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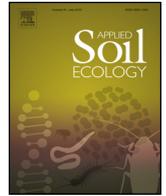
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ABSTRACT

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, Pristomatolaimidae 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 *Pristomatolaimus* 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⁻¹, 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⁻¹). 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⁻¹ yr⁻¹; 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⁻¹ yr⁻¹), and the ConMin system is based on the application of mineral fertilizers only (1250 kg EOM ha⁻¹ yr⁻¹, 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⁻¹ yr⁻¹, 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⁻¹ yr⁻¹.

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.

2.5. Quantitative PCR-based analysis of nematode communities

Nematode suspensions were concentrated and lysed (Holterman et al., 2006). DNA extracts were purified using a glass fibre column-based procedure (Ivanova et al., 2006; Vervoort et al., 2012). For a nematode biodiversity check, 1 µl subsamples were taken from each of the purified DNA extracts, and mixed. All purified DNA extracts were stored at –20 °C until further use.

Overall purified DNA extracts were used as template in qPCR using 51 nematode taxon-specific primer sets. Overall, 33 taxa were shown to be present, and 15 abundant taxa were selected for further analysis (Table S1). In total 17 primer sets were used: 15 taxon-specific, one to assess total nematode density and one external control (to compensate for losses during sampling handling). qPCR reactions were executed and Ct values (the number of PCR cycles that were needed to reach a threshold) were converted to nematode densities by making use of the known linear relationships between Ct values and 10^{\log} (number of target nematodes) (Vervoort et al., 2012). The absolute value of the first mathematic derivative of the melting curve was checked to confirm the correct nature of the amplicon. N/A (non applicable) was used to indicate that no (correct) amplicon was formed.

2.6. Data analysis

Generalized linear models (GLZ) with a Poisson-distributed dependent variable were used to separate effects of farming system and crop as well as their interaction. The outcome of both identification methods was compared. To meet the assumption of normality of residuals, standardized residuals were tested on normality using a Kolmogorov–Smirnov test. To inspect whether the different farming system and crop type affect nematode community compositions, nonmetric multidimensional scaling (NMDS) analyses were conducted on qPCR as well as microscopy data. Degrees of stress in NMDS plots indicate the reliability of the outcome, where a lower s-stress corresponds to a higher reliability (Oksanen, 2015). The dissimilarity matrices in the three different analyses were based on field-mean abundances of all taxa within the community. A Bray–Curtis dissimilarity metric was used to determine distances between the sampling points, for which the metaMDS function was used (R vegan package; Oksanen, 2015; R Development Core Team, 2011). Spearman's ρ was used as a nonparametric measure to determine correlations between densities of taxa obtained by microscopy and qPCR. All statistics were performed using Statistica 9.0 and R version 2.15.1.

3. Results

3.1. Effects of prolonged exposure to distinct farming systems.

Twelve years of three different organic matter input regimes (ConMin, ConSlu and Organic) resulted in a significant increase in the organic matter contents ($OM\%_{ConMin} < OM\%_{ConSlu} < OM\%_{Organic}$), and an elevated moisture content in case of the Organic management

regime (Table 1). Regarding effective organic matter (EOMs) inputs, it should be noted that the annual 1250 kg EOMs input per hectare in the regime with “just mineral fertilizer” (ConMin) consists of an estimation of the average input of crop residues. Also for the other regimes, estimated inputs in the form of crop residues were taken into account. Prolonged input of additional organic matter, pig slurry in case of ConSlu, and cattle manure in case of Organic, resulted in significant differences in organic matter contents, being lowest in ConMin and highest in Organic (Table 1). Only for the Organic fields, a significant increase in the moisture content was detected (Table 1).

