiment characteristics and design, sampling method, and soil properties analyses

The EPN activity and natural occurrence was evaluated for two consecutive years (2017 and 2018) on *Vitis vinifera* var Tempranillo (clon RJ-26, rootstock '110Richter'), in an experimental vineyard located in La Grajera (Logroño, Spain, 42°26′N, 2°30′W, 455 masl, ~8.5% slope), and belonging to the Government of La Rioja. The vineyard training systems were bush (goblet) and double Guyot (trunk height ~40 cm, 1.15 m between vines/2.90 m between rows), set in a plot characterized by loam and sandy-loam soils with low content of soil organic matter (0.88% on average in 2016; data reported by Sáenz-Romo et al., 2019a). The region is located in a warm-summer Mediterranean climate (classified as Csb by the Köppen-Geiger system), with continental influence, characterized by average annual data of 13.9 °C, 67% relative humidity, and 405 mm of total precipitations (Spanish Meteorological Agency, AEMET; years 1981–2010). Monthly climatological data for the two sampling years were recorded (Agro-climatic Information Service in La Rioja, SIAR; Supplementary data 1, Table S1).

The experiment compared the use of three different cover crops (seeded with *B. catharticus* (Poaceae), flower-driven, and spontaneous) *versus* a regular tillage practice. Settled in 1995, the vineyard was fully managed by tilling until the cover crops were implemented in 2016. The selected covers were selected to study the impact of the use of different cover crops on the abundance and biodiversity of above-ground insect communities (Sáenz-Romo et al., 2019a). The grass/graminaceous *Bromus catharticus* Vahl cv. Samson was chosen for grass-seeded covers, a species adapted to drought and the basic pH levels registered in the experimental vineyard, but also easy to implement and develop due to its high self-seeding capacity (Ibáñez-Pascual, 2014).

After removing spontaneous vegetation by using an agriculture rotary tiller (AGRIC® GLF-70-BM, 5–10 cm depth), *B. catharticus* was mechanically seeded 1 − 2 cm depth with a mechanical seed drill (AGRIC® KSA/HER-70) twice a year (early autumn and late winter). The flower-driven covers were manually seeded once a year (early spring, 20 kg/ha) without any previous inter-row management to disturb as little as possible soil structure. The seed commercial mixture (Deco Vignes Anuelles, Nova Flore) was selected for its spread over time/gradually flowering. The spontaneous covers were designated as areas without any tillage or seeds application, allowing the natural bank of seeds to growth. The weeds community composition of flower-driven and spontaneous covers were estimated in mid-May of each year (see Sáenz-Romo et al., 2019a). No herbicides were applied during conducting the study, but acaricides and fungicides to control *Eotetranychus carpini* (Acari: Tetranychidae), and fungal diseases such as powdery (Oidium) and downy mildew, respectively (see Sáenz-Romo et al., 2019b for further information). The insect pest *Lobesia botrana* (Lepidoptera: Tortricidae) was controlled by a mating disruption technique applied throughout the sampling area (Palacios Ruiz et al., 1995). Sáenz-Romo et al. (2019a) described more details of the experimental design, treatments and aboveground fauna.

Treatments were placed on the experimental vineyard following a completely randomized design, three plots of 1200 − 2100 m^2 (~360 vine stocks) per treatment (Fig. 1). To ensure balanced spatial distribution, we divided each plot in two areas and we collected two samples in each subarea (one pair's row/inter-row per subarea). The samples were taken in the middle rows to avoid as much as possible border effects. Therefore, we obtain four samples per plot and a total of 48 samples per sampling time (early autumn and late winter 2017 and 2018). Each sample was composed of 12 single soil cores (2.5 cm ⌀ × 20 cm DP., ca. ~1200 cm^3), pooled in individual plastic bags. The samples were stored at 4 °C in the dark until processed (within 2–4 days). In the laboratory, each sample was manually homogenized and divided in three soil subsamples of 200 g (fresh weight). Following the protocol described by Campos-Herrera et al. (2019), two of the subsamples were used (i) to characterize EPNs and associated organisms through sucrose-gradient centrifugation technique and qPCR identification, and (ii) to determine the soil suppressive capacity by using the traditional insect-bait method (see detailed descriptions below). To express the number of organisms identified per 100 g of dry soil, the third set of aliquots was dried at 40 °C for one week to measure the water content. Thereafter, 200 g of oven-dried soil, result of combining the samples from each subareas (n = 24 per sampling time), were used for the analyses of soil properties: pH (Millennia and Markewitz, 2004), electric conductivity, organic matter (Walkley and Black, 1934), and micro-nutrient elements (P, K, Mg, Ca, Zn, Mn, Fe, and Cu) (Mehlich 3 acid extraction/ICAP for elements; Mehlich, 1978, 1984) (Supplementary data 1, Table S2). The soil texture (sand, silt and clay percentages) (Bouyoucos, 1936) was analyzed only once (Supplementary data 1, Table S3). All soil analyses were performed by Laboratorio Regional del Gobierno de La Rioja, La Grajera (Logroño, Spain).

2.2. Characterization of entomopathogenic nematodes and associated organisms, and estimation of soil activities against insect larvae

Following Campos-Herrera et al. (2019), nematodes and other organisms were co-extracted from 200 g fresh soil samples (Wiesel et al., 2015) by sucrose-gradient centrifugation (Jenkins, 1964), retrieving the nematode community in 50 mL Falcon tubes (Becton Dickinson Labware, USA). After allowed all the organisms be placed in the bottom of the tubes (4 °C overnight), each bottom content (organisms and some rest of soil) was transferred to 1.5 mL Eppendorf tubes by successive centrifuge-evacuation steps. The final concentred material was stored at − 20 °C until DNA extraction procedures.

Following the protocol described by Bedding and Akhurst (1975) with few modifications (see Campos-Herrera et al., 2019), each sample of the second set of 200 g fresh soil was baited with 20 final instar of *Galleria mellonella* (Lepidoptera: Pyralidae) larvae (reared at ICVV) in two independent rounds (n = 10 larvae per round) to allow nematodes to become active (Griffin, 2015). After four days of exposure (22–24 °C in the dark), larval mortality was assessed and dead larvae recovered, rinsed with tap water, and individually placed in White traps (White, 1927). Live larvae were additionally incubated for 24 h to record possible late mortality. The percentage of dead larvae marked the total suppressive capacity of the soil for each sample (hereafter Total-act). Dead larvae were checked every 2–3 days under the stereoscope for about a month to verify nematode occurrence. The percentage of larvae that recorded any nematode emergency indicated the soil activity associated with nematodes (hereafter Nem-act). Emerging nematodes were recovered in tap water (hereafter RO aliquots) and stored (14 °C in the dark). Their entomopathogenic activity was tested by performing the Koch's postulates (Stock and Goodrich-Blair, 1997). Briefly, two Petri dishes lined with filter paper and three *G. mellonella* larvae were inoculated with 0.5 mL of each RO aliquots. This procedure was repeated when not nematode progeny was reported to avoid false negatives. The emerging nematodes were recovered in tap water (hereafter RM aliquots) and stored at 14 °C in the dark. The percentage of larvae that recorded positive Koch's postulates indicated the soil entomopathogenic activity (hereafter EPN-act). Besides, 1 mL of concentrated nematode suspension of both RO and RM aliquots was saved (-20 °C) for later analysis. Besides, we recovered and maintained (Woodring and Kaya, 1988) some of the emerging nematodes to establish laboratory cultures.

