Prevalence and activity of entomopathogenic nematodes and their antagonists in soils that are subject to different agricultural practices

Geoffrey Jaffuel^a, Paul Mäder^b, Ruben Blanco-Perez^a, Xavier Chiriboga^a, Andreas Fliessbach^b, Ted C.J. Turlings^a, Raquel Campos-Herrera^{a, 1*}

^a FARCE Laboratory, University of Neuchâtel, Emile-Argand 11, Neuchâtel CH 2000 (Switzerland)

^b Department of Soil Sciences, Research Institute of Organic Agriculture (FiBL), Ackerstrasse 113, Postfach 219, CH-5070 Frick (Switzerland)

¹ Present address: Centro para os Recursos Biológicos e Alimentos Mediterrânicos (MeditBio), Campus Gambelas, Edif. 8, FCT, Universidade do Algarve, 8005-139, Faro (Portugal)

*** Corresponding author:** Raquel Campos-Herrera, FARCE Laboratory, University of Neuchâtel, Emile Argand 11, Neuchâtel CH 2000 (Switzerland)

Email: raquel.campos@unine.ch; rcherrera@ualg.pt

Abstract

Agricultural management practices can modify soil properties in ways that may disrupt the abundance and activity of beneficial organisms in the soil. We assessed the impact of different soil management practices on entomopathogenic nematodes (EPN), which have great potential as biological control agents against root-feeding insects. Soils were sampled during spring and autumn 2013 in all 96 plots of a long-term Swiss field trial (DOK experiment) in a seven-year grass-clover ley rotation. By combining a traditional insect-baiting technique and real-time qPCR analyses, we identified and quantified over 20 soil-dwelling species (or genera). This allowed us to investigate how communities of natural EPN populations and their associated natural enemies and competitors are affected by (i) three crop types (wheat, maize and grass-clover ley) and (ii) farming systems, i.e. conventional, organic and biodynamic, which differed in fertilization, and pesticide use. We also determined the effects on soils' microbial biomass in terms of carbon (C_{mic}) and nitrogen (N_{mic}) and applied spatial distribution analysis (SADIE) to uncover patterns of aggregations and associations of the study organisms. Although manure based farming systems increased microbial biomass, the systems did not influence the presence of EPN or their antagonists. EPN were more abundant in winter-wheat plots after potatoes than in maize and grass-clover ley plots. Overall, very low numbers of EPN were recorded, implying that their natural presence would not be sufficient to have a satisfactory suppressive effect on root-feeding pests and the application of EPN would therefore be an appropriate measure to protect yields in case of root pest outbreaks.

Keywords: agricultural practices, annual crop, entomopathogenic nematodes, farming systems, inter-guild competition, quantitative real-time PCR

1. Introduction

Agricultural production relies on healthy soils that guarantee essential soil functions such as carbon, nutrient, and water cycling. Traditionally, soil quality has been characterized by the presence of nutrients and water and their availability to the crop (Patzel et al., 2000). The development of agricultural practices such as fertilization and irrigation have largely enhanced crop yield; yet, protecting the crops against herbivores and diseases is also a fundamental aspect of these practices, as pests can reduce yields by up to 30% (Oerke, 2005). Crop rotation, cover crops and organic amendments in cropping systems aim at indirectly controlling pests and diseases; however, due to economic pressure, these strategies are often neglected in highly specialized agricultural cropping systems. Instead, different types of pesticides are readily applied. Soil pests are hard to reach with pesticides and exceedingly large amounts need to be applied in order to be effective. This has led to major environmental concerns, and an increasing number of pesticides are being banned (Pimentel, 1995; van der Werf, 1996). Alternatives are badly needed. As a first step, the current study explores the presence of natural biological control agents in agricultural soils in order to estimate their potential to suppress soil pest populations.

A large diversity of microorganisms have the potential to protect plants against pests and diseases if applied in an appropriate manner (Lacey et al., 2015). Efforts to develop biocontrol methods by augmenting beneficial soil organisms or by promoting their natural occurrence have been successful, but are still unsuitable for most types of large-scale agriculture (Bale et al., 2008; Lacey et al., 2015). The Swiss National Research Program 68 (NPR68; <http://www.nfp68.ch/>) aims to enhance the use of soil natural resources that can improve plant health and, thus, favor plant protection and yield. As a first step towards the development of new biological control methods for sustainable agriculture, a comprehensive inventory of selected beneficial organisms of a given agroecosystem is required, together with knowledge about the factors that might determine their abundance.

Entomopathogenic nematodes (EPN) of the families Steinernematidae and Heterorhabditidae are obligate parasites of insects and are considered excellent biological control agents (Georgis et al., 2006; Kaya et al., 2006; Lewis et al., 2006; Dolinski et al., 2012; Campos-Herrera, 2015; Lacey et al., 2015). Indeed, their infective juveniles (IJs) are able to kill an insect host within 2-3 days. The *modus operandi* of IJs is to actively seek and penetrate a suitable host. Once inside, IJs release their mutualistic γ -proteobacteria in the insect hemocoel. Toxins produced by the bacteria result in the death of insect host by septicemia. Thereafter, EPN and bacteria reproduce for several generations until the resources inside the host are fully consumed. Thousands of newly produced IJs then exit the cadaver and start a new life cycle (Adams and Nguyen, 2002; Dillman et al., 2012).

In the agroecosystem, EPN are affected by various abiotic soil properties such as soil texture, moisture, temperature, and soil organic matter, which might be drastically altered by agricultural management practices, as well as biotic factors such as competitors and natural enemies (Stuart et al., 2006, 2015; Lewis et al., 2015). Understanding these interactions is essential to reveal ways to enhance the potential of EPN as biocontrol agents in a particular soil type and agricultural scenario. Indeed, this knowledge should allow us to apply EPN more judiciously and to increase their efficacy against targeted insect pests. Several studies have demonstrated negative effects of intensive soil management on EPN (Hummel et al., 2002; Campos-Herrera et al., 2008, 2010, 2014). A few studies even found a positive effect of organic soil managed on EPN populations (Briar et al., 2007; Campos-Herrera et al., 2008, 2010). However, we cannot state this as a general rule, since a lack of such effects or even contrary findings have also been reported (Ferris et al., 1996; Bell and Raczkowski, 2008). This calls for more research on the factors that determine EPN occurrence in agricultural fields, but also on other members of the associated food web that may compete, kill or otherwise interact with EPN. Indeed, the distribution of EPN is also affected by the presence of other important organisms in the soil. For example, predators such as microarthropods or nematophagous fungi (NF), ectoparasitic bacteria or natural competitors such as free living nematodes (FLN) have a significant impact on the population

dynamics of EPN (El-Borai et al., 2005; Enright et al., 2005; Jabbour and Barberchek, 2011; Campos-Herrera et al. 2012, 2013a; Pathak et al., 2012). These organisms have been shown to be spatially associated with EPN in the field (Campos-Herrera et al., 2013a). Besides their association with EPN, NF and FLN are also sensitive to abiotic factors (Persmark et al., 1996; Jaffee et al., 1998; Neher, 1999, 2010; Campos-Herrera et al., 2015a). Learning more about the factors that determine the prevalence of the organisms that are directly associated with EPN, can provide additional information on how farming practices might contribute to this important feature of soil health. Hence, the aim of this study was to investigate the effects of farming practices and crop type on the prevalence of EPN and their antagonists. We also investigated the microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) in the soils of each farming system. The overall expectation was that soils with high levels of natural organic matter and minimal soil disturbance sustain larger numbers of soil microorganisms, including EPN and associate organisms. To test this hypothesis we combined traditional insect baits and new molecular methods to screen for the presence and abundance of organisms belonging to the EPN soil food web as proposed by Campos-Herrera et al. (2015a). We analyzed samples coming from a long-term field experiment that started in 1978, which aims at studying the effect of biodynamic, bio-organic and conventional farming systems in a seven-year crop rotation system (Mäder et al., 2002).

