

## RESEARCH ARTICLE

# Effect of tillage, subsidiary crops and fertilisation on plant-parasitic nematodes in a range of agro-environmental conditions within Europe

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## Keywords

Compost; conservation tillage; cover crops; fertiliser; living mulches; plant-parasitic nematodes.

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## Abstract

The overall goal in nematode management is to develop sustainable systems where nematode populations are kept under the economic damage threshold. Conservation tillage and subsidiary crops, applied as cover crops and living mulches, generally improve soil health by increasing soil organic matter content and stimulating soil microbial activity. However, more permanent crop and weed cover associated with subsidiary crops and noninversion tillage, respectively, may benefit plant-parasitic nematodes with broad host spectra such as *Meloidogyne* and *Pratylenchus*. These genera are major constraints to many field crops throughout Europe and there is a need to identify effective and reliable management options that can be applied to avoid excessive infestations. The dynamics of the indigenous fauna of plant-parasitic nematodes were studied in eight coordinated multi-environment field experiments (MEEs) under four agro-environmental conditions in Europe (Continental, Nemoral, Atlantic North and Mediterranean North). The MEEs consisted of a 2-year sequence of wheat combined with a living mulch or subsequent cover crops and second main crops maize, potatoes or tomatoes depending on site. Additionally, the effects of inversion tillage using the plough were compared with various forms of conservation tillage (no-tillage, shallow and deep noninversion tillage). Overall, *Helicotylenchus*, *Paratylenchus*, *Pratylenchus* and *Tylenchorhynchus* were the most frequent genera across sites while *Meloidogyne* occurred only in Germany at very low densities. During the wheat–maize sequences in Switzerland, the populations of *Pratylenchus* increased from 63 to 146 nematodes per 100 mL soil and *Helicotylenchus* from 233 to 632 nematodes per 100 mL soil. The effects of tillage on plant-parasitic nematodes were generally minor, although no tillage in Italy supported higher densities of *Pratylenchus* (184 nematodes per 100 mL soil) than inversion tillage (59 nematodes per 100 mL soil). Furthermore, *Pratylenchus* densities were 160 nematodes per 100 mL soil when leguminous subsidiary crops were grown, 122 nematodes per 100 mL soil in the green fallow and 84 nematodes per 100 mL soil after growing black oat (*Avena strigosa*) or oilseed radish (*Raphanus sativus*). The differences were greatest in Italy, in a sandy soil with low organic matter. Application of compost or nitrogen fertiliser had no consistent effects on plant-parasitic nematodes. We conclude that crop rotations including specific subsidiary crops are prominent factors affecting the indigenous nematode community, while tillage and fertiliser are of lower importance.

## Introduction

Noninversion and no-tillage systems, permanent soil cover and diversified crop rotations are the three principles of conservation agriculture for improving soil fertility and soil health (Hobbs *et al.*, 2008). However, permanent soil cover by subsidiary crops, which are included in the rotation for their agro-environmental benefits, such as living mulches (undersown in main crops) or cover crops (growing in between main crops), as well as higher weed infestations, might form a 'green bridge' for certain pests or diseases that can accumulate over time.

Obligate plant pests such as plant-parasitic nematodes react to changes in crop rotation and tillage intensity and are therefore good indicators to use when evaluating farming systems (Neher, 1999; Fu *et al.*, 2000; Berkelmans *et al.*, 2003; van Capelle *et al.*, 2012). For example, the generally more diverse crop rotations used in organic farming systems often support a high diversity of plant-parasitic nematodes with a broad host spectrum such as *Meloidogyne*, *Pratylenchus* and *Tylenchorhynchus* (van Diepeningen *et al.*, 2006; Hallmann & Kiewnick, 2015). In contrast, conventional management systems with narrower rotations generally cause a reduction in the diversity of plant-parasitic nematode species and host specific taxa such as *Heterodera* and *Globodera* might increase (Briar *et al.*, 2007; Hallmann & Kiewnick, 2015). Furthermore, increasing densities of plant-parasitic nematodes were frequently reported to occur in noninversion and no-tillage systems with or without subsidiary crops (Govaerts *et al.*, 2007; Okada & Harada, 2007; Carter *et al.*, 2009; Smiley *et al.*, 2014). This is especially the case when those subsidiary crops are good hosts for certain plant-parasitic nematodes, such as legumes that are commonly good hosts for root-knot (*Meloidogyne* spp.) and root-lesion (*Pratylenchus* spp.) nematodes (Riggs & Niblack, 1993).