3.2. Nematode assemblages affected by farming system and crop

To make a selection of the most abundant nematode taxa, lysates from nematode assemblages were analysed using 51 taxon-specific primer combinations (Table S1). Thirty-three taxa were shown to be present. Based on density, trophic diversity, and molecular detectability (some classical taxonomic groups such as the family Rhabditidae appeared to be poly and/or paraphyletic, and can not easily be detected using molecular methods), 15 taxa abundant taxa were selected for further analysis. Nematode community compositions as determined by taxon-specific qPCRs show that nematode assemblages in the organic farming were distinct from the ones found under conventional farming irrespective of the current crop (Fig. 1A). No clear difference in clustering was observed between the two types of conventional farming: fields receiving solely mineral fertilizer (ConMin), or fields receiving pig slurry in addition to mineral fertilizer (ConSlu). Analysis of in parallel-generated microscopic community data did not confirm this farming system-based separation of the nematode assemblages (Fig. 1B).

qPCR data revealed clear crop related effects on community composition. Distinct nematode assemblages were observed in potato and maize fields (Fig. 1C) and rather similar communities in barley and pea fields, which were positioned between communities of potato and maize. Microscopic data showed similar, though less pronounced separations of crop-specific nematode assemblages (Fig. 1D). Abiotic factors (organic matter and moisture contents) were positively correlated with the nematode communities in the organic system, as illustrated by the arrows in the NMDS plots (Fig. 1A, C). A similar, but less pronounced, trend was observed for the microscopic data (Fig. 1B, D).

3.3. Responses of individual nematode taxa to farming system and crop

Total nematode densities were not different between farming systems, but significant effects were found for crop type and farming system \times crop type interactions (Table 2). qPCR data showed significant effects of farming system on six out of thirteen nematode taxa (Table 2). Most notably Pristomatolaimidae (a family consisting of a single genus, *Pristomatolaimus*) was relatively common in Organic fields whereas nearly absent from both conventional systems. Among the fungivores *Diphtherophora* was

Table 1
Farming systems: conventional farming with mineral fertilizer (ConMin), conventional farming with mineral fertilizer supplemented with pig slurry (ConSlu), and an organic farming with high organic matter inputs (cattle manure) and no mineral fertilizer (Organic). Different letters indicate significant differences ($P < 0.05$). EOM stands for effective organic matter, i.e. the amount of organic matter still present one year after incorporation into the soil. This is assessed by using standard parameters for every type of organic matter (Sukkel et al., 2008).

Farming system	Annual input	Organic matter		Moisture	
	(kg EOM ha ⁻¹ yr ⁻¹)	(% ± SE)	Significance	(% ± SE)	Significance
ConMin	1250	4.5 ± 0.1	A	7.1 ± 0.3	A
ConSlu	1950	5.1 ± 0.1	B	7.5 ± 0.3	A
Organic	3050	5.7 ± 0.2	C	10.3 ± 0.4	B