2. Material and methods

2.1. Field experiment design and soil sampling

The “DOK” experiment (Dynamisch-Organisch-Konventionell, in German) is located in Therwil, Switzerland (7° 33'E, 47° 30' N). The field experiment is managed by Agroscope (Reckenholz) and the Research Institute of Organic Agriculture (FiBL). The DOK field experiment consists of 96 plots, assigned to eight different treatments (12 replicates per treatment) corresponding

to four farming practices and two different levels of organic fertilizer use (Table 1, Fig. S1). The conventional systems are managed in accordance with Swiss standards for integrated farming, applying crop rotation, cover crops and catch crops, integrated nutrient management, and use of pesticides according economic thresholds (integrated pest control).

In the DOK field, a seven-year crop rotation has been applied since 1978 (Mäder et al., 2002; Fliessbach et al., 2007). It changed in the course of time: in the fifth rotation from 2006 to 2012 it consisted of maize (*Zea mays* L.), winter wheat 1st year (*Triticum aestivum* L.) followed by catch crop rye (*Secale cereale* L.), soybean (*Glycine max* L.) followed by catch crop rye, potato (*Solanum tuberosum* L.), winter wheat 2nd year and two years of grass clover ley, standard mixture STM 330: *Trifolium pratense* L. 6%; *T. repens* L. 12%; *Dactylis glomerata* L. 17%; *Festuca pratensis* Huds. 36%; *Phleum pratense* L. 8%; *Lolium perenne* L. 21%). This crop rotation was applied on three parallel main plots in the DOK experiment, displaced in time. In 2013, the year that we did the soil sampling, the crops planted were: maize var. Colisée (pre-crop grass-clover 2nd year), winter wheat 2nd year, var. Runal (pre-crop potato) and a grass-clover ley in its 1st year (pre-crop winter wheat 2, grass-clover planted in August 2012, standard mixture STM 330) with 32 replicates (plots) per crop (Fig. S1). The different management applied to the plots in 2012 and 2013 are presented in Table S1 and S2.

On April 23rd and October 16th 2013, we randomly collected 20 soil cores per plot (2.5 cm diam. x 20 cm depth), which were pooled in a plastic bag to obtain a composite sample of ~ 3 kg of soil per plot. The plastic bags were transported to the laboratory and kept at 4°C until use.

2.2. Nematode extraction, estimation of the potential of the soil to suppress pest insects, and assessment of microbial biomass-C (*C_{mic}*) and biomass-N (*C_{mic}*)

Soil was thoroughly mixed in the laboratory and stored at 4°C in the dark before further processing. Nematodes were extracted no later than 2-4 days after collection, in such an order as to

ensure that no temporal bias was introduced, following protocols described by Campos-Herrera et al. (2015a). Briefly, three fresh subsamples of 500 g were prepared from each of the 96 soil composite samples. One of these 500 g soil aliquots was used to extract nematodes and other associated organisms by sucrose centrifugation (Jenkins, 1964) for real-time qPCR analyses (Campos-Herrera et al., 2015a). A second 500 g fresh soil aliquot was baited with *Galleria mellonella* (Lepidoptera: Pyralidae) to test the suppressive potential of the soil. Following Bedding and Akhurst (1975) with few modifications, two portions of 250 g soil of the subsamples from each plots were baited with 5 final instar *G. mellonella* larvae (commercial stock, Au Pêcheur SARL Neuchâtel, Switzerland) in two independent rounds. Conditions and processing were carried out following Campos-Herrera et al. (2015a). After exposure for 4 days, the *G. mellonella* cadavers were recovered from the soil, thoroughly rinsed with tap water, and individually placed in White traps, which are routinely used to collect emerging nematodes (White 1927). Every 2-3 days, we checked for the organisms responsible for the mortality of *G. mellonella* larvae under the stereoscope. In case of nematode infection, nematodes were recovered at emergence. After a month of incubation, cadavers for which no obvious cause of death could be discerned were discarded after dissection. We confirmed EPN infection by performing Koch's postulate (Lacey, 1997). Each nematode isolate that was obtained from the soil samples (Original isolated from *G. mellonella*, hereafter OG) was placed in a 2 × 5.5 cm Petri dish containing four *G. mellonella* larvae. Larval mortality was assessed twice a week and emerging nematodes recovered (Multiplication of nematodes in *G. mellonella* larvae, hereafter, MG). A second round was performed to confirm the negative results (Campos-Herrera et al., 2015a).

The third aliquot of 500 g fresh soil was used to measure soil microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}), which was determined by chloroform-fumigation-extraction (CFE) following Fließbach et al. (2007). Triplicates of 20 g (dry soil) per sample were fumigated with chloroform and subsequently extracted in 80 mL of 0.5 M K_2SO_4 solution. In order to be able to relate our findings to a

given amount of dry soil, we dried 180 g aliquots of wet soil of all the samples at 40°C for 3-4 days and estimated the water content.

2.3. Identification and quantification of entomopathogenic nematodes and members of their soil food web

The cultures of the organisms used as positive controls, the subsequent DNA extraction, and the standard curves establishment for the different targeted organisms were generated following Campos-Herrera et al. (2015a). We employed the Power Soil DNA Isolation Kit (MoBio laboratories, Inc.) for the DNA extraction of both soil samples and pure cultures when living material was available. In some cases (see Table S3), when no living material was available, we employed plasmids with the whole sequence of interest to establish our positive control (see Campos-Herrera et al., 2015a). Aliquots of DNA samples not used in the study were stored at –80 °C. The quality and quantity of each DNA sample was analyzed using 1 µL per duplicate in a Nanodrop 1000 (Thermo Scientific, Wilmington, DE, USA).

The identification and quantification of a total of 24 organisms comprising EPN and members of the same soil food web (Table S3) was performed using real-time PCR protocols following the MIQE procedure (Bustin et al. 2009). The species were selected based on previous surveys of Swiss soils, plus we added some species with a high potential to be found in temperate climates (Steiner, 1996; Kramer et al., 2001; Hominick, 2002; Campos-Herrera et al., 2015a). Specifically, we screened for a total of 13 EPN species (*Heterorhabditis megidis*, *H. bacteriophora*, *H. zealandica*, *Steinernema feltiae*, *S. carpocapsae*, *S. glaseri*, *S. kraussei-silvaticum*, *S. affine*, *S. bicornotum*, *S. intermedium*, *S. weiseri*, *S. poinari*, and *S. intermedium* group), six nematophagous fungi (NF) (*Arthrobotrys oligospora*, *A. dactyloides*, *A. musiformis*, *Purpureocillium lilacinus*, *Hirustella rhossiliensis*, and *Catenaria* sp.), four free-living nematodes (*Acrobeloides*-group, *Oscheius tipulae*, *Oscheius onirici* and

191 *Oscheius* sp. 3) and one ectoparasitic bacterium (*Paenibacillus nematophilus*) (Atkins et al., 2005;
192 Zhang et al., 2006; Torr et al., 2007; Pathak et al., 2012; Campos-Herrera et al., 2011a, 2011b, 2012,
193 2015a, 2015b). In some cases, we described the maximum taxonomical resolution that our molecular
194 tools can provide, which concerns the data for *S. kraussei-silvaticum* or *Acrobelloides*-group, as
195 discussed in Campos-Herrera et al. (2012, 2015a).