If subsidiary crops are used in noninversion and no-tillage systems, care needs to be taken that those crops do not build up nematode densities to levels causing economic damage in the main crop. This can be achieved by either resistant cultivars or poor and nonhost crops. Several fodder radish (*Raphanus sativus*) cultivars are widely used to control certain species of *Meloidogyne* and *Heterodera schachtii* in temperate climates, while sun hemp (*Crotalaria juncea*) is used to control *Meloidogyne* spp., *Rotylenchus reniformis* and *Helicotylenchus multincinctus* in warmer regions (Hallmann & Kiewnick, 2015). However, if poor or nonhost subsidiary crops are grown, sufficient weed control is important as most of the common weeds are good hosts for plant-parasitic nematodes (Viaene *et al.*, 2013; Visser & Molendijk, 2015).

In the long-term, noninversion and no-tillage combined with subsidiary crops should increase the organic matter content in the soil (Tebrügge & Düring, 1999) and thus, the antagonistic potential, which might increase soil suppressiveness towards plant-parasitic nematodes. Although the general mechanisms leading to nematode suppressive soils are still unclear, natural enemies, such as nematode trapping fungi, predatory nematodes and other nonspecific organisms, are likely to increase under such conditions. Furthermore, the mortality rates of plant-parasitic nematodes may be increased throughout the decomposition process of organic matter, for example, due to toxic metabolites released by organic matter degrading microbes (Viaene *et al.*, 2013; Stirling, 2014; Hallmann & Kiewnick, 2015). Additional application of compost might enhance these processes due to maintenance of an abundant and active soil microbial community (Pocasangre *et al.*, 2015).

We hypothesized that the adoption of agricultural practices, such as cover cropping as well as noninversion and no-tillage, could lead to an increase of plant-parasitic nematodes. However, pests and diseases may be diversely affected by agricultural management according to site-specific climate, soil conditions and rotational crops (Strand, 2000). To address such questions, European scientific networks have been established aiming for a holistic examination of cropping system functions (Lechenet *et al.*, 2017) whose 'complexity is more than the sum of its parts' (Drinkwater, 2002). With such holistic approaches, which are also addressed in our study, impacts of cropping systems can be generalised while taking into account year and environmental effects as well as site-specific system adaptations (Lechenet *et al.*, 2017).

Therefore, the overall objective of this study was to investigate the effect of noninversion and no-tillage combined with subsidiary crops on the short-term population dynamics of plant-parasitic nematodes in coordinated multi-environment experiments (hereafter called MEEs) in four agro-environmental zones in Europe ranging from Nemoral (Sweden) to Mediterranean North (Italy). The specific objectives of this study were to evaluate: (a) the initial effects of noninversion and no-tillage practices compared to inversion tillage on plant parasitic nematodes as affected by pedoclimatic conditions; (b) the effect of leguminous and nonleguminous subsidiary crops in crop rotations versus green fallow on locally occurring plant parasitic nematodes; and (c) the effect of nitrogen fertiliser or yard waste compost application on plant-parasitic nematodes.

## Materials and methods

The MEEs were conducted at four sites within Europe representing the following agro-environmental zones

**Table 1** Soil characteristics and climate of the four agro-environmental sites

Site	% Clay	% Silt	% Sand	Soil Type (USDA)	Soil pH	% Organic Matter	Temp <sup>a</sup> (°C)	Ppt. <sup>a</sup> (mm)
Switzerland	21	35	44	Hapludalf	7.0	2.1	10.0	1185
Sweden	16	64	20	Inceptisol	5.9	4.4	7.7	562
Germany	13	84	3	Typic Hapludalf	6.0	2.0	9.8	636
Italy	19	21	60	Typic Xerofluvent	6.7	1.2	11.4	729

<sup>a</sup>Annual means during the experiment: Temp., temperature; Ppt., precipitation.