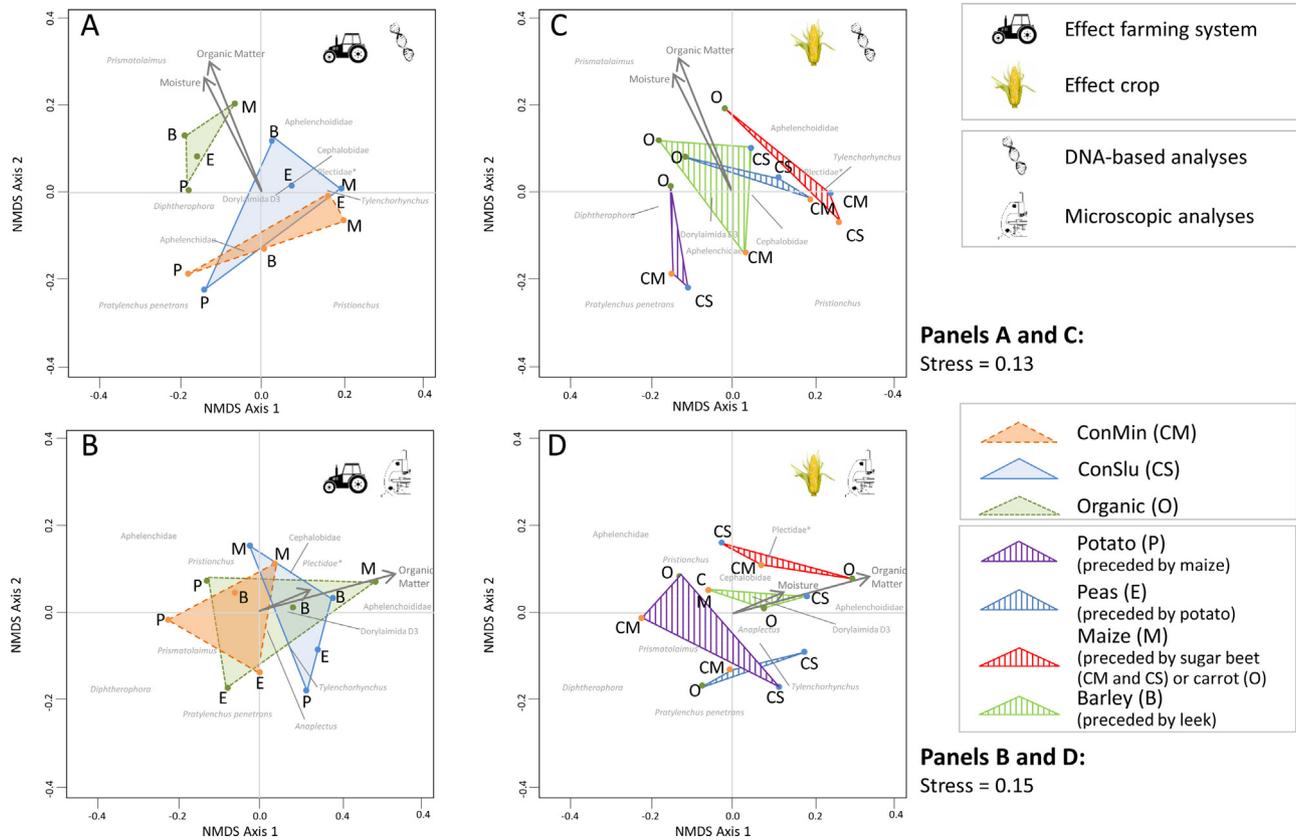


Fig. 1. NMDS showing nematode community compositions per farming system (indicated by tractor icon; A and B) and crop type (indicated by a maize cob; C and D) based on qPCR data (indicated by a DNA strand; A and C) and microscopic data (indicated by a microscope icon; B and D). Dissimilarities between community compositions were determined using a Bray–Curtis dissimilarity index. Points further apart are more dissimilar. Goodness of fit is expressed by a stress value. Polygons in different colors indicate farming system and crop type (see ppt file).

Table 2

Effects of farming system, crop and the interaction between both variables on the nematode community. Nematodes communities were analysed using taxon-specific molecular assays ($n = 72$), and approximately half of the samples were analysed microscopically in parallel ($n = 38$). Single letters are used to indicate food preferences of nematode taxa: (f): fungivores, (b): bacterivores, (pp): plant parasites, (omni): omnivores. *Pristionchus* (*): qPCR specifically detected representatives of the genus *Pristionchus*, in case of microscopic analysis all members of the family Neodiplogasteridae were included. *Pratylenchus* (**): was analysed at species level, microscopic analysis allowed for quantification at genus level only. For all taxa, generalized linear models (GLZ) with a Poisson-distributed dependent variable were used to analyse densities. P values ≤ 0.05 are given in bold.