196 We employed optical 100-well gene disc (Biolabo, scientific instruments, Switzerland)
197 reaction plates on the Corbett Research real time PCR machine. All samples were run in duplicates
198 (unknown, positive and negative controls) in all the reactions. Final reaction, concentrations, and
199 protocols have been described by Campos-Herrera et al. (2015a). The soil DNA extractions were
200 individually screened with the corresponding unique species-specific primers/probes combination for
201 each species, which represent >80 full-plate reactions (including some re-runs for confirmation).
202 Numbers of nematodes collected from the soil samples were assessed from a 10-fold dilution of the
203 DNA, whereas total DNA without dilutions was used for the identification and quantification of NF
204 and ectoparasitic bacteria using the adapted qPCR procedure as described for EPN (see details in
205 Campos-Herrera et al., 2015a). A correction factor was derived from the dilution series to transform
206 qPCR data to numbers of IJs. Similarly, aliquots of nematodes corresponding to all the MG isolates
207 were used for DNA extraction, but using QIAamp DNA mini kit (Qiagen). DNA quality and quantity
208 was verified (Nanodrop system) and samples were diluted to the range of 0.5-1 ng/μL.

209 Real time qPCR was also used to assess the abundance and identity of the progeny of each
210 MG, screening for all the EPN and FLN species that were initially detected in the soil samples. In this
211 case, aliquots of these samples were pooled four by four and adjusted to have a final 10-fold dilution
212 volume. When a species was detected in the pooled sample, we analyzed the corresponding samples
213 individually to identify the exact sample(s) that contained the species. In addition to this, the sequences
214 corresponding to the ITS region of selected MG isolates were amplified and sequenced as described by
215 Campos-Herrera et al. (2015a) to confirm species identity.

2.4. Statistical analysis

Data from the *G. mellonella* baits allow us to estimate the suppressive capacity of each sampled plot (here called “activity” and defined as the percentage of larval mortality per plot, averaged by treatment). The activity was determined with respect to the total mortality, caused by any agent (i.e. nematode, bacteria, fungi, virus, etc.) and caused by nematodes or EPN only. A General Linear Model (GLM) fitting a binomial distribution was used to analyze the data obtained by *G. mellonella* baits using management practices, crop and the period of sampling as factors.

All the organisms’ variables quantified by using qPCR were expressed per 100 g of dry soil. Thereafter, data were transformed for further analyses following Campos-Herrera et al. (2015a). The parasitism of nematodes by NF was determined by dividing the DNA quantity of each species by the total amount of DNA and expressed as “infection rate” (Campos-Herrera et al., 2012; Duncan et al., 2013). To estimate the total FLN and NF, we standardized the units of measurement among species to be on a scale of 0 to 1, by dividing all data within a species by the highest measurement for that species (de Rooij van der Goes et al., 1995). EPN abundance was expressed as number of IJs per 100 g of soil. We also calculated the EPN species richness, equivalent to the total number of species per plots.

C_{mic} and N_{mic} values obtained by CFE were analyzed without any transformation. A linear model with stepwise procedure was used to analyze qPCR and CFE data, using (i) farming system, (ii) crop and (iii) period of sampling as factors and every sample as a random factor to account for repeated measurements. Normality and equal variances of the data were assessed, but most of the data failed those assumptions, thus the reported F-values were obtained from the original model whereas *P*-values were obtained by 1000 permutations using the “pbkrtest” package of R 3.1.0 (CRAN). Data are presented as mean $\pm$ SEM of the untransformed values.

The competition among nematodes (FLN and EPN) for a cadaver (MG samples) was determined using relative proportion of Ct values obtained from the qPCR for each species. Because a low Ct value indicates a high concentration and vice versa, we subtracted the total number of cycles run during the qPCR process ($n = 36$) to the final Ct value to obtain a proxy of relative proportion per nematode species in each of the cadaver (Campos-Herrera et al., 2015b). In addition to this, we used Cts obtained from both nematodes isolated from the soil and nematodes that emerged from the subsequent Koch's postulate larvae. We compared the proportion of nematodes detected in these cadavers to determine species prevalence.

As suggested by Campos-Herrera et al. (2013a), we employed SADIEshell (v2.0) (Kelvin F. Conrad & IACR-Rothamsted 2001), to explore the spatial distribution of the different investigated trophic guilds. SADIEshell uses the measurements of abundance in relation to the coordinates. This provides the degree of clustering of the data (patches or gaps) that can be associated with the species distribution and the association or dissociation of two trophic guilds populations. The resulting cluster indices were used to draw contour maps that represent the distribution of the targeted species. We focused on the "index of aggregation" (I_a), explained as follows: value of $I_a = 1$ suggests a spatially random pattern, $I_a > 1$ suggests an aggregated pattern and $I_a < 1$ indicates a regular pattern (Perry, 1998). In addition to this, we obtained the index of association (X) among selected organisms. This index describes the degree of association or dissociation of two variables, with a positive X index suggesting a local association, and a negative value indicating a local disassociation. Coordinates (x, y) were assigned to the plots and used to perform the different analyses described above. The analyses were carried out using qPCR transformed data (for NF, EPN and FLN) and values obtain from CFE (for C_{mic} and N_{mic}).

3. Results

3.1. Measurements by qPCR of entomopathogenic nematodes and members of their soil food web

EPN were detected in 63.5% of the plots. Overall, we detected 6 out of the 13 targeted EPN species: *S. affine*, *S. carpocapsae*, *S. feltiae*, *S. poinari*, *H. bacteriophora* and *H. megidis*. The most common species was *H. megidis*, which was found in 30.2% of the plots, followed by *H. bacteriophora*, detected in 29.1% of the plots, but only in the spring sampling. *S. carpocapsae*, *S. feltiae*, *S. poinari* and *S. affine* were less abundant and were detected in 13.5%, 7.3%, 5.2% and 4.2% of the plots, respectively. Because of the low quantities detected (on average below 1 IJ/ 100g of dry soil), statistical analyses were only performed for the total number of IJs, irrespective of species. The EPN community showed significantly higher number of IJs in October than in April (Table 2). Also, significantly higher numbers of IJs were recorded in plots planted with winter wheat and maize than those in the recently sown artificial grass-clover plots (Table 2, Fig. 1a), whereas farming system did not significantly affect the EPN community (Table 2, Fig. 2a). The EPN richness was only affected by the sampling period ($P < 0.001$) (data not shown).

NF were detected in almost all the plots (98.9 %) and we detected the presence of 4 of the 6 targeted NF species in the soil. The dominant species were *Catenaria* sp. and *H. rhossiliensis*, which were found in 89.6% and 90.6% of the plots, respectively. *P. lilacinus* and *A. oligospora* were less abundant with 52.1% and 72.9% of detection, respectively. *Arthrobotrys dactyloides* and *A. musiformis*, both trapping NF species, were not detected. The NF community was more abundant in October than in April (Table 2). Higher values, but not statistically significant, were recorded in plots planted with winter wheat than in those with grass-clover ley and maize (Table 2, Fig. 1b). Like with the EPN guild, the farming practices did not have a significant impact on the targeted NF community (Table 2, Fig. 2b).

We did not detect the ectoparasitic bacterium *Paenibacillus nematophilus* in any of our sample. By contrast, the free living nematodes (FLN) that compete with EPN for cadavers, namely *Osccheius*

spp. and nematodes belonging to the *Acrobeloides*-group were detected in all of the 96 plots. Out of the 4 species targeted, the only nematode not detected was *Oscheius* sp 3. More precisely, the *Acrobeloides*-group was found in all of the plots, whereas *Oscheius onirici* and *Oscheius tipulae* occurred both in 95.8% of the plots. In agreement with the EPN, the FLN were significantly more abundant in October than in April (Table 2). Similarly to the other trophic guilds, significantly higher values were recorded in plots planted with winter wheat than in those with grass-clover ley and maize (Table 2, Fig. 1c), and again no differences were found among different farming practices (Table 2, Fig. 2c).