(Jongman *et al.*, 2006): Continental: Agroscope, Tänikon, Switzerland (47°30'N, 8°55'E); Nemoral: Swedish University of Agricultural Science, Uppsala, Sweden (59°49'N, 17°42'E); Atlantic North: Kassel University, Witzenhausen, Germany (51°22'N, 9°54'E) and Mediterranean North: Tuscia University, Viterbo, Italy (42°25'N, 12°05'E). At each experimental site, two MEE's were established in successive years, MEE 1 from 2012 to 2014, MEE 2 from 2013 to 2015.

The MEEs all started with wheat, that is, winter wheat (Switzerland, Sweden, Germany) or durum wheat (Italy) in autumn of the first year followed by the summer main crops maize (Sweden, Switzerland), potato (Germany) or tomato (Italy). Wheat was either cultivated as pure crop or undersown with subterranean clover (Switzerland, Italy and Germany) or white clover (Sweden, Germany). The clovers were intended to continue after the wheat was harvested as cover crop. Where no clovers were present, wheat was followed by a cover crop that was established shortly after the harvest of wheat. A green fallow was used as control. The subsidiary crops were terminated before the second main crop was sown in the following spring. For practical and economic reasons, such as precise tillage and sowing operations, experiments at all sites were arranged as split-blocks (see descriptions below). Site-specific climatic conditions and soil characteristics are summarised in Table 1.

### Wheat–maize MEEs

The MEEs in Switzerland were conducted at the Agroscope experimental station in Tänikon (537 m a.s.l.). In the years preceding the experiment, the soil was annually ploughed 20 cm deep and the crops were managed conventionally. Forage pea (*Pisum sativum* subsp. *arvense*) was grown prior to the start of the experiments. The two MEEs representing a wheat (*Triticum aestivum* cv. CH Claro)–maize (*Zea mays* cv. LG 30.222) crop sequence were arranged in a strip-split-block design with four replicates. Factor I (main plots) was tillage intensity: (a) inversion tillage by mouldboard ploughing at about 20 cm soil depth (hereafter called CT), (b) noninversion tillage at about 5 cm soil depth (hereafter called RT) and (c) no-tillage (hereafter called NT), all applied before maize

in the second year of each experiment. Factor II (first split) were subsidiary crops either as: (a) undersown subterranean clover (*Trifolium subterraneum* cv. Campeda) in winter wheat and resown after wheat harvest (LM), (b) leguminous cover crop (L CC), *Vicia villosa* cv. Hungvillosa, (c) nonleguminous cover crop (NL CC), *R. sativus* cv. Pegletta and (d) green fallow as control (C). Both L CC and NL CC were sown after wheat harvest. Factor III (second split) was two levels of ammonium nitrate application to the wheat (70 and 140 kg N ha<sup>-1</sup>) and the maize (45 and 90 kg N ha<sup>-1</sup>). The combination of the three factors resulted in 96 plots per MEE (6 m × 8 m plot size). Weed control in wheat was performed by herbicide application [8.25 g ha<sup>-1</sup> active ingredient (a.i.) iodosulfuron and mesosulfuron each +180 g ha<sup>-1</sup> a.i. fluroxypyr] in the pure wheat treatments (control and both cover crop treatments), whereas no weed control was performed in the LM treatment. Weeds in maize were controlled by herbicides in the CT and NT treatments with a mixture of 105 g ha<sup>-1</sup> a.i. mesotrione and 495 g ha<sup>-1</sup> a.i. terbuthylazine +1.2 kg ha<sup>-1</sup> a.i. S-metolachlor +40 g ha<sup>-1</sup> a.i. nicosulfuron in MEE 1 and 105 g ha<sup>-1</sup> a.i. mesotrione and 495 g ha<sup>-1</sup> a.i. terbuthylazine +36 g ha<sup>-1</sup> a.i. nicosulfuron +96 g ha<sup>-1</sup> a.i. dicamba in MEE 2. The RT treatment was mechanically hoed. Differential tillage was applied to kill subsidiary crops and for seedbed preparation to the maize. This was done by, ploughing (20 cm) and rotary harrowing in CT, three times chisel ploughing about 5 cm deep in RT, and 1.44 kg ha<sup>-1</sup> a.i. glyphosate application and direct sowing in NT, respectively. Maize was sown at the end of May in both MEE's.