2.3. Identification and quantification of entomopathogenic nematodes and associated organisms by real time qPCR

Before DNA extraction, all samples were mechanically

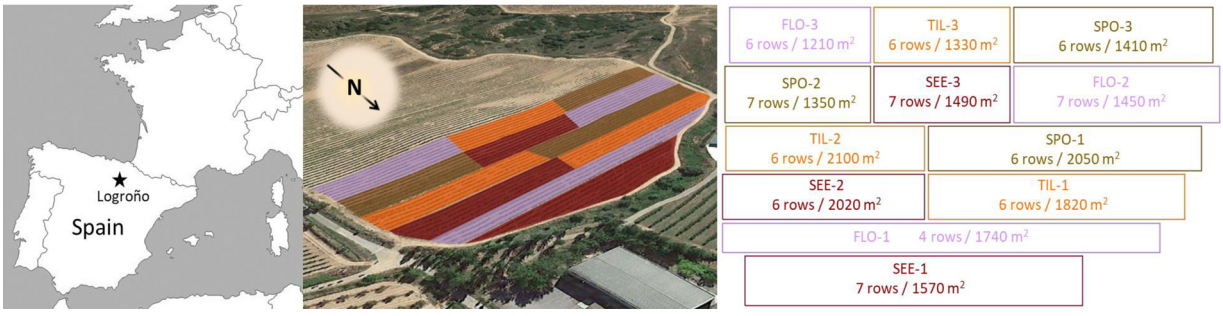

Fig. 1. Experimental design of the experimental vineyard. The experiment was settle on the vine variety Tempranillo (clon RJ-26, rootstock '110-Richter'). The plots consisted of four to seven plant rows (1.15 m between strains/2.90 m between rows). Different treatments (n = 3) are indicated with different colors: tillage (TIL) and three different cover crops, seeded (SEE) with *Bromus* catharticus (Poaceae), flower-driven (FLO), and spontaneous (SPO). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Soil organisms tested and qPCR running conditions.

Type of organism/species	Population	GenBank ACNO ITS Region	Primers/probes final concentration (nM)	Reference for primers and probes sequences
Entomopathogenic nematodes				
Heterorhabditis bacteriophora	(commercial)	KJ938576	400/200	Campos-Herrera et al. (2011b)
Heterorhabditis indica	Btw	KJ938571	400/200	Campos-Herrera et al. (2011a)
Steinernema affine	CH	KJ938567	600/300	Torr et al. (2007)
Steinernema arenarium	SA	KU194615	600/300	Campos-Herrera et al. (2019)
Steinernema carpocapsae	DOK-83	KJ818295	400/200	Campos-Herrera et al. (2011b)
Steinernema feltiae	RS-5	KJ938569	600/300	Campos-Herrera et al. (2011b)
Steinernema kraussei	OS	KJ696686	266/150	Campos-Herrera et al. (2015b)
Steinernema riojaense	RM-30	MK503133	600/300	Current study
Free-living nematodes				
Acrobeloides-group	RT1-R15C	JQ237849	400/200	Campos-Herrera et al. (2012)
Oscheius tipulae	MG68 P29	KJ938579	400/200	Campos-Herrera et al. (2015a)
Oscheius onirici	MG67 P20	KJ938578	400/200	Campos-Herrera et al. (2015a)
Pristionchus maupasi	AM-3	MG551681	400/200	Campos-Herrera et al. (2019)
Nematophagous fungi				
Catenaria sp.	1D	JN585805	400/200	Pathak et al (2012)
Arthrobotrys dactyloides	H55	KJ938574	400/200	Pathak et al (2012)
Arthrobotrys musiformis	11	KJ938572	400/200	Pathak et al (2012)
Arthrobotrys oligospora	8	KJ938573	400/200	Pathak et al (2012)
Hirsutella rhossiliensis	2931	–	400/200	Zhang et al. (2006)
Purpureocillium lilacinum	9357	KJ938575	400/200	Atkins et al. (2005)
Ectoparasitic bacteria				
Paenibacillus nematophilus	NEM2	AF480936	400/200	Campos-Herrera et al. (2011b)
Paenibacillus sp.	SdTc1FEE1	JF317562	400/200	Campos-Herrera et al. (2011b)

disaggregated (15 s) by using sterile blue pestles assembled to a pellet mixer (VWR International, UK). Then, we used the PowerSoilR DNA Isolation Kit (MOBIO Laboratories, Inc, Carlsbad, CA, USA) for the aliquots obtained by sucrose-gradient centrifugation, and the Speedtools tissue DNA extraction kit (Biotools, Spain) for the RO/RM aliquots. Thereafter, DNA extractions were analyzed for quality and quantity using 1 µl per duplicate in a Nanodrop system (Thermo Scientific 2000C spectrophotometer) and then stored at −20 °C until qPCR analysis.

We screened for the occurrence and abundance of 20 soil organism species by using species-specific primers/probe (Table 1), comprising 8 EPNs, 4 FLNs, 6 NF and 2 EcPB. Specifically, soil baits in La Rioja have previously reported three of the EPN species under study: *Steinernema carpocapsae*, *S. feltiae*, and *S. kraussei* (Campos-Herrera et al., 2007, 2008). The EPN species *Steinernema affine*, *S. arenarium*-like, *Heterorhabditis indica*, and *H. bacteriophora* have been also reported in the Iberian Peninsula (Campos-Herrera et al., 2016, 2019; Doucet and Gabarra, 1994; García del Pino and Palomo, 1996; Valadas et al., 2014). We made an additional screening for a new EPN species, *Steinernema riojaense* (Půža et al., 2020), identified from aliquots of the insect-baits conducted in this study, after designing a species-specific primers/probe set (Supplementary material 2). The FLNs screened in this study, *Oscheius tipulae*, *O. onirici*, *Pristionchus maupasi*, and *Acrobeloides* spp. (Campos-Herrera et al., 2012; 2015), were also previously recorded in the Iberian Peninsula, specifically in the Algarve Region, in South Portugal (Campos-Herrera et al., 2019). Moreover, other nematode species (15 EPNs and 9 FLNs) were included in this study to validate the qPCR tools, avoiding cross-amplification and adapting previously published protocols to our experimental conditions (Supplementary data 2, Tables S4 and S5). We maintained pure cultures of most nematode species, EPNs reproduced in *G. mellonella* larvae (Woodring and Kaya, 1988), and FLNs in 1% nutrient agar (NA; Difco, MD, USA) feeding on associated bacteria (R.J. Sommer and R. Campos- Herrera reference laboratory populations). A plasmid containing the complete published ITS region was used when living material was not available (Supplementary data 2). EPN and FLN populations were maintained in tap water and Buffer M9, respectively, and stored at 14 °C in dark conditions.

We screened for fungal species belonging to the three main groups of NF (Nordbring-Hertz et al., 2006): nematode-trapping fungi (*Arthrobotrys dactyloides*, *A. musiformis*, and *A. oligospora*), endoparasites (*Hirsutella rhossiliensis* and *Catenaria* sp.), and eggs-parasitic fungi (*Purpureocillium lilacinum*). These species were also previously recorded in Iberian Peninsula (Campos-Herrera et al., 2016, 2019). We maintained pure cultures of all fungi in Corn Meal Agar (Fluka Analytical, Sigma-Aldrich, CO, USA) (Pathak et al., 2012) except *Catenaria* sp., for which we used as positive control/standard curve a plasmid containing the complete published ITS region (Table 1). Finally, we screened for two bacterial species in the genus *Paenibacillus*: *P. nematophilus* and *Paenibacillus* sp. (Campos-Herrera et al., 2011a, 2019). Because no living material was available, we used a plasmid containing a fragment of the 16S rDNA region (Table 1).