3.2. Soil suppression and microbial biomass

Considering all the plots and both periods of sampling, the mean total activity of the soil, defined as the mean percentage of *G. mellonella* larval mortality by various known causes, including nematode, bacteria and fungi, was 30.7%. The activity was spread over the entire DOK field with 87.3% of the plots showing some activity. Of all larvae, 12.6% were infected by nematodes (FLN+EPN), which is almost half of the total measured activity. Nematode activity was registered in 35.5% of the plots, but EPN were responsible for only half of the nematode activity (5.8%). Total numbers and activity of nematodes differed significantly between the two sampling periods, with a higher percentage of cadavers with nematodes in October than in April (Table 2). The crop type significantly shaped the activity of nematodes, with higher activity observed in the wheat plots than in the plots with the two other crops (Table 2, Fig. 3a), but this was not the case for the total activity (Table 2, Fig. 3b). The farming practices applied to the different plots, however, did not have any influence on the activity of organisms (Table 2, Fig. 4). As far as the microbial biomass proxies C_{mic} and N_{mic} were concerned, the type of crop did not affect C_{mic} or N_{mic} (Table 2). As expected, C_{mic} and N_{mic} were significantly influenced by the farming practices (Table 2, Fig. S2), with the highest values

observed in the biodynamic (N_{mic} and C_{mic}) and organic (only C_{mic}) plots. Finally, the period of sampling only influenced N_{mic} , with higher values in October (Table 2).

3.3. Inter and intra-specific nematode guild competition for the cadavers

The qPCR data collected from Koch's postulate cadavers (MG) revealed the same species as were detected in the soil sample, but with the addition of *S. kraussei-silvaticum*. Mixed nematode progenies (EPN+FLN) were found in 44% of all the MG records ($n = 254$). In total, EPN represented 11.5% of the nematodes reproducing from the Koch's postulate cadavers, whereas free-living *Osccheius* spp. represented 88.5%. In the soil samples, Steinernematidae and Heterorhabditae showed similar proportional occurrences, accounting for 2.6% and 4.3%, respectively, while Heterorhabditae were almost absent in MG cadavers (0.3%) in favor of the Steinernematidae (11.1%). The *Osccheius* spp. were even more dominant in the MG (88.5%) than in the soil samples (66.7%). The *Acrobeloides*-group, very abundant in the soil (26.2%), did not reproduce during any of the subsequent MG steps (Fig. 5).

3.4. Spatial distribution and association

Results from SADIE analyses suggest that EPN were randomly distributed in the DOK field. FLN, C_{mic} and N_{mic} were aggregated and NF followed a regular pattern (Table S4). EPN were associated in the field with all of the four tested parameters (NF, FLN, C_{mic} and N_{mic}) (Table S5). These associations in the distributions of EPN, FLN and NF throughout the DOK field were transposed to a contour map in Surfer 12 (Trial version, Golden Software, 2015), (Fig. S3).

4. Discussion

Entomopathogenic nematodes, which can serve as biological control agents, were found to be very rare in the soil of the DOK field (less than 1 IJs per 100 g of dry soil). With the exception of *S. poinari*, all of the EPN species that we detected have previously been reported for Swiss soils (Steiner, 1996; Kramer et al., 2001; Hominick, 2002; Campos-Herrera et al., 2015a). Interestingly, and contrary to our hypothesis, differences in farming system did not affect the presence or abundance of EPN or any of the associated food web members included in this study, except for the microbial biomass measurement. On the other hand, the cropping sequence (pre-crop) strongly affected EPN density. For most of the species, only trace levels were detected. The low numbers were similar to those recorded in other long-term Swiss field experiments dealing with other agronomic treatments, such as arable land under various tillage practices (Campos-Herrera et al., 2015a). The current study, as well as the findings by Campos Herrera et al. (2015a), support the idea that the disturbance caused in the soil as consequence of arable crop production negatively affects the natural occurrence of EPN to the extent that it might mask possible additional detrimental effects caused by other soil modifications. It is also possible that the low EPN numbers are caused by an absence of suitable insect hosts due to effective crop rotation strategies applied in Switzerland. The DOK experiment started 35 years ago and involves a seven-year crop rotation, well balanced between cereals, root crops, leguminous crops and grass-clover ley. Our experiment was also preceded by crop rotations, including three years of grass-clover. There was no indication that any of these treatments affected EPN presence. It should be noted that the overall scarcity of the EPN might have been the main reason for the fact that we did not find any difference between the practices. At higher EPN densities these practices may have an effect and EPN scarcity could be simply explained by the general absence of insect hosts (Stuart et al. 2015). Several studies have correlated the abundance of hosts with the abundance of EPN, but found contrasting findings. Those correlations are difficult to interpret as EPN infect a wide variety of insects, but also have more or less suitable hosts (Půža and Mráček, 2005, Mráček et al. 1999, 2005). It likely that in the

DOK field management practices, as well as the lack of hosts, interferes with the proliferation of EPN. Moreover, the EPN were out-numbered by competing FLN species, in particular two species from the *Oscheius* genus, *O. tipulae* and *O. onirici*. Nematodes emergence from *G. mellonella* cadavers by EPN (5.8%) was similar to the one by FLN (6.8%). Cadavers that were obtained following Koch's postulate, i.e. killed by nematode offspring that originated from the cadavers collected from the original soil samples, showed a dominance of the two *Oscheius* spp. These FLN have been recently reported as important competitors of the EPN for the cadavers in a species-specific and density-dependent manner (Campos-Herrera et al., 2015b). This strong competition for the cadavers by the FLN limits the reproduction potential of EPN under those circumstances, as FLN may displace EPN from the cadaver. Nematodes from the *Acrobeloides* group can interfere with the reproduction of EPN, but this potential interference is EPN species-dependent (Campos-Herrera et al., 2012). It seems that *Acrobeloides* nematodes can reproduce concomitantly with EPN-cadavers, but since they cannot kill the insect they will not contribute, but rather interfere with pest control (Campos-Herrera et al., 2012). For *Oscheius* spp. this is less clear (Dillman et al., 2012; Campos-Herrera et al., 2015b) and needs to be further investigated.

Despite the low numbers of IJs, the results showed a relatively high diversity of EPN species, with a total of seven species detected at the DOK site. But there was no difference in species richness among the different farming systems nor was there a difference among crop type. Under different circumstances (i.e. other crop included in rotations, type of soil, climate conditions) with more insect hosts and/or fewer competing FLN, the EPN diversity might be considerably higher.

The long-term conventional application of fertilizers had no impact on the abundance or the activity of nematodes, nor did it affect the associated organisms. In a comparable study in La Rioja (Spain) by Campos-Herrera et al. (2008, 2010), no EPN were detected in annual arable crops, independently of whether organic or conventional management was applied. Similarly, Jaffee et al. (1998) observed that the use of chemical fertilizers in the conventional systems do not have any effect

on EPN. But, some other studies in different types of crops have shown that the effect of conventional soil management depends on the period of sampling (Campos-Herrera et al. 2010), or did not detect any effect (Bell and Raczkowski, 2008). The only parameters that were influenced by the farming practices were those of the microbial biomass, with both C_{mic} and N_{mic} showing the highest values in the biodynamic plots, and increased values for C_{mic} in organic plots. These findings are consistent with what has been found before in the same field site over a period of 21 years (Fließbach et al., 2007; Esperschütz et al., 2007), and with the recent evaluation of the soil microbial diversity under long-term organic and conventional farming (Hartmann et al., 2015). The microbial biomass has followed the same pattern over many years in the DOK field, highlighting the consistency of plot management. It should be noted that in addition to the agricultural practices and crops that were planted, plots differed in soil clay content due to a specific gradient of clay that is present in the DOK field (P. Mäder, personal communication). However, we did not find any correlation between clay content and the presence of any of the organisms (data not shown) and conclude that the clay content was not one of the main factors shaping the distribution of the studied species in the DOK field.