Nematode taxon	Molecular analysis		Microscopic analysis			
	Farming system	Crop	Farming system * Crop	Farming system	Crop	Farming system * Crop
Aphelenchoididae (f)	NS	$P = 0.02$	$P < 0.001$	NS	$P = 0.013$	$P < 0.001$
Aphelenchidae (f)	NS	$P = 0.01$	$P = 0.004$	NS	$P < 0.001$	$P = 0.02$
Cephalobidae (b)	$P = 0.01$	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	NS
Plectidae minus Anaplectus (b)	NS	NS	$P < 0.001$	NS	NS	$P = 0.01$
Anaplectus (b)	NS	$P < 0.001$	NS	NS	$P = 0.02$	NS
Filenchus (pp & f)	NS	$P = 0.003$	NS	$P = 0.02$	$P < 0.001$	NS
<i>Pristionchus</i> (*) (b)	$P < 0.001$	$P < 0.001$	NS	$P = 0.02$	$P = 0.001$	NS
<i>Diphtherophora</i> (f)	$P = 0.01$	NS	$P < 0.001$	$P < 0.001$	$P = 0.03$	$P = 0.006$
<i>Mesorhabditis</i> (b)	$P = 0.002$	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$
<i>Prismatolaimus</i> (b)	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$
<i>Tylenchorhynchus</i> (pp)	NS	$P < 0.001$	NS	NS	$P < 0.001$	NS
<i>Pratylenchus</i> (**) (pp)	NS	$P < 0.001$	$P < 0.001$	NS	$P = 0.002$	$P = 0.02$
<i>Dorylaimida D3</i> (omni)	$P < 0.001$	$P < 0.001$	$P = 0.02$	NS	NS	NS
Total count	NS	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	$P = 0.002$
** <i>Pratylenchus penetrans</i>	NS	$P < 0.001$	$P < 0.001$	Routine light microscopic analysis (no morphometrics) does not offer sufficient resolution to distinguish individual <i>Pratylenchus</i> species		
<i>P. crenatus</i>	$P < 0.001$	$P < 0.001$	$P = 0.002$			
<i>P. neglectus</i>	Only present in block 2 (See Fig. S1)					

slightly but significantly more abundant in the organic fields, whereas the two other fungivorous taxa, Aphelenchidae and Aphelenchoididae, were not affected by the farming system.

Remarkably, *Pristionchus* a necromenic bacterivore and facultative predator was significantly more abundant in ConMin (Table 2). No significant effect of farming system was found for the dominant

plant-parasitic taxa *Pratylenchus* (three species; only for one species, *P. crenatus*, a significant effect was observed) and *Tylenchorhynchus* spp., and this outcome in part contrasts with results from a previous study by Neher & Olson (1999).

For a few other taxa, farming system-related shifts could not be confirmed by microscopic analysis (criterion: $P \leq 0.05$). The omnivorous Dorylaimida D3 (mainly *Thonus*, *Enchodelus*, *Eu-*, *Epi-* and *Prodorylaimus* (see Holterman et al., 2008 hard to distinguish from other Dorylaimids in routine micropic analyses) was more abundant in organic fields. Among the bacterivores, representatives of the opportunistic genus *Mesorhabditis* were significantly more abundant in the ConSlu fields, whereas Cephalobidae were present at higher densities in the ConMin fields (Table 3).

Eleven out of 13 taxa (including total nematode densities) showed a significant response to crop type, irrespective of the nematode community analysis method used (Table 2). Total nematode densities were significantly higher in barley and maize than in pea and potato (Table 3). This difference can be attributed mainly to the significantly higher densities of relatively abundant taxa *Pristionchus* and Cephalobidae in these crops. In comparison to the farming systems, the impact of crop on fungivorous taxa was reversed: both Aphelenchidae and Aphelenchoididae numbers were increased in a crop-specific manner, whereas *Diphtherophora* remained unaffected (Tables 2 and 3). Regarding plant parasitic taxa, *Tylenchorhynchus* densities increased in maize and pea fields. *Pratylenchus* spp. were more abundant in pea and potato fields (Tables 2 and 3).