The two MEEs in Sweden were conventionally managed and set up as strip-blocks with four replicates. Pre-crops were winter wheat in MEE 1 and summer oilseed rape in MEE 2. Factor I (main plots) was tillage intensity: (a) CT at about 20 cm soil depth applied late in autumn; and (b) an RT noninversion tillage system where weeds and subsidiary crops were killed with 1.2 kg ha<sup>-1</sup> a.i. glyphosate applied in early spring and maize (*Z. mays* cv. Activate) was direct drilled in MEE 1 and sown after stubble cultivation in MEE 2. Direct drilling was not considered feasible in MEE 2 due to large amounts of crop residues. NT was not applied. Factor II (split plots) were subsidiary crops either applied as: (a) LM of white clover

(*T. repens* cv. Klondike) undersown in wheat (*T. aestivum* cv. Olivin), (b) L CC, *V. villosa* cv. Minnie, (c) NL CC, *R. sativus* cv. Doublet and (d) green fallow (weeds) as control (C). Both L CC and NL CC were sown after wheat harvest. In total, the experiments consisted of 32 plots (9 m × 12 m plot size). Weeds in wheat were not controlled in MEE 1, but controlled in MEE 2 by 1 kg ha<sup>-1</sup> a.i. bentazone. A mixture of 45 g ha<sup>-1</sup> a.i. mesotrione, 7.5 g ha<sup>-1</sup> a.i. foramsulfuron + 0.025 g ha<sup>-1</sup> a.i. iodosulfuron-methyl-sodium + 7.5 g ha<sup>-1</sup> a.i. isoxadifen-ethyl (safener) and 0.67 L ha<sup>-1</sup> maize oil was applied twice yearly to the maize.

### Wheat–potato MEEs

The organically managed MEEs in Germany were also set up as strip-split-blocks with four replicates. The MEEs were started after 2 years of regularly mulched grass–clover as pre-crop. Factor I (main plots) was either: (a) CT at about 25 cm soil depth after one to two times chisel ploughing at about 10 cm depth before wheat and potatoes, respectively or (b) RT with two to three times chisel ploughing at about 10 cm depth prior to wheat and at about 15 cm prior to potato cultivation. In addition, potatoes in RT were mulched with an 8–10 cm layer of a winter pea–rye (C/N ratio = 27.5; approximately 30% legumes) and triticale–vetch mixtures (C/N ratios = 22.5; approximately 60% legumes) 4 weeks after planting. This translates into about 150 and 360 kg nitrogen ha<sup>-1</sup> (in MEE 1 and MEE 2, respectively) that were applied in the noninversion tillage system. Based on the C/N ratio of the mulch, the model of Laber (2002) suggests that 0 and 54 kg of this N became plant available during the potato season in MEE 1 and MEE 2, respectively. Although this potentially affected potato yields between both MEEs, the effects on plant-parasitic nematodes were assumed low due to the relatively low difference in nitrogen available to the plants. The mulch application was part of the RT treatment as recommended by the manufacturer (Friedrich Wenz GmbH, Schwanau, Germany) of the RT equipment. NT was not applied. Factor II (split-plots) was subsidiary crops applied as: (a) a LM of either subterranean clover cv. Dalkeith or white clover cv. Huia undersown in wheat (cv. Achat), (b) L CC, *V. sativa* cv. Berninova grown after wheat and (c) NL CC, *R. sativus* var. *oleiformis* cv. Kompass/*Avena strigosa* cv. Pratex mixed 1:4, grown after wheat. Due to complete failure of both undersown clover species in both experimental years, the LM treatments are considered as green fallow in the data analysis (i.e. control C). Factor III (split-split plots) was either: (a) manual application of 5 and 10 t ha<sup>-1</sup> dry matter of a yard waste compost before sowing wheat or planting potatoes, respectively; or (b) mineral fertilisation consisting of potassium (approximately 167 kg ha<sup>-1</sup>

