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Effects of cover crops on the overwintering success of entomopathogenic nematodes and their antagonists

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ABSTRACT

Conservation agriculture is arising as an alternative to conventional agriculture with the aim to have a reduced impact on the environment. This includes the use of cover crops to conserve soil quality by limiting soil erosion, adding organic matter, and enhancing soil nutrient content, as well as water availability, which are all factors that can greatly influence the presence of soil organisms. In the current study, we investigated whether winter cover crops can enhance the persistence of entomopathogenic nematodes (EPN) over the winter season. In a first trial we augmented EPN populations in plots without (bared) and with the cover crops, pea (*Pisum sativum*) or mustard (*Brassica juncea*). In late autumn, individual mini-plots in each of the three treatments were supplemented with infective juveniles (IJs) of either *Heterorhabditis bacteriophora* or *Steinernema feltiae*. In a second trial we studied naturally occurring EPN in plots without (bared) and with the cover crop pea (*Pisum sativum*) followed by planting of winter wheat. To identify and quantify EPN, we analyzed soil samples using quantitative real time PCR (qPCR) at three time points over the winter season. We also measured the activity of augmented EPN by baiting the soil with wax moth larvae, *Galleria mellonella*. In addition, we used the qPCR method to investigate the presence of nematophagous fungi (NF), free-living nematodes (FLN) and ectoparasitic bacteria, all of which can interfere with EPN performance. Numbers of naturally occurring EPN in the investigated fields were very low (<1 EPN per 100 g of soil). The cover crops only had a significant positive effect on the numbers of augmented *S. feltiae* found in early winter. No striking effect was found for *H. bacteriophora*. Yet, augmentation was found to enhance the insect-suppressiveness of the soil, as the recorded EPN infectivity after the winter was slightly higher than what was observed in autumn, one month after application. The numbers of FLN, which compete for insect cadavers, was higher in spring than in early winter. These FLN and other antagonists may be important in reducing EPN numbers. In conclusion, the effect of cover crops on EPN persistence was only evident during early-winter and was only significant in the plots augmented with *S. feltiae*. Moreover, we found that higher numbers of EPN in agricultural soils do not necessarily translate into high infectivity, which is the key factor determining their effectiveness in controlling soil pests.

1. Introduction

Improving soil quality and enhancing agricultural production in a sustainable manner is essential to face the challenge of reducing the impact of agriculture on the environment. Problems such as contamination of the environment with agrochemicals, depletion of natural resources and soil erosion call for novel strategies to optimize sustainable crop production (Abdollahi and Munkholm 2014; Adl, 2016; Komatsuzaki and Ohta, 2007; Lal, 2009). Concepts such as conservation agriculture, through adapted tillage, residue management and crop rotation, are key elements to improve modern agriculture (Verhulst et al., 2010).

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One promising strategy is to use winter cover crops after summer productions, as these may help to diminish nutrient losses, reduce soil erosion and positively influence beneficial soil organisms (Dabney et al., 2001; Ewing et al., 1991; Fageria et al., 2005; Gómez et al., 2009; Hargrove, 1991; Schipanski et al., 2014; Snapp et al., 2005). Yet, the impact of cover crops on the soil biota, in general, and on soil beneficial organisms such as biological control agents, in particular, is poorly understood.

Entomopathogenic nematodes (EPN) are obligate parasites of insects and excellent biocontrol agents (Lacey et al., 2015). Infective juveniles (IJs) actively seek for a host that they invade and once inside they release their symbiotic γ–Proteobacteria, which produce toxin(s) that generates a general septicemia in the host. Their high infectiousness and ability to kill an insect within 2–3 days and their relatively low impact on non-target species make EPN good candidates for sustainable pest management (Piedra-Buena et al., 2015). However, the use of EPN in large-scale agriculture faces several constraints that make their application rarely cost-effective (Lacey et al., 2015).

In agroecosystems, soil properties may be severely altered by agricultural management practices and EPN will be challenged with drastic changes of various abiotic and biotic factors (Lewis et al., 2015; Stuart et al., 2006, 2015). As part of a research consortium that explores how soil health can be improved by applying ecological and rational approaches (NRP68: http://www.nrp68.ch), we studied how soil food webs, including EPN, can be better exploited to control soil-dwelling insect pests in annual crops. There is increasing interest in the factors that determine the presence and the dynamics of EPN populations, as well as their competitors and natural enemies, such as free-living nematodes, nematophagous fungi and bacteria (Campos-Herrera et al., 2013a, 2015a; Griffin 2015; Lewis et al., 2015).