Biosearch Technologies (supplied by Biotools, Spain) synthesized species-specific primers and TaqMan® probes. The probes were labeled at the 5′ end with the fluorogenic reporter dye FAM and the 3′ end with the quencher BQH-1. We established positive controls by the combination of three independent DNA extractions, using Power SoilR DNA Isolation Kit for each pure culture or QIAprep Spin Miniprep for plasmid. In all the preliminary checks and optimization protocols, DNA concentration was standardized to correspond to 30 IJs (for EPNs), 1 ng/µL of pure culture (for FLNs and NF), and 0.1 ng/µl when plasmids were used (Supplementary material 2). For the quantifications, the standard curves were obtained by 10-fold serial dilutions (n = 5 points), starting with the highest concentration employed in the pre-screenings. Real-time qPCR tests and quantifications were performed with the Quantimix Easy Probe mix (Biotools, Spain) in Applied Biosystems® MicroAmp™ Optical 96-Well reaction plates on the Applied Biosystems® 7500 Real-Time PCR System. We run 38 cycles for nematodes species and 50 cycles for NF and EcPB species at 60 °C annealing temperature (Table 1; see Supplementary material 2 for more details). The DNA samples obtained by sucrose-gradient centrifugation were adjusted to 10 ng/µl before qPCR tests, employing a correction factor to transform qPCR data to original quantities per sample. Similarly, the quantification of the DNA samples obtained from the RO/RM aliquots was estimated by using a dilution between 0.5 and 1 ng/µL, recording the threshold cycle (Ct) values for estimation of the relative abundance of nematodes emerged per insect larva.

2.4. Statistical analyses

We ran generalized linear mixed models (GLMMs) testing the effect of the use of cover crops pair-treatment compared with regular tillage (fixed factor) on numbers and frequencies of occurrence of EPNs, FLNs, NF, EcPB, and soil activity measures, performed with SPSS 25.0 (SPSS Statistics, SPSS Inc., Chicago, IL, USA). Sampling area into plots (pair's rows/inter-rows) was included as subjects of the models since no significant differences for any of the variables under study were observed (data not shown). In each model, we also accounted for yearly and seasonally repeated measures as random factors.

We adjusted all organism quantifications from DNA samples obtained by sucrose-gradient centrifugation to express the corresponding quantities per 100 g of dry soil. Thus, EPN and EcPB abundances were expressed as number of IJs and copies of plasmids per 100 g of dry soil, respectively. Numbers of FLNs and NF were standardized to a 0–1 range by dividing all values recorded for a particular species (n_i) by the largest number (n_{max}) of that species, according to $100 \times n_i/n_{max}$ (Jongman et al., 1995). By this standardization, the data set is liberated from variation caused by the intrinsic differences such as large variations recorded for the copy numbers between the FLN and NF species analyzed. Since many NF species can survive in soil saprophytically (Nordbring-Hertz et al., 2006), we can considerate that most of the NF species isolated by sucrose-gradient centrifugation were in the infectious phase. Thus, NF numbers were expressed as infection rate (IR), determined by dividing the DNA quantity of each NF species by the total amount of DNA (Campos-Herrera et al., 2012). Finally, we evaluated the soil activities and nematode occurrence in the RO/RM aliquots as larval percentages recorded in insect-baits.

Prior statistical analysis, variables expressed as a percentage and quantitative variables were arcsine and $\log(x + 1)$ transformed, respectively. For the quantification of soil organisms and soil activities, we used a GLMM with a gamma distribution (log-link function), and for the frequencies of occurrence a binomial distribution (logit-link function). Besides, we looked for significant differences among treatments in the abiotic soil parameters analyzed. For the soil texture (sand, silt and clay percentages), we run one-way ANOVA and Tukey's HSD test SPSS 25.0 (Table S3), and for the rest of soil properties, we used a linear mixed models (LMM) in SPSS 25.0 within pair-treatment comparisons of soil tillage management and each cover crop treatment (Fig. 2; Table S2). We initially included as covariates in the GLMM all soil parameters for which we recorded significant differences, and then we removed all predictors that were not significant from those statistical models. Thus, for the pair-treatment comparisons between tillage and grass-seeded cover crop treatments, we included soil organic matter to test for differences of total NF numbers. We used least square means ± SE as descriptive statistics.

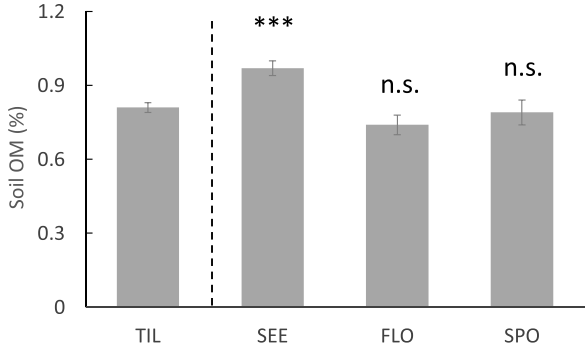

Fig. 2. Linear mixed models testing for differences of the percentage soil organic matter (OM) between till (TIL) and no-till soils but the implementation of the cover crops grass-seeded (SEE), flower-driven (FLO), and spontaneous (SPO). Asterisks indicate significant differences within pair-treatment comparisons at $^{***}P < 0.001$, and n.s., not significant. Values are least-square means ± SE.

3. Results

3.1. Soil properties analysis

The experimental field was homogeneous for most of the soil properties (Supplementary data 1, Tables S1 and S2), except for few variables in specific treatments when compared with the tillage management. In the case of the grass-seeded cover, only soil organic matter (Fig. 2), and Mg content (Table S1) resulted higher than in the tillage plots. In the flower-driven cover, the water content and electroconductivity (Table S2) registered lower values than in the tillage treatment. Finally, the spontaneous cover only had a significantly superior value of Zn content than the control (Table S2). Among all these variables, only the soil organic matter (Fig. 2) were included in the NF presence pair-treatment comparisons between tillage and grass-seeded cover crop treatments.

3.2. Numbers and frequency of occurrence of the soil organisms identified by sucrose-gradient centrifugation

The total IJ numbers on grass-seeded and spontaneous cover crops were significantly higher than recorded on tillage management (Fig. 3A; Supplementary data 3, Table S6). However, no differences for flower-driven cover crops were observed, nor for the frequency of EPN occurrence among treatments (Fig. 3B). We detected five out of the eight EPN species screened: the heterorhabditids *H. bacteriophora* and *H. indica*, and the steinernematids *S. feltiae*, *S. affine*, and the new species *S. riojaense* (Fig. 3; Table 2). The only EPN species detected for all treatments were *H. indica* and *S. feltiae*. While no difference was observed for *H. indica* among treatments, compared to tilled soils *S. feltiae* was found in lower numbers on grass-seeded and spontaneous covers, and higher numbers on flower-driven covers (Table 2). However, the frequency of *S. feltiae* occurrence was similar for all treatments except grass-seeded covers, that it was significantly lower than tillage (Table 2). The presence of *H. bacteriophora* explained the differences for EPN numbers among treatments, higher on grass-seeded and spontaneous covers compared to tilled soils, and absent on flower-driven covers, while no significant differences were observed among treatments for it frequency of occurrence (Table 2). *S. riojaense* was detected on all cover crops but not on tilled soils, and *S. affine* only on grass-seeded covers and in very low numbers (without significant differences among treatments for any of the two species; Table 2).