Potato fields typically had relatively high levels of the omnivores group Dorylaimida D3. Barley and pea fields were characterized by relatively high densities of *Filenchus* group 3 (predominantly root-hair feeders and/or fungivores) and fungivorous *Aphelenchoides* species. Other remarkable observations were the low incidence of *Pristionchus* species in pea fields, and – to a lesser extent – low *Mesorhabditis* densities in potato (Table 3). Significant interaction effects were common, illustrating that, for the majority of nematode taxa, crop response is farming system dependent. The total nematode densities are significantly distinct

between the four crops (Tables 2 and 3). As samples were collected early in the growing season, observed changes could relate to the previous crop, the kind of cover crop used during wintertime, and the actual crop (and not solely to the actual crop).

3.4. (In) consistencies between qPCR and microscopy-based quantitative community analyses

To see whether qPCR-based analyses could be confirmed by the most common approach for nematode community characterization, microscopic analysis, nematode suspensions were split and analyzed with both methods. Due to practical limitations, viz. the time required to microscopically analyse nematode community samples, not all 72 samples were analyzed. Half of the samples ($n=38$) were investigated using both methods. From the 42 test results, 83% led to similar or comparable P-values and hence concurrent conclusions about effects of farming system, crop types or interactions of these (Table 2, see Table S2). Poor correlation coefficients were found for (occasionally) rare taxa such as *Aphelenchoides*, Plectidae, *Anaplectus*, *Aphelenchus*, *Pristionchus*, *Diphtherophora* and *Tyloilaimophorus* (Table S2 and Fig S4). Doubling of efforts by microscopy resulted in structurally higher correlations (t -test for dependent samples: $P=0.03$) (Table S2).

4. Discussion

Long-term organic farming resulted in overall shifts in the nematode community; three out of 13 nematode taxa under investigation showed a significant farming system effect, while differences between the two conventional practices were less pronounced. Strongest effects were observed for *Prismatolaimus*, which was relatively common in organic fields and nearly absent in conventional fields, and *Pristionchus*, which was abundant in conventional fields and nearly absent in organic fields. Crop type affected more nematode taxa than farming system; 11 out of 13 nematode taxa showed a significant response to crop. Significant interaction effects for numerous taxa suggested that the effect of crop type is farming system dependent.

Table 3
Densities of individual nematode taxa as determined by quantitative PCR. Densities are expressed as number of individuals per 100 g soil (fresh weight). With regard to crop it should be that nematode communities were sampled early in the growing season. In case of barley, radish was grown as cover crop in wintertime, in maize plots barley was used as cover crop, in pea plots ryegrass was grown as cover crop, and in case of potato the cover crop was barley.