To our knowledge, this is the first large scale, replicated study to investigate whether crop type influences the distribution of nematodes and their associated food web members at the same field site. The EPN, as the other associated organisms investigated were found to be most numerous in wheat plots (after potatoes as preceding crop). In accordance, the highest activity of nematodes was recorded in the wheat plots. Interpretation should be done with caution since we were investigating a field subjected to crop rotation and it is well possible that the preceding crop (Fig S1), at least in part, influenced the observed pattern. Records from the DOK field suggest a generally low prevalence of root-feeding insects in wheat plots, but an infestation by wireworms was noticed on the preceding potatoes (P. Mader, personal communication). It is, therefore, indeed possible that rotation and the soil fauna established during the earlier cultivation had an effect on EPN presence in the following winter wheat plots. Nevertheless, differences in rhizosphere structure and soil community among wheat,

maize and grass-clover fields (Demarta et al., 2014), as well as differences in the complexity of these habitat (Jabbour and Barbercheck, 2008) may explain the pattern observed for EPN.

We found seasonal variability in the abundance of all the organisms that were investigated, densities and activities being higher in autumn (October sampling) than in spring (April sampling). While this inference is only based on two sampling events, the phenology we observed is consistent with data reported by other studies (Efron et al., 2001, Campos-Herrera et al., 2008, 2010, 2015a). The advantage of repeating our measurements two times over the year, one in spring and another in autumn, is that we can assess if the differences due to the crop and the management were consistent over time. It is remarkable that even with the expected temporal differences, we obtain very consistent pattern in response to the management practices and the crop type, for both sampling periods.

Using SADIE analyses, we showed how EPN presence is associated in the field with the other trophic guilds (FLN, NF and microbial biomass). EPN are likely to suffer from predation and competition, which may significantly decrease their efficiency as biocontrol agents (Stuart et al., 2015). This is relevant for crop protection strategies that included EPN augmentation, as the response of FLN and NF populations will play a key role in the successful establishment of EPN populations. These results and those of studies in other crop systems start to provide evidence that the trophic cascades in the soil can impact crop health and even yield (Campos-Herrera et al., 2013b, 2014; Duncan et al., 2013). Our results further confirm that qPCR data and traditional baiting results can be combined to assess the spatial distribution of trophic groups from soil samples (Campos-Herrera et al., 2013a).

Overall, we found a high proportion of mixed progeny of nematodes emerging from Koch's postulate cadavers, with FLN species and different EPN species co-existing in the same cadaver. Studies on interspecific (Kondo, 1989; Alatorre-Rosas and Kaya, 1990 reviewed in 1991; Koppenhöfer and Kaya, 1996; Koppenhöfer et al., 1995; Duncan et al., 2003, Půza and Mráček, 2009) and intraspecific competition (Selvan et al., 1993, Koppenhöfer and Kaya, 1995; O'Callaghan et al., 2014) among EPN show that cadavers can produce mixed progenies, but that in some cases one of the species

is more suppressed than the other (e.g: Půža and Mráček, 2009) or the numbers of both species are dramatically reduced (e.g: Koppenhöfer and Kaya, 1996). Competition of EPN with FLN within cadavers has also been reported, but has been less extensively studied than competition between EPN species (Campos-Herrera et al., 2012; Duncan et al., 2003). Recently, relying on molecular data, Campos-Herrera et al. (2015a) found that several EPN and FLN species can coexist in the same cadaver. In a subsequent study, by using qPCR assays Campos-Herrera et al. (2015b) showed that two FLN species, *Oscheius tipulae* and *Oscheius onirici*, were unable to kill a *G. mellonella* larvae by themselves, but were readily able to reproduce in larvae co-infected with EPN. The current data support these observations, i.e. FLN species belonging to *Oscheius* spp. were able to reproduce and could be maintained over several generations on *G. mellonella* larvae, as long as they were in the presence of EPN. More importantly from an agronomic perspective, our results suggest that in the DOK field, EPN may be prevented from reproducing to their full potential due to strong competition with FLN. Their main competitors (*Oscheius* spp.) were much more abundant than EPN in both the soil and the cadavers used to fulfill Koch's postulate.

5. Conclusions

The differences in fertilization methods and plant protection for the four farming systems evaluated in this study had no impact on the abundance or the activity of nematodes, nor did they affect the associated organisms. However, crop type and the crop history (crops in rotation) might have an important effect. EPN, as well as the associated organisms were found to be most numerous in wheat plots (after potatoes), which is in agreement with the highest activity of nematodes recorded in the plots with wheat. We consider that under this ecological scenario, the biocontrol service offered by the native EPN might be very limited, as they occurred only in very low numbers, showed weak activity, and were in strong competition with other nematode guilds. It may therefore be advisable to use an

augmentative approach in the case of a serious root pest outbreak, but further research is needed to confirm the effectiveness of such a strategy. It also remains to be determined whether the association of EPN species with *Oscheius* species and other FLN is detrimental or beneficial, this is the subject of ongoing studies. EPN release as biocontrol appears to be most promising in intensive conventional crop rotation with mono-cultures, but there is also great potential in organic and integrated farming for specific crops such as potatoes, as they suffer from severe wireworm infestations.

Acknowledgements

We thank the members of the FARCE laboratory for their frequent assistance and relevant discussion, in particular, Jinwon Kim and Ilham Sbaiti for their help during different steps of the measurements. We especially thank our field technicians at FiBL for their precious help during the sampling, and Anton Kuhn and Munyangabe Adolphe for lab analyses. We also thank Corentin Corblin and Maud Sivera for spending part of their weekend recovering cadavers from soil samples. and Thomas Degen for his comments on an earlier version of the manuscript. This work was supported by an economic stimulus grant (51NF40-144621) and by the National Research Program 68 (NRP68) grant (406840_143065) from the Swiss National Science Foundation. GJ was supported by an assistantship from the University of Neuchâtel and XC was supported by a PhD fellowship from the Commission Fédérale des Bourses pour Etudiants Etrangères CFBE (Confédération Suisse).

References

- Adams, B.J., Nguyen, K.B., 2002. Taxonomy and systematics. In: Gaugler, R. (Ed.). Entomopathogenic Nematology CABI Publishing, Wallingford, UK, pp. 1-34.
- Alatorre-Rosas, R., Kaya. H.K., 1990. Interspecific competition between entomopathogenic nematodes in the genera *Heterorhabditis* and *Steinernema* for an insect host in sand. J. Invertebr. Pathol. 55, 179–188.

490 Atkins, S.D., Clark, I.M., Pande, S., Hirsch, P.R., Kerry, B.R., 2005. The use of real-time PCR and
 491 species-specific primers for the identification and monitoring of *Paecilomyces lilacinus* FEMS
 492 Microbiol. Ecol. 51, 257–264.

493 Bale, J.S., van Lenteren, J.C., Bigler, F., 2008. Biological control and sustainable food production.
 494 Philos Trans. R. Soc. Lond. B Biol. Sci. 363, 761–776.

495 Bedding, R.A., Akhurst, R.J., 1975. A simple technique for the detection of insect parasitic rhabditid
 496 nematodes in soil. Nematologica 21, 109–110.

497 Bell, M.C., Raczowski, C.W., 2008. Soil property indices for assessing short-term changes in soil
 498 quality. Renew Agr. Food Syst. 23, 70–79.