Overall, FLNs were more abundant on cover crops than tilled soils, both in numbers and frequency of occurrence (Fig. 4), but with no significant differences. We detected two out of the four FLNs screened: *P. maupasi* and *Acrobeloides* spp., both present on all treatments but with no significant differences even if proportional *P. maupasi* numbers were higher on tillage and grass-seeded treatments (Fig. 4A; Table 2). The NF IR was only significantly higher on grass-seeded covers than tilled soils, both for the frequency of occurrence and numbers (Fig. 5; Table S6), in the latter case mediated by the higher percentages of soil organic matter found for grass-seeded covers (Fig. 2; Table S6). We detected five out of the six NF species screened: *A. oligospora*, *A. dactyloides*, *H. rhossiliensis*, *Catenaria* sp., and *P. lilacinum*. The NF *A. oligospora* was the only species identified on all treatments. No records of *A. dactyloides* and *H. rhossiliensis* were observed on grass-seeded and flower-driven covers, respectively. *Catenaria* sp. was the less abundant NF, only identified on grass-seeded and flower-driven covers (Fig. 5). However, the only significant difference was recorded for the frequency of occurrence of *P. lilacinum*, absent on bare soils (Table 2). Finally, we only detected one out of the two EcPB screened, *Paenibacillus* sp., in higher numbers and frequency of occurrence in cover crops than tilled soils but with no significant differences (Fig. 6).

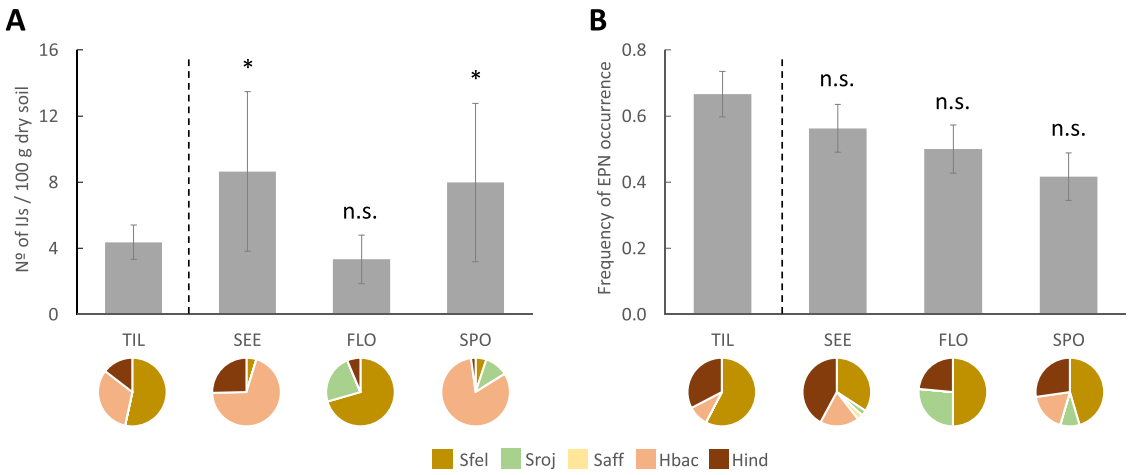

Fig. 3. Effect of the use of the cover crops grass-seeded (SEE), flower-driven (FLO), and spontaneous (SPO), on (A) number of infective juveniles (IJs) and (B) frequency of occurrence of the entomopathogenic nematode (EPN) species identified: *Steinernema feltiae* (Sfel), *S. affine* (Saff), *S. riojaense* (Rroj), *Heterorhabditis bacteriophora* (Hbac), and *H. indica* (Hind). Asterisks indicate significant differences from generalized linear mixed models testing within pair-treatment comparisons of tillage management (TIL) and each cover crop at $^{*}P < 0.05$ and n.s., not significant. Values are least-square means ± SE. EPN species averages are represented in pies (see Table 2 for statistics).

Table 2
Effect of the implementation of the cover crops grass-seeded (SEE), flower-driven (FLO), and spontaneous (SPO), on quantification and frequency of occurrence of the isolated species by sucrose-gradient centrifugation of entomopathogenic nematodes (EPNs) and the associated organisms free-living nematodes (FLNs) and nematophagous fungi (NF). Results from generalized linear mixed models testing within pair-treatment comparisons of tillage management (TIL) and each cover crop. Asterisks indicate significant at $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, n.s., not significant, and n.d., no data.

Dependent variable	Quantification			Frequency of occurrence		
	TIL vs SEE (χ^2, *P*)	TIL vs FLO (χ^2, *P*)	TIL vs SPO (χ^2, *P*)	TIL vs SEE (χ^2, *P*)	TIL vs FLO (χ^2, *P*)	TIL vs SPO (χ^2, *P*)
Total EPNs – nº IJs						
S. feltiae	13.16 ***	4.17 *	6.72 ***	1.78 *	n.s.	n.s.
S. riojaense	n.d.	n.s.	n.s.	n.d.	n.s.	n.s.
S. affine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H. bacteriophora	123.40 ***	n.d.	47.02 ***	n.s.	n.d.	n.s.
H. indica	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Total FLNs – ratio 0 – 1						
P. maupasi	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Acrobeloides ssp.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Total NF – IR ratio 0 – 1						
A. oligospora	n.s.	n.s.	48.53 ***	n.s.	n.s.	n.s.
A. dactyloides	33.32 ***	n.s.	n.d.	n.s.	n.s.	n.s.
P. lilacinum	n.s.	n.s.	n.s.	2.71 *	2.08 *	2.30 *
H. rhossilliensis	n.d.	n.d.	n.d.	n.d.	n.d.	n.s.
Catenaria ssp.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

3.3. Soil activities recorded in insect-baits

Compared to tillage treatment, we recorded significant differences for the EPN-act registered for flower-driven and spontaneous covers (Fig. 7A; Table S6), while no differences for the frequencies of occurrence of any soil activity tested (total activity, Nem-Act nor EPN-act) were obtained (Fig. 7B; Supplementary material 3, Fig. S1). We identified the same nematode species from the RO/RM aliquots than through sucrose centrifugation technic except for *S. carpocapsae*, registered instead of *S. affine*, also in very low numbers. On the contrary, the nematode species *H. bacteriophora*, *S. feltiae*, *S. riojaense*, and *Acrobeloides* spp. were the nematode species identified for all treatments. However, we did not observe any significant differences for the Nem-act nor EPN-act among treatments for any nematode species (Fig. 7; Fig. S1).

4. Discussion

According to our hypothesis, we observed evidence of some positive effects on EPN populations resulting from the use of cover crops, although with reservations. The spontaneous cover was the only no-till treatment that recorded both numbers and activity of EPNs significantly higher than conventional soil management (Fig. 3 and 7). Conversely, grass-seeded and flower-driven covers showed opposite patterns with each other. We found high EPN abundance but low activity on grass-seeded cover crops, while high pathogenicity rates but low EPN numbers characterized flower-driven covers. Compared to other surveys performed by insect baiting in the Iberian Peninsula, we recorded similar frequencies of EPN occurrence (or slightly higher) than registered before in La Rioja and Catalonia regions (Campos-Herrera et al., 2007; García del Pino and Palomo, 1996). However, Valadas et al. (2014) and Campos-Herrera et al. (2019) reported completely different numbers for two surveys conducted in continental Portugal, approximately three times lower (~ 7%) and three times higher (~ 60%), respectively. Regarding the EPN abundance, we recorded lower numbers than Campos-Herrera et al. (2019) did in the Algarve region (South Portugal) using the same methodology, specifically for citrus and oak groves (~ 25 IJs/100 g of dry soil).