	Farming system						Crop								
	ConMin(n=24)		ConSlu (n=24)		Organic (n=24)		Barley (n=18)		Maize (n=18)		Pea (n=18)		Potato (n=18)		
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	
Organic matter (w/w%)	4.5	0.5	5.1	0.5	5.7	0.7	5.1	0.8	5.2	0.7	5.1	0.9	5.0	0.6	
Moisture content (w/w%)	7.1	1.4	7.5	1.5	10.4	1.8	8.3	2.2	8.6	1.4	8.9	2.7	7.7	1.9	
Nematode taxon															
Aphelenchoididae (f)	37.4	93.9	19.7	21.7	42.8	89.3	60.0	104.7	12.4	10.6	7.5	8.6	53.4	101.4	
Aphelenchidae (f)	0.6	0.7	1.2	1.8	0.8	1.9	0.5	0.5	1.2	2.1	0.5	0.5	1.3	2.1	
Cephalobidae (b)	236.8	122.7	188.7	123.7	204.1	92.4	292.2	104.3	273.1	58.4	120.2	56.1	154.1	116.8	
Plectidae (except <i>Anaplectus</i>) (b)	2.9	2.6	3.6	3.4	4.3	3.1	3.7	2.8	4.1	3.4	3.3	1.9	3.4	4.0	
<i>Anaplectus</i> (b)	34.1	24.4	31.8	39.1	28.4	30.7	34.0	24.4	55.7	46.5	17.6	13.0	18.6	15.2	
<i>Filenchus</i> group 3 (pp & f)	7.2	7.9	1.2	2.5	13.1	17.3	14.0	19.0	3.3	5.1	10.5	9.6	1.0	2.0	
<i>Pristionchus</i> (b)	616.4	1802.5	145.9	221.7	2.5	6.4	594.7	2001.4	353.0	695.7	9.9	17.5	62.0	167.0	
<i>Diphtherophora</i> (f)	0.9	1.0	0.7	1.0	1.6	1.8	1.1	1.1	1.3	2.2	0.8	0.8	1.1	1.0	
<i>Mesorhabditis</i> (b)	6.1	6.0	12.1	7.9	7.4	4.4	13.0	8.6	7.5	4.8	9.5	6.0	4.0	3.0	
<i>Prismatolaimus</i> (b)	0.1	0.5	0.1	0.1	19.3	30.6	8.8	27.3	9.8	22.1	4.0	16.3	3.3	8.9	
<i>Tylenchorhynchus</i> (pp)	3.7	2.9	2.6	2.4	1.7	1.5	1.7	1.5	4.1	3.0	3.7	2.3	1.1	1.1	
<i>Pratylenchus</i> (pp)**	30.3	62.9	16.5	12.5	15.2	13.9	9.6	7.9	8.3	10.7	44.6	69.2	20.3	12.8	
Dorylaimida D3 (omni)	66.7	69.0	43.2	40.7	116.6	116.7	37.2	40.7	90.9	77.8	50.9	49.9	123.2	126.5	
Total nematode densities	2959	3131	2134	1478	2352	982	3211	3186	3614	1585	1777	998	1325	720	
** <i>Pratylenchus penetrans</i>	9.1	14.3	8.9	10.1	5.9	6.8	7.4	7.0	2.3	5.4	4.8	7.2	17.4	14.7	
<i>P. crenatus</i>	19.9	62.43	2.4	2.7	9.2	12.2	2.2	2.5	5.9	10.4	31.2	70.4	2.8	5.0	
<i>P. neglectus</i>	1.3	3.1	5.2	11.6	0.1	0.2	0.1	0.2	0.1	0.3	8.6	12.5	0.0	0.0	

4.1. The absence of farming system-related effects on total nematode densities

In our study, total nematode densities in ConMin, ConSlu and Organic fields were not significantly different. This corresponds with data presented by Van Diepeningen et al. (2006) and Pan et al. (2010), but contrasts with findings in numerous other studies where higher nematode densities were reported for organic systems (for review see Hole et al., 2005). Apparently, a long-term increase of OM inputs by organic farming does not necessarily result in increased nematode densities.

Our results suggest that crop type is a major short-term determinant of total nematode abundances (reflected by a steep density increase of a few taxa only). Likewise, large crop effects were reported by Berkelmans et al. (2003) and Neher (1999) implying that crop type is a major driver of nematode abundances.

4.2. Possible biological explanations for the farming system-specific impact on individual nematode taxa

For three taxa pronounced farming system effects were observed: relatively steep increase in *Prismatolaimus* densities, as well as a slight increase in the *Diphtherophora* levels were found in soils under organic management, whereas an opposite trend was observed for *Pristionchus*. Higher levels of *Prismatolaimus* in fields under organic management for a prolonged period were previously reported by Neher (1999) in a range of locations varying in organic matter content (1.9–5.6%) and soil type (both sand and clay sites were represented). Yeates et al. (1997) also found higher densities of this particular genus under organic management on silt and loam, however an opposite tendency was observed on sandy soils. Usually, members of the genus *Prismatolaimus* are considered to be bacterivores, but the presence of teeth in the stoma could point at more non-selective feeding habits (Ferris et al., 1996), suggesting that they may be facultative predators as well. The fact that a similar phenomenon has been described now by various laboratories under a range of soil conditions could justify additional efforts to ecologically understand this relationship. As compared to *Prismatolaimus*, only slightly higher *Diphtherophora* densities were observed in fields under organic farming. Similar observations have been reported before: in a meta-analysis on the effects of a range of disturbances in terrestrial nematode communities, Zhao and Neher (2013) reported significantly lower levels of *Diphtherophora* in fields under conventional cultivation (as compared to e.g. low tillage).