499 Briar, S.S., Grewal, P.S., Somasekhar, N., Stinner, D., Miller, S.A., 2007. Soil nematode community,
 500 organic matter, microbial biomass and nitrogen dynamics in field plots transitioning from
 501 conventional to organic management. Appl. Soil Ecol. 37, 256–266.

502 Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Hugget, J., Kubista, M., et al., 2009. The MIQE
 503 guidelines: Minimum information for publication of quantitative real-time PCR experiments.
 504 Clínica/ Chemistry 55, 611–622.

505 Campos-Herrera, R. (ed.). 2015. Nematode Pathogenesis of Insects and Other Pests. Springer
 506 International Publishing Switzerland.

507 Campos-Herrera, R., Gómez-Ros, J.M., Escuer, M., Cuadra, L., Barrios, L., Gutiérrez, C., 2008.
 508 Diversity, occurrence, and life characteristics of natural entomopathogenic nematode populations
 509 from La Rioja (Northern Spain) under different agricultural management and their relationships
 510 with soil factors. Soil Biol. Biochem. 40, 1474–1484.

511 Campos-Herrera, R., Piedra-Buena, A., Escuer, M., Montalbán, B., Gutiérrez, C., 2010. Effect of
 512 seasonality and agricultural practices on occurrence of entomopathogenic nematodes and soil
 513 characteristics in La Rioja. Pedobiologia 53, 253–258.

514 Campos-Herrera, R., Johnson, E.G., Stuart, R.J., Graham, J.H., Duncan, L.W., 2011a. Long-term
 515 stability of entomopathogenic nematode spatial patterns in soil as measured by sentinel insects and
 516 Real-Time PCR assays. *Ann. Appl. Biol.* 158, 55–68.

517 Campos-Herrera, R., El-Borai, F.E., Stuart, R.J., Graham, J.H., Duncan, L.W., 2011b.
 518 Entomopathogenic nematodes, phoretic *Paenibacillus* spp., and the use of real time quantitative
 519 PCR to explore soil food webs in Florida citrus groves. *J. Invertebr. Pathol.* 108, 30–39.

520 Campos-Herrera, R., El-Borai, F.E., Duncan, L.W., 2012. Wide interguild relationships among
 521 entomopathogenic and free-living nematodes in soil as measured by real time qPCR. *J. Invertebr.*
 522 *Pathol.* 111, 126–135.

523 Campos-Herrera, R., Ali, J.G., Diaz, B.M., Duncan, L.W., 2013a. Analyzing spatial patterns linked to
 524 the ecology of herbivores and their natural enemies in the soil. *Front. Plant Sci.* 4 (September), 1–
 525 18.

526 Campos-Herrera, R., Pathak, E., El-Borai, F.E., Schumann, A., Abd-Elgawad, M.M.M., Duncan, L.W.,
 527 2013b. New citriculture systems suppresses native and augmented entomopathogenic nematodes
 528 *Biol. Control* 66, 183–194.

529 Campos-Herrera, R., El-Borai, F.E., Ebert, T.E., Schumann, A., Duncan, L.W., 2014. Management to
 530 control citrus greening alters the soil food web and severity of a pest-disease complex. *Biol.*
 531 *Control* 76, 41–51.

532 Campos-Herrera, R., Jaffuel, G., Chiriboga, X., Blanco-Pérez, R., Fesselet, M., Pûža, V., Mascher, F.,
 533 Turlings, T.C.J., 2015a. Traditional and molecular detection methods reveal intense interguild
 534 competition and other multitrophic interactions associated with native entomopathogenic
 535 nematodes in Swiss tillage soils. *Plant Soil* 389, 237–255.

536 Campos-Herrera, R., Puza, V., Jaffuel, G., Blanco-Pérez, R., Čepulytė Rakauskienė, R., Turlings,
 537 T.C.J., 2015b. Unraveling the intraguild competition between *Osccheius* spp. nematodes and

538 entomopathogenic nematodes: implications for their natural distribution in Swiss agricultural soils.
539 J. Invertebr. Pathol. 132, 216–227.

540 Demarta, L., Hibbard, B.E.O. Bohn, M., Hiltbold, I., 2014. The role of root architecture in foraging
541 behavior of entomopathogenic nematodes. J. Invertebr. Pathol. 122, 32–39.

542 de Rooij-van der Goes, P.C.E.M., van der Putten, W.H., van Dijk, C., 1995. Analysis of nematodes and
543 soil-borne fungi from *Ammophila arenaria* (Marram grass) in Dutch coastal foredunes by
544 multivariate techniques. Eur. J. Plant Pathol. 101, 149–162.

545 Dillman, A.R., Chaston, J.M., Adams, B.J., Ciche, T.A., Goodrich-Blair, H., Stock, S.P., Sternberg,
546 P.W., 2012. An entomopathogenic nematode by any other name. PLoS Pathogens 8, e1002527.

547 Dolinski, C., Choo, H.Y., Duncan, L.W., 2012. Grower acceptance of entomopathogenic nematodes:
548 case studies on three continents. J. Nematol. 44, 226–235.

549 Duncan, L.W., Dunn, D.C., Bague, G., Nguyen, K., 2003. Competition between entomopathogenic and
550 free-living bacterivorous nematodes in larvae of the weevil *Diaprepes abbreviatus*. J. Nematol. 35,
551 187–193.

552 Duncan, L.W., Stuart, R.J., El-Borai, F.E., Campos-Herrera, R., Pathak, E., Giurcanu, M., Graham,
553 J.H., 2013. Modifying orchard planting sites conserves entomopathogenic nematodes, reduces
554 weevil herbivory and increases citrus tree growth, survival and fruit yield. Biol. Control. 64, 26–
555 36.

556 Efron, D., Nestel, D., Glazer, I., 2001. Spatial analysis of entomopathogenic nematodes and insect
557 hosts in a citrus grove in a semi-arid region in Israel. Environ. Entomol. 30, 254–261.

558 El-Borai, F.E., Duncan, L.W., Preston, J.F., 2005. Bionomics of a phoretic association between
559 *Paenibacillus* sp. and the entomopathogenic nematode *Steinernema diaprepesi*. J. Nematol. 37,
560 18–25.

561 Enright, M.R., Griffin, C.T., 2005. Effects of *Paenibacillus nematophilus* on the entomopathogenic
562 nematode *Heterorhabditis megidis*. J. Invertebr. Pathol. 88, 40–48.

563 Ferris, H., Venette, R.C., Lau, S.S., 1996. Dynamics of nematode communities in tomatoes grown in
 564 conventional and organic farming systems, and their impact on soil fertility. *Appl. Soil Ecol.* 3,
 565 161–175.

566 Fließbach, A., Oberholzer, H.-R., Gunst, L., Mäder, P., 2007. Soil organic matter and biological soil
 567 quality indicators after 21 years of organic and conventional farming. *Agric. Ecosyst. Environ.*
 568 118, 273–284.

569 Georgis, R., Koppenhöfer, A.M., Lacey, L.A., Bélair, G., Duncan, L.W., Grewal, P.S., Samish, M.,
 570 Tan, L., Torr, P., van Tol, R.W.H.M., 2006. Successes and failures in the use of parasitic
 571 nematodes for pest control. *Biol. Control* 38, 103–123.

572 Hartmann, M., Frey, B., Mayer, J., Mäder, P., Widmer, F., 2015. Distinct soil microbial diversity under
 573 long-term organic and conventional farming. *ISME J.* 9, 1177–1194.

574 Hominick, W.M., 2002. Biogeography. In: Gaugler, R. (Ed.), *Entomopathogenic Nematology*, CABI
 575 Publishing, Wallingford, UK, pp. 115-143.

