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

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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). 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 was more abundant in winter-wheat plots 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.

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

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(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). Few studies even found a positive effect of organic soil management 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 and Griffin, 2005; Jabbour and Barbercheck, 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, abundance and activity 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 with 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 5th rotation from 2006 to 2012 it consisted of maize (*Zea maize* 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 perennne* 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 potatoes) 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

Table 1
Treatments corresponding to the four farming practices, with the different levels of organic 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