The abundance and frequency of occurrence of all soil organisms evaluated were not affected by the sampling location within the vineyard (row/inter-row), suggesting that attending to the soil microhabitat, the plots comprise a homogeneous set. The prevalent EPN species in Europe *S. feltiae* and *H. bacteriophora* (Hominick, 2002) were the most commonly found in our study. In particular, previously mentioned findings in the Iberian Peninsula reported more evidence of *S. feltiae* activity, in a wide variety of habitats (Campos-Herrera et al., 2007; García del Pino and Palomo, 1996; Valadas et al., 2014), and more numerous (Campos-Herrera et al., 2019), than other EPN species. Overall, we also found *S. feltiae* in higher frequencies of occurrence, but, except for flower-driven covers, it seems to be replaced by *H. bacteriophora* on cover crop treatments. Bare soils easily suffer abrupt temperature changes that affect various EPN biological processes such as IJ infectiveness, development to adults within the host, and productiveness, and thus, could reduce the persistence of sensitive EPN species during the spring and autumn seasons. According to Grewal

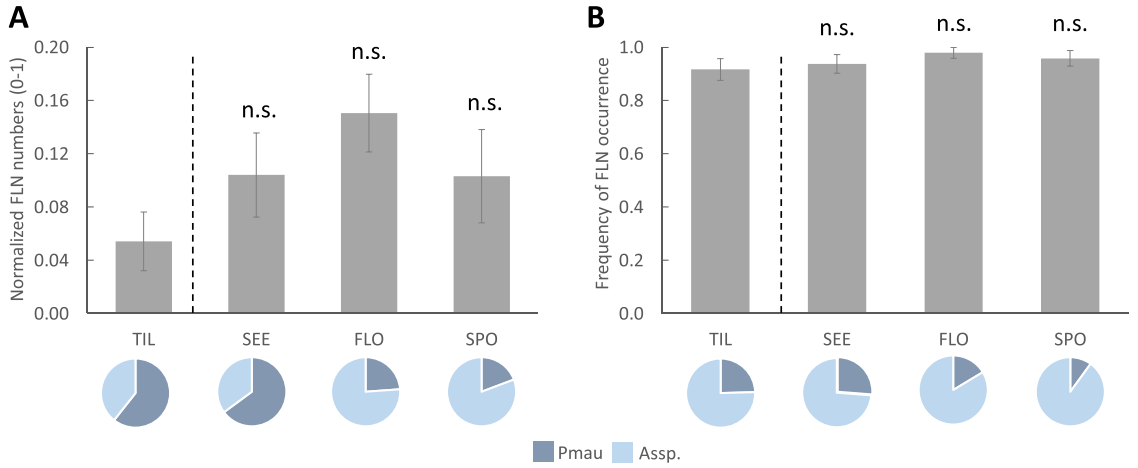

Fig. 4. Effect of the use of the cover crops grass-seeded (SEE), flower-driven (FLO), and spontaneous (SPO), on (A) quantification and (B) frequency of occurrence of the free-living nematodes (FLNs) identified: *Pristionchus maupasi* (Pmau) and *Acrobeloides* ssp. (Assp.). Not significant (n.s.) differences were found from generalized linear mixed models testing within pair-treatment comparisons of tillage management (TIL) and each cover crop. Values are least-square means ± SE. FLN species averages are represented in pies.

et al. (1994), *S. feltiae*, with thermal niche breadths between 8 – 25 °C, tolerates low temperatures much better than *H. bacteriophora*, that establish and reproduce in *G. mellonella* larvae up to 14 °C. Hence, the use of cover crops could favor cold-sensitive EPN species as *H. bacteriophora*. The new EPN species identified in this study, *S. riojaense*, was also more abundant on no-till soils, particularly on flower-driven and spontaneous covers, the managements that less disturb the soil structure. However, additional studies to verify the ecological characteristics of this new EPN species are needed. On the other hand, it is worth noting the presence of *H. indica* in our samples, recently identified for the first time in the Iberian Peninsula (Campos-Herrera et al., 2016, 2019), and now reported in a high-latitude region that still within the marginal ecoclimatic suitability for *H. indica* predicted by Kour (2017).

The numbers reported for other organisms linked to EPN soil food web may explain, partially at least, our observations. In particular, spontaneous and grass-seeded covers, similar for the abundance of IJs, remarkably differ for the frequency of *G. mellonella* larvae killed by EPNs after baiting, probably due to the differences in the abundance of EPN antagonist organisms observed between treatments. Indeed, spontaneous covers registered, despite the lack of significant differences, the lowest numbers of NF, FLN, and EcPB species among the no-till treatments, while just the opposite trend characterized grass-seeded covers. It is likely that not only abundance but also the proportions of the identified NF species could be one of the keys to explaining these observations. We reported significantly higher presence of NF-IR on grass-seeded covers, the treatment that recorded the highest content in organic matter and the lowest sand percentages, two of the key factors that favor NF occurrence on soils (Nordbring-Hertz et al., 2006; Pathak et al., 2017). Endoparasitic fungi (*H. rhossilliensis* and *Catenaria* sp.) were well represented on grass-seeded covers and bare soils, but almost absent on spontaneous and flower-driven covers, the treatments with higher evidence of EPN activity, and for which nematode-trapping fungi in the genus *Arthrobotrys* were the predominant NF species. Altogether, the results obtained in this study suggest the possibility that the endoparasitic NF species identified were more efficient in killing EPNs than the trapping NF species. Timper and Kaya (1989) pointed out the protective role of the second-stage cuticle of heterorhabditid IJs (third-stages) against endoparasitic NF by preventing the penetration of adhering fungal spores within the nematode body cavity. Consequently, Timper et al. (1991) confirmed that *H. rhossiliensis* infects ensheathed *H. bacteriophora* IJs less efficiently than steinernematid IJs, a supposal that support our observations of both the large numbers recorded for

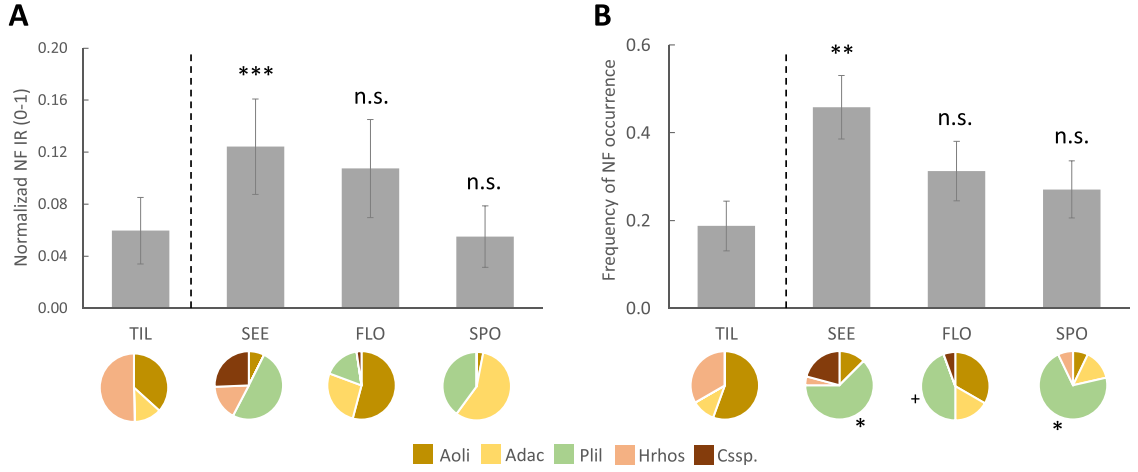

Fig. 5. Effect of the use of the cover crops grass-seeded (SEE), flower-driven (FLO), and spontaneous (SPO), on (A) quantification and (B) frequency of occurrence of nematophagous fungi (NF) infection ratio (IR) for the identified species: *Arthrobotrys oligospora (Aoli), A. dactyloides (Adac), Purpureocillium lilacinum (Plil), Hirsutella rhossiliensis (Hrhos), and Catenaria* sp. (*Csp.*). Asterisks indicate significant differences from generalized linear mixed models testing within pair-treatment comparisons of tillage management (TIL) and each cover crop at ***$P < 0.001$, **$P < 0.01$, and n.s., not significant. Values are least-square means ± SE. NF species averages are represented in pies (see Table 2 for statistics).

