



## Organic farming practices result in compositional shifts in nematode communities that exceed crop-related changes



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### ARTICLE INFO

#### Article history:

Received 20 August 2015

Received in revised form 23 October 2015

Accepted 26 October 2015

Available online 6 November 2015

#### Keywords:

Organic farming

Effective organic matter

*Prismatolaimus*

*Pristionchus*

Microscopic analysis

Quantitative PCR

Bio-indicators

Soil health

### ABSTRACT

Intensification of conventional agriculture has resulted in a decline of soil ecosystem functioning. Organic agriculture intends to manage soil biota in a manner that is more geared towards adequate cycling of nutrients with minimal losses. Ecological interpretation of agricultural practices-induced shifts in primary decomposers, bacteria and fungi, is non-trivial due to their enormous biodiversity. Bacterivorous and fungivorous nematodes feed selectively on these microorganisms, and we intended to test whether farming system effects are mirrored in compositional changes in nematode communities. Therefore, we analysed the impact of three farming systems, conventional (ConMin), integrated (ConSlu) and organic (Organic), on nematode communities in the southeastern part of The Netherlands on a sandy soil with 3–5% organic matter. Effects of each farming system were assessed for four different crops (barley, maize, pea or potato) by a series of taxon-specific quantitative PCRs (qPCR). Changes in community structure analysed by nonmetric multidimensional scaling (NMDS) showed that organic farming resulted in specific shifts in nematode community composition exceeding crop-related assemblage shifts. Three out of thirteen quantified nematode taxa showed significant farming system effects. Strongest effects were observed for the (putative) bacterivore *Prismatolaimus*, which was relatively common in Organic fields and nearly absent in ConMin and ConSlu fields. A reverse effect was observed for *Pristionchus*; this necromenic bacterivore and facultative predator made up about 21% and 7% of the total nematode community in respectively ConMin and ConSlu fields, whereas it was nearly absent from Organic fields. The observed farming system effects suggest that specific nematode taxa might be indicative for the impact of farming practices on soil biota.

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## 1. Introduction

Soil organisms are essential for the decomposition of organic matter from plant or animal origin (Janzen, 2006). In (agro-) ecosystems plants benefit from the biological degradation of various types of organic matter as soil biota mediate the bio-availability of e.g. carbon, nitrogen and phosphorous. Other ecosystem services delivered by soil biota are the build-up of soil organic matter, the improvement of soil structure (Six and Paustian, 2014), and the promotion of disease suppressiveness (Van Bruggen and Semenov, 2000; Wagg et al., 2014).

Intensification of agriculture has led to a decline of soil biodiversity (Tsiafouli et al., 2014) and a general decline in soil ecosystem functioning (de Vries et al., 2012). Organic agriculture aims at more sustainable food production through application of multiple types of organic fertilizers and reduction of pesticide use (Mäder et al., 2002). Long-term effects of organic farming generally result in higher organic matter levels (Gattinger et al., 2012), increased soil biodiversity and aboveground pest suppression (Mäder et al., 2002; Birkhofer et al., 2008).

To evaluate effects of farming on soil quality, various biotic indicators of soil quality have been identified (Doran and Zeiss, 2000). However, due to the overwhelming biodiversity, and the poor ecological characterisation of numerous constituents, it is hard to relate composition, diversity and abundance to ecological functioning of soils (Giller et al., 1997; Thiele-Bruhn et al., 2012). The use of nematodes as bio-indicators to monitor the impact of

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farming strategy and crop types has received some attention (Berkelmans et al., 2003; Neher, 1999; Van Diepeningen et al., 2006). Nematodes are present in high densities in virtually any soil, and their communities are species-rich with representatives in all trophic layers of the soil food web. Moreover, nematodes show distinct sensitivities towards various kinds and levels of environmental stressors (Bongers, 1990; Bongers and Ferris, 1999; Yeates, 2003). Despite these advantageous biological characteristics and the fact that nematodes can be easily separated from the soil matrix, the use of nematodes as indicators of soil quality is not widespread. This is mainly due to difficulties with identification as a result of the scarcity of informative morphological characters. Routine microscopic analyses are therefore time-consuming and require ample training. The resolution offered the ribosomal DNA (rDNA) locus is relatively high, thus enabling DNA-based identification of nematode communities. Several quantitative ( $q$ ) PCR-based methods have been developed for the characterisation of nematode assemblages (Floyd et al., 2002; Holterman et al., 2008; Vervoort et al., 2012), but hitherto, such methods have not been frequently used for impact assessments.

Long-term (>10 years continued treatment) effects of organic and conventional farming practices on nematode assemblages have been investigated in various experimental settings. Studies showed a negative effect of tillage on food web complexity (Ugarte et al., 2013), an increase in overall abundance of nematodes in response to organic matter inputs (Li et al., 2014), and strong correlations between soil nutrient status and the number of bacterivores (Berkelmans et al., 2003; Pan et al., 2010). Crop type was shown to affect composition of nematode assemblages to a larger extent than farming system (Berkelmans et al., 2003; Neher, 1999). In four locations in North Carolina (USA), Neher (1999) investigated in detail the effects of conventional and organic farming practices during more than eight years of farming strategy transition. Three free-living nematode families were shown to be more abundant in organically managed soils; Plectidae, Pristionchidae and Tylencholaimidae. Due to our superficial knowledge about the feeding preferences within these groups, plausible mechanistic explanations for the promotion of these trophically distinct families by organic farming are lacking. At the same time, these studies illustrate the potential for using nematode communities to test the impact farming systems on the soil biological condition.