576 Hummel, R.L., Walgenbach, J.F., Barbercheck, M.E., Kennedy, G.G., Hoyt, G.D., Arellano, C., 2002.
 577 Effects of production practices on soilborne entomopathogens in Western North California
 578 vegetable systems. *Environ. Entomol.* 31, 84–91.

579 Jabbour, R., Barbercheck, M.E., 2008. Soil and habitat complexity effects on movement of the
 580 entomopathogenic nematode *Steinernema carpocapsae* in maize. *Biol. Control* 47, 235–243.

581 Jabbour, R., Barbercheck, M.E., 2011. Soil microarthropod response to the application of
 582 entomopathogenic nematode-killed insects in maize and flower strip habitats. *Pedobiologia* 54,
 583 243–251.

584 Jaffee, B.A., Ferris, H., Scow, K.M., 1998. Nematode-trapping fungi in organic and conventional
 585 cropping systems. *Phytopathology* 88, 344–350.

586 Jenkins, W.R., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant*
 587 *Dis. Rep.* 48, 492.

588 Kaya, H.K., Aguillera, M.M., Alumai, A., Choo, H.Y., de la Torre, M., Fodor, A., Ganguly, S., Hazir,
589 S., Lakatos, T., Pye, A., Wilson, M., Yamanaka, S., Yang, H., Ehlers, R.-U., 2006. Status of
590 entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of
591 the world. *Biol. Control*. 38, 134–155.

592 Koppenhöfer, A.M., Kaya, H.K., 1995. Density-dependent effects on *Steinernema glaseri* (Nematoda :
593 Steinernematidae) within an insect host. *J. Parasitol.* 81, 797–799.

594 Koppenhöfer, A.M., Kaya, H.K., 1996. Coexistence of two steinernematid nematode species
595 (Rhabditida : Steinernematidae) in the presence of two host species. *Appl. Soil Ecol.* 4, 221–230.

596 Koppenhöfer, A.M., Kaya, H.K., Shanmugam, S., Wood, G.L., 1995. Interspecific competition
597 between steinernematid nematodes within an insect host. *J. Invertebr. Pathol.* 66, 99–103.

598 Kondo, E., 1989. Studies on the infectivity and propagation of entomogenous nematodes *Steinernema*
599 spp. (Rhabditida: Steinernematidae) in the common cutworm, *Spodoptera litura* (Lepidoptera:
600 Noctuidae). *Agrie. Bull. Saga Univ.* 67, 1–87.

601 Kramer, I., Hirschy, O., Grunder, J.M., 2001. Survey of baited insect parasitic nematodes from the
602 Swiss lowland. In: *Developments in Entomopathogenic Nematode/bacterial Research. Proceedings of the Workshop at National University of Ireland Maynooth. 13-15 April 2000.* EUR
603 19696-COST ACTION 819.

604

605 Lacey, L.A., (ed.). 1997. *Manual of techniques in insect pathology*. San Diego: Academic Press.

606 Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., Goettel, M.S., 2015.
607 Insect pathogens as biological control agents: Back to the future. *J. Invertebr. Pathol.* 132, 1–41.

608 Lewis, E.E., Campbell, J., Griffin, C., Kaya, H.K., Peters, A., 2006. Behavioral ecology of
609 entomopathogenic nematodes. *Biol. Control* 38, 66–79.

610 Lewis, E.E., Hazir, S., Hodson, A., Gulcu, B., 2015. Trophic relationships of entomopathogenic
611 nematodes in agricultural habitats. In: Campos-Herrera, R. (Ed.). *Nematode Pathogenesis of*
612 *Insects and Other Pests*, Springer International Publishing, Switzerland, pp. 139-163.

613 Mäder, P., Fließbach, A., Dubois, D., Gunst, L., Fried, P., Niggli, U., 2002. Soil fertility and
 614 biodiversity in organic farming. *Science* 296, 1694–1697.

615 Mráček, Z., Becvár, S., Kindlmann, P., Jersakova, J., 2005. Habitat preference for ento-mopathogenic
 616 nematodes, their insect hosts and new faunistic records for the Czech Republic. *Biol. Control* 34,
 617 27–37.

618 Mráček, Z., Becvár, S., Kindlmann, P., 1999. Survey of entomopathogenic nematodes from the
 619 families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) in the Czech Republic.
 620 *Folia Parasit.* 46, 145–148.

621 Neher, D.A., 1999. Nematode communities in organically and conventionally managed agricultural
 622 soils. *J. Nematol.* 31, 142–154.

623 Neher, D.A., 2010. Ecology of plant and free-living nematodes in natural and agricultural soil. *Ann.*
 624 *Rev. Phytopathol.* 48, 371–394.

625 O’Callaghan, K.M., Zenner, A.N.R.L., Hartley, C.J., Griffin, C.T., 2014. Interference competition in
 626 entomopathogenic nematodes: male *Steinernema* kill members of their own and other species. *Int.*
 627 *J. Parasitol.* 44, 1009–1017.

628 Oerke, E.C., 2005. Crop losses to pests. *J. Agric. Sci.* 144, 31–43.

629 Pathak, E., El-Borai, F.E., Campos-Herrera, R., Johnson, E.G., Stuart, R.J., Graham, J.H., Duncan,
 630 L.W., 2012. Use of real-time PCR to discriminate parasitic and saprophagous behaviour by
 631 nematophagous fungi. *Fun. Biol.* 116, 563–573.

632 Patzel, N., Sticher, H., Karlen, D.L., 2000. Soil fertility - Phenomenon and concept. *J. Plant Nutr. Soil*
 633 *Sci.* 163, 129–142.

634 Perry, J.N., 1998. Spatial analysis by distance indices. *J. Ani. Ecol.* 64, 303–314.

635 Persmark, L., Banck, A., Jansson, H.-B., 1996. Population dynamics of nematophagous fungi and
 636 nematodes in an arable soil: vertical and seasonal fluctuations. *Soil Biol. Biochem.* 28, 1005–1014.

637 Pimentel, D., 1995. Amounts of pesticides reaching target pests: environmental impacts and ethics. J.
638 Agric. Environ. Ethics 8, 17–29.

639 Půža V, Mráček Z., 2005. Seasonal dynamics of entomopathogenic nematodes of the genera
640 *Steinernema* and *Heterorhabditis* as a response to abiotic factors and abundance of insect hosts. J.
641 Invertebr. Pathol. 89, 116–22.

642 Půža, V., Mráček, Z., 2009. Mixed infection of *Galleria mellonella* with two entomopathogenic
643 nematode (Nematoda: Rhabditida) species: *Steinernema affine* benefits from the presence of
644 *Steinernema kraussei*. J. Invertebr. Pathol. 102, 40–43.

645 Selvan, S., Campbell, J.F., Gaugler, R., 1993. Density-dependent effects on entomopathogenic
646 nematodes (Heterorhabditidae and Steinernematidae) within an insect host. J. Invertebr. Pathol. 62,
647 278–284.

648 Esperschütz, J, Gattinger, A., Mäder, P., Schlöter, M., Fließbach, A. 2007. Response of soil microbial
649 biomass and community structures to conventional and organic farming systems under identical
650 crop rotations. FEMS Microbiol. Ecol. 61, 26–37

651 Steiner, W.A., 1996. Distribution of entomopathogenic nematodes in the Swiss Alps. Rev. Suis. Zool.
652 103, 439–452.

653 Stuart, R.J., Barbercheck, M.E., Grewal, P.S., Taylor, R.A.J., Hoy, C.W., 2006. Population biology of
654 entomopathogenic nematodes: concepts, issues, and models. Biol. Control 38, 80–102.