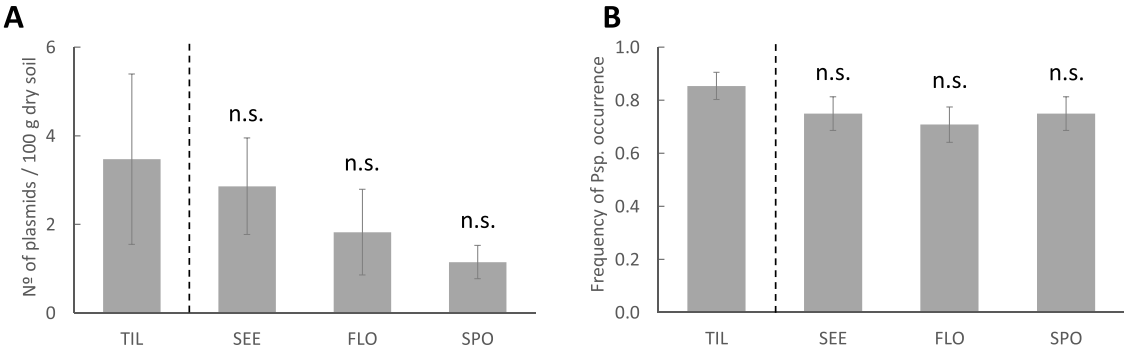

Fig. 6. Effect of the use of the cover crops grass-seeded (SEE), flower-driven (FLO), and spontaneous (SPO), on (A) number of plasmids and (B) frequency of occurrence of the ectoparasitic bacterial species *Paenibacillus* sp. (Psp.). Not significant (n.s.) differences were found from generalized linear mixed models testing within pair-treatment comparisons of tillage management (TIL) and each cover crop. Values are least-square means ± SE.

this EPN species and the low presence of steinernematids on grass-seeded covers. However, *in vitro* experiments reported that two endoparasitic NF species *Catenaria* sp. *Myzocytium* sp. exposed to ensheathed IJs did not reduce the prevalence of *H. indica* but *H. zealandica* (El-Borai et al., 2009), and propose that the NF efficacy in killing EPNs could be more species-specific dependent. For example, large and highly motile IJs will encounter more *H. rhossiliensis* conidia than less motile nematodes (Timper et al., 1991). Similarly, big nematodes might able to escape more readily from adhesive or constricting hyphal structures than small nematodes (El-Borai et al., 2009). In agreement with these observations, we reported higher occurrence and numbers of *S. riojaense*, characterized by large IJs (up to 1000 µm), on flower-driven and spontaneous covers, where trapping-nematode fungi were the predominant NF species, than on grass-seeded covers or bare soils, more abundant in endoparasitic NF species.

Various studies have addressed the competitive relationships among EPN and FLN species (Blanco-Pérez et al., 2019; Campos-Herrera et al., 2012, 2015b; Duncan et al., 2003), but the nature of possible occurring interaction between these two groups of nematodes seems to be much more uncertain. For example, Blanco-Pérez et al. (2017) suggest that, in particular cases, the presence of bacteriophagous nematodes within the insect's cadaver could assist EPN development. Of the two identified FLN species in this study, *P. maupasi* co-emerged more frequently with EPNs from insect larvae baited on soil samples collected in the Algarve region (Campos-Herrera et al., 2019). We found *P. maupasi* in higher and lower numbers on grass-seeded and spontaneous covers, respectively, similarly than observed for the other EPN antagonism organisms tested (NF and EcPB). However, *in vitro* experiments conducted by Blanco-Pérez et al. (2019) discarded any pathogenic capacity of a Portuguese strain of *P. maupasi* against *G. mellonella* larvae, and concluded that IJ infectiveness and reproduction for the EPN species *S. feltiae* were not affected by the presence of this particular FLN strain even in large numbers. Besides, in our insect-baits, we did not report many emergences for *P. maupasi*, but nematodes in the *Acrobeloides*-group. Surprisingly, we did not found evidence for the presence of nematodes in the genus *Oscheius*, commonly existing in soils (Félix et al., 2001), so it seems plausible that other FLN species may occupy their ecological niche. The remarkable abundance of the eggs-trapping fungal species *P. lilacinum* on no-till soils point in this direction and suggests that we may be underestimating the total number of FLNs that these treatments would contain. Expanding the molecular qPCR tools for new FLN species associated with EPNs, such as *Caenorhabditis elegans* (R. Blanco-Pérez, personal observation), or using Next-Generation Sequencing (NGS) analysis to expand the number of species explored (Dritsoulas et al., 2020; Geisen et al., 2018), might provide additional insight on the interactions between these two groups of nematodes in nature.

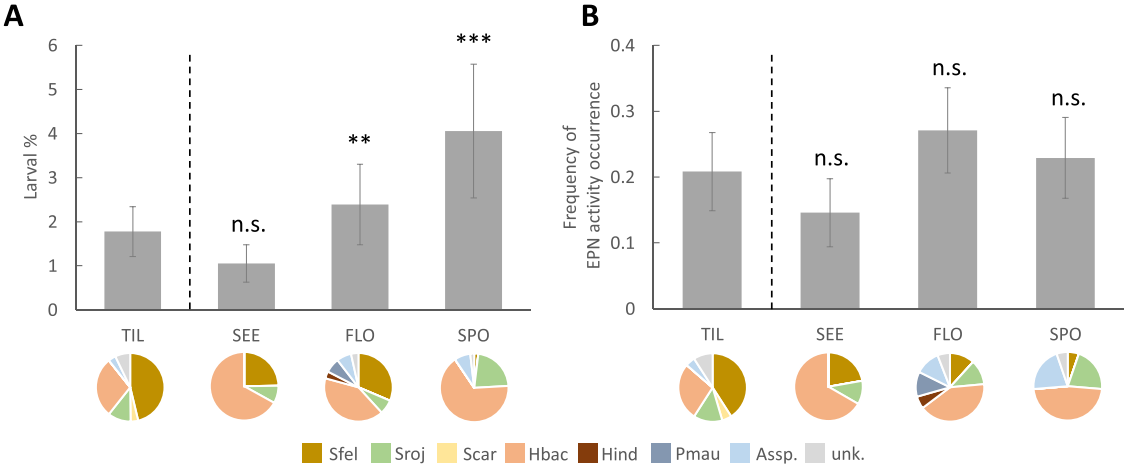

Fig. 7. Effect of the use of the cover crops grass-seeded (SEE), flower-driven (FLO), and spontaneous (SPO), on entomopathogenic nematode (EPN) activity (A) measured as the percentage of *G. mellonella* larvae that recorded positive Koch's postulates nematode emergence, and (B) the frequency of occurrence of EPN activity. Asterisks indicate significant differences from generalized linear mixed models testing within pair-treatment comparisons of tillage management (TIL) and each cover crop at $^{***}P < 0.001$ $^{**}P < 0.01$, and n.s., not significant. Values are least-square means ± SE. Averages of the nematode species identified are represented in pies: *Steinernema feltiae* (Sfel), *S. riojaense* (Rroj), *S. carpocapsae* (Scar), *Heterorhabditis bacteriophora* (Hbac), *H. indica* (Hind), *Pristionchus maupasi* (Pmau), and *Acrobeloides* ssp. (unk., unknown species).