Here, we investigated effects of farming system and crop species on nematode assemblages in a long-term field experiment. Based on the results of a biodiversity inventory with 51 taxon-specific qPCRs, 15 abundant and trophically diverse nematode taxa were selected. qPCR analyses revealed significant impact of crop species and farming system on the nematode community composition. As a verification, subsamples were analysed microscopically in parallel, and this independent methodological approach gave similar, though less pronounced results. On top of the crop-related effects, organic farming practices resulted in significantly higher *Pristionchus* and *Diphtherophora* levels, whereas a strong opposite trend was observed for *Pristionchus*. Finally, possible explanations for the observed farming system-related shifts in nematode communities are presented.

## 2. Materials and methods

### 2.1. Study site

The Vredepeel farm is located in the southeastern part of the Netherlands (Oceanic climate (Cfb); 600–700 mm precipitation year<sup>-1</sup>, mean temperatures of 11 °C) on a sandy soil (93.3% sand, 4.5% silt, 2.2% clay) with moderately high organic matter (OM) levels (3–5%) and high to very high phosphorus contents (~2.2

mg kg<sup>-1</sup>). In 2001, three different farming strategies were installed. Organic farming fields received the highest organic matter inputs (cattle manure and crop residues) and no pesticides were applied (Organic: 3050 kg effective organic matter (EOM) ha<sup>-1</sup> yr<sup>-1</sup>; EOM as defined by Sukkel et al. (2008)). The two types of conventional farming differed in the type and quantity of EOM application. In the ConSlu system, mineral fertilizers were applied in combination with pig and cattle slurry (1950 kg EOM ha<sup>-1</sup> yr<sup>-1</sup>), and the ConMin system is based on the application of mineral fertilizers only (1250 kg EOM ha<sup>-1</sup> yr<sup>-1</sup>, mainly crop residues). Nutritional regimes in each of the systems was designed to keep the total P and K input constant (~50 and 220 kg ha<sup>-1</sup> yr<sup>-1</sup>, respectively), while the active N input in the Organic treatment was 45% of the N inputs in the ConMin and ConSlu treatments. ConMin and ConSlu both received about 180 kg active N ha<sup>-1</sup> yr<sup>-1</sup>.

### 2.2. Set-up field experiment

The field trials are based on a six-year crop rotation with (1) potato, (2) pea, (3) leek, (4) barley, (5) sugar beet (in ConMin and ConSlu) or carrot (in Organic), and (6) maize. For this study, samples were collected from fields with potato, pea, barley and maize. In total 12 rectangular experimental fields (each 180 m by 15m or 18 m) were sampled; four fields for each of the three farming systems. The overall layout of this field experiment is shown in Fig. S1. With regard to the design of this field experiment it should be noted that the European organic farming directive (SKAL) did not allow us to use a completely randomized block design. Therefore, the organic fields had to be placed in one block, whereas the two conventional treatments were mixed on the remaining two blocks. Additional studies have shown that differences between the organic and conventional farming systems could not be related to position effect (see Figs. S2 and S3).

### 2.3. Soil sampling and nematode extraction

Sampling took place on the 1st of May 2013, just prior to the growing season and presumably an ideal period to measure farming system effects, as preceding crop-effects have eroded during the winter period. In each field ( $n=12$ ) samples were collected along six virtual lines parallel to the short end of the rectangle. The spacing between the parallel lines was 30 m. Along each virtual line, one composite soil sample was collected consisting of 12 equidistantly-taken cores ( $\odot$  1.5 cm, depth: 20 cm). Immediately after sampling, the resulting 72 (6 from each of the 12 fields) composite soil samples were stored at 4 °C. Soil samples were homogenised thoroughly and nematodes were extracted from a 100 g subsample using an Oostenbrink elutriator (Oostenbrink, 1960). This amount was chosen because samples smaller than 100 g are less likely to reflect the true community (Wiesel et al., 2015), and since Verschoor and De Goede (2000) found that nematodes were more efficiently extracted from small (50 g) than from large samples (250 g). Nematode suspensions were split into two equal portions. One portion was analysed by a series of quantitative PCR assays, a subsample of the second half was analysed microscopically.

### 2.4. Microscopic analysis of nematode communities

Nematode suspensions were fixed in 8 ml 5% formaldehyde (Seinhorst, 1962) in 38 out of 72 samples. For this study at first 100 individuals were identified to genus level. For taxa represented by fewer than five individuals in any of the samples, another batch of 100 nematodes was examined. To estimate the nematode density 1/10 of each sample was counted under a dissecting microscope.

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