Recent surveys of Swiss agricultural soils have shown that the density of naturally occurring EPN is very low, independently of management practices (Campos-Herrera et al., 2015a; Jaffuel et al., 2016). Therefore, EPN application to augment their numbers may be a promising strategy to improve the control of root pests. Overwintering ability of augmented *Heterorhabditis megidis* was assessed in field studies in Switzerland and was found to be poor (Imperiali, Chiriboga et al., unpublished), which is in agreement with the natural population dynamics observed for the native populations by Campos-Herrera et al. (2015a) and Jaffuel et al. (2016). Yet, several other studies show that EPN can persist and remain infectious after overwintering (Cappaert and Koppenhöfer, 2003; Duncan et al., 2013; Elmowitz et al., 2013), which implies that persistence is context and climate-dependent. The aim of the current study was to explore ways to improve overwintering persistence of EPN. Several previous studies used Baermann funnel extraction to survey the nematode community under cover crops. Overall, they found positive effects of cover crops on populations of different nematode genera, even in very different cropping systems (DuPont et al., 2009; Ferris et al., 2012; Ito et al., 2015a, 2015b). The Baermann funnel technique mostly recovers long nematodes that actively move, and therefore, may miss certain nematodes. A study using insect baiting in different crops found a better persistence of *H. bacteriophora* in bean cultivation after red clover was used as a cover crop (Susurluk and Ehlers, 2008). On the other hand, only using insect baits provides information on infectivity, but not on overall EPN presence.

Here, we employed two distinct measurement techniques (traditional baiting with wax moth larvae, *Galleria mellonella,* and quantitative real time PCR analyses) to investigate whether certain cover crops can help to maintain higher densities of active EPN in agricultural soils. We chose two commonly used cover crops (Lu et al., 2000): (i) pea (*Pisum sativum*), which we assumed to have a positive impact, as its presence may modify the soil properties in a way that can favor nematodes (increased moisture, host insects, root system, UV protection) (Stuart et al., 2015) and (ii) mustard (*Brassica juncea*), which potentially has a negative impact, as mustard is known to produce bio-fumigants that may be detrimental to soil organisms, including nematodes (Lu et al., 2000; Henderson et al., 2009; Ramírez et al., 2009). We performed two independent field experiments. The first experiment was conducted to test how survival and activity of both native and augmented EPN populations persist under cover crops (pea or mustard) as compared to bare soil. The second experiment allowed us to evaluate how the naturally occurring EPN population persisted under pea as an early-winter cover crop compared to bare soil followed by sowing of winter wheat. This was combined with a comparison between tillage *versus* direct seeding management, as well as different soil textures. For both field experiments we also surveyed other key members of the soil food web (free-living nematodes, nematophagous fungi and bacteria). Thus, we also established how EPN application may affect the population dynamics of these associated organisms and how they in turn may (or may not) affect EPN persistence. For the first experiment, we also measured the microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) in the soils as an additional proxy for soil health.

2. Material and methods

2.1. Field experiments design

We surveyed two ongoing field trials located in the Experimental Station Agroscope Changins, near Nyon (Switzerland, 46° 24′ N, 06° 14′ E, 430 m above sea level). To take into account possible harsh environmental conditions occurring during our studies, we obtained the temperature of the soil (−10 cm) and air (2 m) registered by a weather station located on the site (http://www.agrometeo.ch/fr/station/3) (Supplementary data (Appendix A, Fig. A1)).

A first experiment was conducted in field CC27, where we evaluated the impact of two types of cover crops. One was Indian mustard (*Brassica juncea* var. Vitasso) with a possible deleterious effect on nematodes (Ramírez et al., 2009) and the other pea (*Pisum sativum* var. Arkta), which potentially has a positive impact on EPN. Both crops were sown August 6th 2014. Bare soil plots were considered as control plots. Each plot was 8 m × 1.5 m, and the treatments were distributed following a randomized block design with four replicates. The field is characterized by a soil composition of of 24.6% clay, 29.7% sand and 45.7% silt, a pH of 7 (1:2.5 suspension of soil:destilled water) and a soil organic matter content of 2.6%. We also evaluated how augmenting plots with EPN before the start of winter affected EPN numbers throughout the winter. For this, we applied the following three treatments with nematodes to 1 m × 1 m split-plots (within the cover crop plots): (1) no augmentation (control), (2) application of *Steinernema feltiae* RS-5 (native) and (3) application of *Heterorhabditis bacteriophora* Andermatt (commercial, http://www.andermattbiocontrol.com). Both species of nematodes were previously detected in the same area (Campos-Herrera et al., 2015a). The mini-plots were separated by 60–90 cm buffer zones.

The native population of *S. feltiae* was cultured in the laboratory using *Galleria mellonella* (Lepidoptera: Pyralidae) as host (obtained from Au Pêcheur sàrl, Neuchatêl). Nematodes within two weeks after emergence or delivery (for *H. bacteriophora,* the commercial species) were employed in the augmentation plots. For both nematode species, water suspensions were prepared the night before application to obtain the equivalent of 50 IJs/cm^2 in the field (Grewal and Peters 2005). We verified infectivity in the laboratory for both species by infecting larvae of *G. mellonella.* In the field, the application was performed by using watering cans (one per