5. Conclusion

Pest prevention is one of the main aims of current agriculture. Natural enemies occurring in crop soils allow for protection against pests and pathogens if conditions favor their survival. Overall, our results confirm a tendency of the positive impact of the implementation of specific cover crops on natural occurrence and activity of EPNs, which should be an indicator of more high-grade soil health. But the proper selection of alternative management strategies in agroecology is essential to maximize benefits and reduce potential problems (Provost and Pedneault, 2016), and viticulture is not an exception. For example, we observed that the use of grass-seeded covers seems to enhance IJ abundance, but also endoparasitic NF proliferation that might reduce their activity, perhaps by accumulating more organic matter than other no-till managements. Conversely, the spontaneous cover showed an equilibrium in the abundance and activity of the EPN species naturally occurring, while maintaining low levels of abundance for antagonistic organisms. Besides, the evaluation of above-ground arthropods in the same experimental vineyard revealed that several predator taxa, including ants, ground beetles, earwigs, and vespoid wasps, were also significantly better represented on spontaneous cover crops (Sáenz-Romo et al., 2019b). Remarkably, this particular treatment should not contribute to increasing viticulture costs. These promising results can contribute to expanding the use of strategies that enhance the occurrence of natural enemies of arthropods potentially damaging to vineyards at a reasonable cost for the farmers. Thus, future studies to confirm this general trend and unravel the factors associated with the beneficial effect of cover crop implementations in vineyards will be needed.

Intellectual property

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

Research ethics

We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript (not applicable to this work).

Authorship

The International Committee of Medical Journal Editors (ICMJE) recommends that authorship be based on the following four criteria:

Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND

Drafting the work or revising it critically for important intellectual content; AND

Final approval of the version to be published; AND

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding

Funding was received for this work. All of the sources of funding for the work described in this publication are acknowledged below:

Personal help: Dr. Xoaquín Moreira (Misión Miológica de Galicia, CSIC, Spain) for his help with the statistical analysis, and members of Vitigen Lab (ICVV) for kindly sharing their equipment.

Grants funding: Ministry of Economy and Competitiveness (AGL2014-53336R)

The Government of Spain with Ramón y Cajal grant (RYC-2016-19939).

Contracts and fellowships awarded by the authors that supported their work: R.B.P. is financed by the Department of Economic Development and Innovation, Government of La Rioja (Spain) with a predoctoral contract (CAR-2018)(2018-2022)

MGSR was supported by FPI-UR 2015 (University of La Rioja)(2015-2019)

IVD awarded the ADER I + D+i (2019) fellowship by the Riojan Agency of Economic Development (La Rioja, Spain) (2019–2020).

RCH is awarded by Ramon y Cajal contract award (RYC-2016-19939) from the Government of Spain (2018–2023).

Declaration of Competing Interest

No conflict of interest exists.

Acknowledgments

The authors thank Dr. Xoaquín Moreira (Misión Miológica de Galicia, CSIC, Spain) and Laura Barrios (Área de Informática Científica, CSIC, Spain) for their help with the statistical analysis, and members of Vitigen Lab (ICVV) for kindly sharing their equipment. This study was grant supported by the Ministry of Economy and Competitiveness, Goverment of Spain (AGL2014-53336R). The pre-doctoral contracts CAR-2018 (Department of Economic Development and Innovation of the Government of La Rioja) and FPI-UR 2015 (University of La Rioja) support RBP and MGSR, respectively. IVD awarded the ADER I+D+i (2019) fellowship by the Rioja Agency of Economic Development (La Rioja, Spain). The Government of Spain currently supports RCH with a Ramón y Cajal, government of Spain contract award (RYC-2016-19939).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2020.107028.

References

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. In: Paoletti, M.G. (Ed.), Invertebrate Biodiversity as Bioindicators of Sustainable Landscapes. Elsevier B.V., Amsterdam, The Netherlands, pp. 19–31. https://doi.org/10.1016/b978-0-444-50019-9.50005-4.

Altieri, M., Nicholls, C., 2004. Biodiversity and Pest Management in Agroecosystems, Biodiversity and Pest Management in Agroecosystems. CRC Press, Boca Raton. https://doi.org/10.1201/9781482277937.

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.

Bedding, R.A., Akhurst, R.J., 1975. A simple technique for the detection o f insect paristic rhabditid nematodes in soil. Nematologica 21, 109–110. https://doi.org/10.1163/187529275X00419.

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, I., 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.

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.

Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends Ecol. Evol. (Amst.) 14, 224–228. https://doi.org/10.1016/S0169-5347(98)01583-3.

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.

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., 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., 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., 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., 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., Jaffuel, G., Chiriboga, X., Blanco-Pérez, R., Fesselet, M., Půža, V., Mascher, F., Turlings, T.C.J., 2015a. 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., Půža, V., Jaffuel, G., Blanco-Pérez, R., Čepulyte-Rakauskiene, R., Turlings, T.C.J., 2015b. 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., 2019. 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.
Coll, P., Le Cadre, E., Blanchart, E., Hinsinger, P., Villenave, C., 2011. Organic viticulture and soil quality: a long-term study in Southern France. Agric., Ecosyst. Environ., Appl. Soil Ecol. 50, 37–44. https://doi.org/10.1016/j.apsoil.2011.07.013.
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.
Doran, J.W., 1980. Soil microbial and biochemical changes associated with reduced tillage. Soil Sci. Soc. Am. J. 44, 765–771. https://doi.org/10.2136/sssaj1980.03615995004400040022x.
Doucet, M.M.Ade, Gabarra, R., 1994. On the occurrence of *Steinernema glaseri* (Steiner, 1929) (Steinernematidae) and *Heterorhabditis bacteriophora* Poinar, 1976 (Heterorhabditidae) in Catalogne, Spain. Fundam. Appl. Nematol. 17, 441–443.
Dritsoulas, 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. 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 bactivorous nematodes in larvae of the weevil *Diaprepes abbreviatus*. J. Nematol. 35, 187.
DuPont, S.T., Ferris, H., Van Horn, M., 2009. Effects of cover crop quality and quantity on nematode-based soil food webs and nutrient cycling. Agric., Ecosyst. Environ., Appl. Soil Ecol. 41, 157–167. https://doi.org/10.1016/j.apsoil.2008.10.004.
El-Borai, F.E., Duncan, L.W., Preston, J.F., 2005. Bionomics of a phoretic association between *Paenibacillus* sp. and the entomopathogenic nematode *Steinernema diaprepesi*. 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.
Enright, M.R., Griffin, C.T., 2005. Effects of *Paenibacillus nematophilus* on the entomopathogenic nematode Heterorhabditis megidis. J. Invertebr. Pathol. 88, 40–48. https://doi.org/10.1016/j.jip.2004.10.002.
FAOSTAT, 2018. World Food and Agriculture 2018: Statistical Pocketbook. Food and Agriculture Organization of the United Nations.
Félix, M.A., Vierstraete, A., Vanfleteren, J., 2001. Three biological species closely related to *Rhabditis* (*oscheius*) *pseudodolichura* Körner in Osche, 1952. J. Nematol. 33, 104–109.
Ferris, H., Sánchez-Moreno, S., Brennan, E.B., 2012. Structure, functions and interguild relationships of the soil nematode assemblage in organic vegetable production. Agric. Ecosyst. Environ. Appl. Soil Ecol. 61, 16–25. https://doi.org/10.1016/j.apsoil.2012.04.006.
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.
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/jipa.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.
Grewal, P.S., Selvan, S., Gaugler, R., 1994. Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction. J. Therm. Biol. 19, 245–253. https://doi.org/10.1016/0306-4565(94)90047-7.
Griffin, C.T., 2015. Behaviour and population dynamics of entomopathogenic nematodes following application. 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. 57–95. https://doi.org/10.1007/978-3-319-18266-7.
Hiltpold, I., 2015. Prospects in the application technology and formulation of entomopathogenic nematodes for biological control of insect pests. 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. 187–205. https://doi.org/10.1007/978-3-319-18266-7_7.
Hominick, W.M., 2002. Biogeography. Entomopathogenic Nematology. CABI, Wallingford, UK. https://doi.org/10.1079/9780851995670.0035. pp. 155–143.
House, G.J., Stinner, B.R., 1983. Arthropods in no-tillage soybean agroecosystems: community composition and ecosystem interactions. Environ. Manage. 7, 23–28. https://doi.org/10.1007/BF01867037.
Ibáñez-Pascual, S., 2014. Gestión Del Suelo En Viñedo Mediante Cubiertas Vegetales. Incidencia Sobre El Control Del Rendimiento Y El Vigor. Aspectos Ecofisiológicos, Nutricionales, Microclimáticos Y De Calidad Del Mosto Y Del Vino. Universidad de La Rioja, Logroño, Spain ISBN: 978-3-639-55293-55295.
Ito, T., Araki, M., Komatsuzaki, M., Kaneko, N., Ohta, H., 2015. Soil nematode community structure affected by tillage systems and cover crop managements in organic soybean production. Agric. Ecosyst. Environ. Appl. Soil Ecol. 86, 137–147. https://doi.org/10.1016/j.apsoil.2014.10.003.
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., Büchi, L., Mäder, P., Fließbach, A., Charles, R., Degen, T., Turlings, T.C.J., Campos-Herrera, R., 2017. Effects of cover crops on the overwintering success of entomopathogenic nematodes and their antagonists. Agric. Ecosyst. Environ. Appl. Soil Ecol. 114, 62–73. https://doi.org/10.1016/j.apsoil.2017.02.006.
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.
Jenkins, W.R., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Report. 48, 692. https://doi.org/10.1007/s10333.
Jongman, R.H.G., Braak, C.J.F., Ter, Tongeren, van, O.F.R., 1995. Data Analysis in Community and Landscape Ecology, Data Analysis in Community and Landscape Ecology. Cambridge University Press, Cambridge, UK. https://doi.org/10.1017/cbo9780511525575.
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/jipa.1996.0092.
Kour, S., 2017. Occurrence and Characterization of Entomopathogenic Nematodes in Fiji Islands and Their Potential Application in Biocontrol. The University of the South Pacific, Fiji Islands.
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.
Liang, W., Zhang, X., Li, Q., Jiang, Y., Ou, W., Neher, D.A., 2005. Vertical distribution of bacterivorous nematodes under different land uses. J. Nematol. 37, 254–258.
Marquez, J., 2017. Evaluating Effects of No-till Cover Cropping Systems on Indigenous Entomopathogenic Nematodes and Fungi. University of Hawai'i at Mānoa, USA.
McGourty, G.T., Reganold, J.P., 2005. Managing vineyard soil organic matter with cover crops. Soil Environ. Vine Miner. Nutr. 145–150.
Mehlich, A., 1978. New extractant for soil test evaluation of phosphorus, potassium, magnesium, calcium, sodium, manganese and Zinc1. 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.
Millennia, O., Markewitz, D., 2004. Book reviews. Soil Sci. Soc. Am. 104, 681–682. https://doi.org/10.1016/j.agee.2004.07.007.