655 Stuart, R.J., Barbercheck, M.E., Grewal, P.S., 2015. Entomopathogenic nematodes in the soil
656 environment: distributions, interactions and the influence of biotic and abiotic factors. In:
657 Campos-Herrera, R. (Ed.), Nematode Pathogenesis of Insects and Other Pests. Springer
658 International Publishing, Switzerland, pp. 97-137.

659 Torr, P., Spiridonov, S.E., Heritage, S., Wilson, M.J., 2007. Habitat associations of two
660 entomopathogenic nematodes: a quantitative study using real-time quantitative polymerase chain
661 reactions. J. Ani. Ecol. 76, 238–245.

662 van der Werf, H.M.G., 1996. Assessing the impact of pesticides on the environment. *Agric. Ecosyst.*
663 *Environ.* 60, 81–96.

664 White, G.F., 1927. A method for obtaining infective nematode larvae from cultures. *Science* 66, 302–
665 303.

666 Zhang, L.M., Liu, X.Z., Zhu, S.F., Chen, S.Y., 2006. Detection of the nematophagous fungus
667 *Hirsutella rhossiliensis* in soil by real-time PCR and parasitism bioassay. *Biol. Control* 36, 316–
668 332.

669

Figure legend

Fig. 1. Influence of crop type (maize, winter wheat, grass-clover ley) on natural occurrence of entomopathogenic nematodes (EPN) along with selected key members of their soil food web: nematophagous fungi (NF) and free-living nematodes (FLN). Data show the average value for the combined species-specific quantification of each of the organisms in the DOK field trial combining two different sampling periods in 2013. (a) Total number of EPN infective juveniles (IJs) of the species *Steinernema affine* (Saff), *S. carpocapsae* (Sca), *S. feltiae* (Sf), *S. poinari* (Spoin), *Heterorhabditis bacteriophora* (Hb), *H. megidis* (Hm). (b) Total NF infection rate (IR) proportion for the species *Purpureocillium lilacinus* (Plil), *Catenaria* sp. (Cat), *Hirsutella rhossiliensis* (Hrhos), and *Arthrobotrys oligospora* (Ao). (c) Total proportional occurrence of FLN for the *Acroboides*-group (Acrob), *Osccheius onirici* (O.oni) and *Osccheius tipulae* (O.tip). The proportional presence of each EPN, NF or FLNs species is shown with the corresponding proportion of the pie graph above each column. Data are shown as means $\pm$ SEM. Lowercase letters indicate statistical differences.

Fig. 2. Influence of management practices on the natural occurrence of entomopathogenic nematodes (EPN) along with selected key members of their soil food web: nematophagous fungi (NF) and free-living nematodes (FLN). Data present the average value of the combined species-specific quantification of each of the organisms in the DOK field trial combining two different sampling points in 2013. (a) Total number of EPN infective juveniles (IJs) of the species *Steinernema affine* (Saff), *S. carpocapsae* (Sca), *S. feltiae* (Sf), *S. poinari* (Spoin), *Heterorhabditis bacteriophora* (Hb), *H. megidis* (Hm). (b) Total NF infection rate (IR) proportion for the species *Purpureocillium lilacinus* (Plil), *Catenaria* sp. (Cat), *Hirsutella rhossiliensis* (Hrhos), and *Arthrobotrys oligospora* (Ao). (c) Total proportional occurrence of FLN for the *Acroboides*-

group (*Acrob*), *Oscheius onirici* (*O.oni*) and *Oscheius tipulae* (*O.tip*). The proportional presence of each EPN, NF or FLNs species is shown with the corresponding proportion of the pie graph above each column. Data are shown as means $\pm$ SEM. Lowercase letters indicate statistical differences.

Fig. 3. Influence of crop type (maize, winter wheat, grass-clover ley) on activity recorded from *Galleria mellonella* baiting as expressed by the average percentage of larval mortality. (a) Mean activity of nematodes (EPN + FLN) and (b) mean activity of all organisms (EPN + FLN + Other organisms, mainly bacteria and fungus). Data are shown as means $\pm$ SEM. Lowercase letters indicate statistical differences.

Fig. 4. Influence of management practices (biodynamic 0.7 LU/ha and 1.4LU/ha; organic 0.7 LU/ha and 1.4LU/ha; conventional 0.7 LU/ha and 1.4LU/ha; Mineral fertilizer; No fertilizer, biodynamic) on activity recorded from *Galleria mellonella* baiting expressed as the average percentage of larvae mortality. (A) Mean activity of nematodes (EPN + FLN) and (b) mean activity of all organisms (EPN + FLN + Other organisms, mainly bacteria and fungus). Data are shown as means $\pm$ SEM. Lowercase letters indicate statistical differences.

Fig. 5. Relative proportions of emerging EPN species in both soil samples and MG samples (Koch's postulate cadavers) after *Galleria mellonella* baiting. Proportions were obtained from Ct values of qPCR species-specific quantification of each of the organisms in the DOK field trial combining two different sampling points in 2013.

1 **Table 1**

2 Treatments corresponding to the four farming practices, with the different levels of organic
3 fertilizer use per hectare. Livestock Unit (LU) are average stocking density in Switzerland.

Management	Code	Organic fertilizer use	Other fertilizer use
Organic	O2	1.4 LU/ha	-
	O1	0.7 LU/ha	-
Dynamic	D2	1.4 LU/ha	-
	D1	0.7 LU/ha	-
Conventional	K2	1.4 LU/ha	Mineral
	K1	0.7 LU/ha	Mineral
No fertilization, biodynamic	N	-	-
Only Mineral fertilizers (since 1985)	M	-	Mineral

1 **Table 2**
2 Statistical analysis of activity measured by *Galleria mellonella* bait data, natural occurrence of the organisms (in 100 g of dry soil) detected by qPCR in the
3 soils and microbial biomass detected by CFE. Data are presented as Chisq_{df} (for binomial analyses), F_{df} (for permutation analyses) and probability levels: * *P* <
4 0.05, ** *P* < 0.01, *** *P* < 0.001, n.s., no significant.

	Farming system (F)	Crop type (C)	Period (P)	F*C	C*P	F*P	F*C*P ⁵
GM bait data							6
All organisms							7
Activity (%)	n.s.	n.s	307.24 ₁ ***	n.s.	n.s.	n.s.	38.2 ₁₄ *** 8
Entomopathogenic nematodes							9
Activity (%)	n.s.	n.s.	110.6 ₁ ***	n.s.	n.s.	n.s.	29.6 ₁₄ **
Nematodes (all)							10
Activity (%)	n.s.	9.04 ₂ *	165.4 ₁ ***	n.s.	n.s.	n.s.	28.08 ₁₄ ** 11
qPCR data							12
Entomopathogenic nematodes							13
Total IJs	n.s.	4.3 ₂ *	5.0 ₁ *	n.s.	4.0 ₂ *	n.s.	1.6 ₁₄ *.
Free-living nematodes							14
ng DNA, (0-1) scaled	n.s.	20.9 ₂ ***	6.1 ₁ *	n.s.	21.4 ₂ ***	n.s.	n.s. 15
Nematophagous fungi							16
Total IR ¹	n.s.	n.s.	6.8 ₁ **	n.s.	n.s.	n.s.	n.s. 17
CFE data							18
Cmic (mg/kg)	23.2 ₇ ***	n.s.	8.5**	n.s.	4.7 ₂ *.	n.s.	n.s. 18
Nmic (mg/kg)	11.4 ₇ ***	n.s.	219.4 ₁ ***	n.s.	n.s.	n.s.	n.s. 19

20

21 ¹ IR, infection rate for the nematophagous fungi (NF), equivalent to pg NF DNA / total DNA

Figures

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5