Multiple explanations can be proposed for the low *Pristionchus* densities in fields under organic management. *Pristionchus* is described as a bacterivore and facultative predator (Yeates et al., 1993). Members of this genus live in close association with beetles (mainly Scarabaeidae), and their main food source consists of bacteria that invade the corpus of the beetle upon its death (Rae et al., 2008). So far, *Pristionchus* has been sampled mostly by collecting beetles in the field. In a recent paper on the impact of soil carbon increase on nematode assemblages in subtropical arable soils, *Pristionchus* was identified as one of the most responsive genera (Ito et al., 2015). In our experiment, the organic matter contents (OM%) under the three farming systems differed significantly: Organic fields showing the highest OM%, and ConMin fields the lowest. Seroby et al. (2014) showed the ecological advantage of the ability of *Pristionchus pacificus* to develop different mouth forms. Shortage of bacterial food triggered the formation of individuals with a mouth form more suitable for a predatory lifestyle. The unusual trophic flexibility of this genus could be advantageous in more disturbed habitats such as, in this research, the ConMin fields. As an alternative explanation, the

reduced levels of *Pristionchus* in the Organic fields could be associated with the non-use of insecticides

4.3. Farming system versus crop-related effects on nematode communities

Significant interaction effects (farming system \times crop type) were shown for 6 of 13 nematode taxa. This illustrates that the observed effects of crops on nematode communities are largely farming system dependent. Both qPCR and microscopic analysis revealed interaction effects for all fungivores under investigation, and by qPCR data for individual *Pratylenchus* species. No consistent interaction effects were detected for the remaining 7 taxa including *Pristionchus*, and the polyphagous plant parasite *Tylenchorhynchus*. From studies such as Neher (1999) and Berkelmans et al. (2003), it is known that crops have large effects on individual nematode taxa and the nematode community as a whole.

It should be underlined that the effects of farming system reported in this paper were observed in Spring, at the very beginning of the growing season. Moreover, the experimental fields were located in an area with sandy soils, and fields were exposed to temperate, relatively moist Northwestern European climate conditions. An indication that our observations are not necessarily bound to this soil type and/or climate zone only, was recently presented by Ito et al. (2015). In their study the impact of increased soil carbon levels on nematode communities was studied on an Andosol under humid subtropical conditions. Monitoring nematode assemblages over eight years revealed a similar trend as revealed in the present study: the nematode genera *Prismatolaimus*, *Pristionchus*, and *Pratylenchus* were mostly affected by increased total soil carbon (Ito et al., 2015). These results suggest that amongst the two main organic farming-typifying practices – higher and qualitatively distinct organic matter inputs and the non-use of pesticides – the first one might be the most relevant explanatory factor for the observed nematode community shifts.

5. Conclusion

Our results show that prolonged exposure of agricultural fields to three different farming systems, conventional, integrated and organic, resulted in nematode community shifts that exceeded the crop-related changes. Although our results show clear taxon-specific differences between crops and farming systems at the onset of a new growing season, it should be noted that the fate of these contrasts was not monitored during the growing season. Relatively high levels of *Prismatolaimus* and – to a lesser extent – *Diphtherophora* were observed in soil under organic management, whereas high *Pristionchus* densities were associated with conventional farming practices. It remains to be investigated whether these changes can be attributed to specific organic matter input, the non-use of chemical pesticides or other measure that are typical for organic farming.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.apsoil.2015.10.022>.

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