Nicholls, C.I., Altieri, M.A., Ponti, L., 2008. Enhancing plant diversity for improved insect pest management in Northern California organic vineyards. Acta Hortic. 785, 263–278. https://doi.org/10.17660/ActaHortic.2008.785.32.

Nordbring-Hertz, B., Jansson, H.-B., Tunlid, A., 2006. Nematophagous fungi. Els. John Wiley & Sons, Ltdhttps://doi.org/10.1038/npg.els.0004293.

OIV International organization of vine and wine, 2018. State of the Vitiviniculture World Market April 2018. International Organisation of Vine and Winehttps://doi.org/10.1016/j.psyneuen.2012.01.003.

Palacios Ruiz, E., Gil-Albarellos Marcos, C., Pérez Marín, J., Ortega Sáenz, C., 1995. Un nuevo método de control de la polilla del racimo de la vid: la confusión sexual. Zuba 65–77.

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.

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.

Provost, C., Pedneault, K., 2016. The organic vineyard as a balanced ecosystem: improved organic grape management and impacts on wine quality. Sci. Hortic. (Amsterdam). 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., Nermuť, J., 2020. *Steinernema riojaense* n. sp., a new entomopathogenic nematode (Nematoda: steinernematidae) from Spain. Nematology. https://doi.org/10.1163/15685411-00003343.

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., 2019a. 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.

Sáenz-Romo, M.G., Martínez-García, H., Veas-Bernal, A., Carvajal-Montoya, L.D., Martínez-Villar, E., Ibáñez-Pascual, S., Marco-Mancebón, V.S., Pérez-Moreno, I., 2019b. Effect of ground-cover management on predatory mites (Acari: phytoseiidae) in a Mediterranean vineyard. Vitis - J. Grapevine Res. 58, 25–32. https://doi.org/10.5073/vitis.2019.58.special-issue.25-32.

Shapira, I., Rosenfeld, A., Rothschild, A., Ackerman, M., Eshel, G., Keasar, T., 2017. Herbaceous vegetation enhancement increases biodiversity in a wine-producing vineyard in Israel, promoting shifts in agricultural practices in other vineyards. Conserv. Evid. 14, 10–15. https://doi.org/10.1016/j.agee.2018.08.012.

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.

Stock, P.S., Goodrich-Blair, H., 1997. Nematode parasites, pathogens and associates of insects and invertebrates of economic importance. In: Lacey, L. (Ed.), Manual of Techniques in Insect Pathology. Academic Press, San Diego (USA), pp. 409. https://doi.org/10.1016/b978-0-12-432555-5.x5000-3.

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.

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.

Thies, C., Tscharntke, T., 1999. Landscape structure and biological control in agroecosystems. Science 285, 893–895. https://doi.org/10.1126/science.285.5429.893.

Timper, P., Kaya, H.K., 1989. Role of the second-stage cuticle of entomogenous nematodes in preventing infection by nematophagous fungi. J. Invertebr. Pathol. 54, 314–321. https://doi.org/10.1016/0022-2011(89)90115-8.

Timper, P., Kaya, H.K., Jaffee, B.A., 1991. Survival of entomogenous nematodes in soil infested with the nematode-parasitic fungus *Hirsutella rhossiliensis* (Deuteromycotina: hyphomycetes). Biol. Control 1, 42–50. https://doi.org/10.1016/1049-9644(91)90100-E.

Torr, P., Spiridonov, S.E., Heritage, S., Wilson, M.J., 2007. Habitat associations of two entomopathogenic nematodes: a quantitative study using real-time quantitative polymerase chain reactions. J. Anim. Ecol. 76, 238–245. https://doi.org/10.1111/j.1365-2656.2006.01196.x.

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.

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.

Wheaton, A.D., McKenzie, B.M., Tisdall, J.M., 2008. Management to increase the depth of soft soil improves soil conditions and grapevine performance in an irrigated vineyard. Soil Tillage Res. 98, 68–80. https://doi.org/10.1016/j.still.2007.10.007.

White, G.F., 1927. A method for obtaining infective nematode larvae from cultures. Science 66, 302–303. https://doi.org/10.1126/science.66.1709.302-a.

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.

Woodring, J.L., Kaya, H.K., 1988. Steinernematid and heterorhabditid nematodes: a handbook of techniques, southern cooperative series bulletin. Arkansas Agric. Exp. Sta.

Zhang, L.M., Liu, X.Z., Zhu, S.F., Chen, S.Y., 2006. Detection of the nematophagous fungus *Hirsutella rhossiliensis* in soil by real-time PCR and parasitism bioassay. Biol. Control 36, 316–332. https://doi.org/10.1016/j.biocontrol.2005.08.002.