K<sub>2</sub>SO<sub>4</sub>) and phosphorus (approximately 33 kg ha<sup>-1</sup> rock phosphate) fertiliser matching the concentration of the composts. Total nitrogen in the composts was below 2% with a maximum of 200 mg kg<sup>-1</sup> plant available (mineral) nitrogen and C/N ratios of 16–25. The pH and electrical conductivity in the composts ranged from 6.4 to 8.0 and 500–930 μS cm<sup>-1</sup>, respectively. The total number of plots was 64 (6 m × 15 m plot size). Weeds were only controlled in wheat in MEE 1 via hoeing and harrowing in spring.

### Durum wheat–tomato MEEs

In Italy, the two MEEs were carried out at the conventionally managed, experimental farm of Tuscia University (Viterbo). The experimental fields were kept bare via periodically tilling during 14 months before start of the MEEs with a durum wheat (*T. durum* cv. Claudio) – tomato (*Solanum lycopersicum* cv. San Marzano Kero) crop sequence. The experimental design was a strip-split-block with three factors and four replicates. Factor I (main plots) was subsidiary crops applied either as: (a) LM, that is, undersown subterranean clover (*T. subterraneum* cv. Campeda) in durum wheat, (b) L CC, *V. villosa* cv. Capello and 3) NL CC, *A. strigosa* cv. Pratex and (c) green fallow control (C). Both L CC and NL CC were sown after wheat harvest. Factor II (first split) was soil tillage, managing cover crop aboveground biomass with: (a) CT to a depth of 25 cm and (b) NT with residues left on the soil surface in strips as dead organic mulch. RT was not applied. Factor III (second split) were two levels of nitrogen fertilisation in wheat (60 and 120 kg N ha<sup>-1</sup>) and tomato (75 and 150 kg N ha<sup>-1</sup>). Total number of plots was 64 (12 m × 4 m plot size). Weeds in tomatoes were controlled with 0.5 kg ha<sup>-1</sup> a.i. flufenacet + 17 g ha<sup>-1</sup> a.i. metribuzine applied 30 days after transplanting in May.

### Nematode sampling and assessments

Soil samples for initial and final nematode extraction were collected from the end of August until early November depending on site-specific sowing and harvesting dates. Thus, soil samples for the assessments of initial plant-parasitic nematode densities were taken in late August/early September (Germany), late September (Switzerland) and October (Sweden, Italy). Soil samples for the assessments of final nematode densities were taken in September/October (all sites), except of MEE 1 in Switzerland (early November). In Switzerland, initial plant-parasitic nematode densities were assessed from 48 plots that were thereafter divided in two subplots for the two nitrogen fertilisation levels resulting in 96 plots for the assessment of final population densities.

In Germany in MEE 2, 32 plots before the application of tillage and compost were sampled for the assessment of initial plant-parasitic nematode densities. In all other cases, all plots were sampled for the assessment. Soil samples were collected from the upper 20 cm soil using an auger with 2–3 cm diameter. To maintain a representative sample, compensating for the heterogeneous distribution of plant-parasitic nematodes within the soil, a total of 30 cores per experimental plot were taken following a zigzag pattern. The soil was collected in a bucket, thoroughly mixed and an aliquot of 500 mL soil was filled in plastic bags, labelled and shipped within 1 week to the Julius Kühn Institute in Münster, Germany (JKI). At JKI, soil samples were stored at 6°C until evaluation.

### Nematode evaluation

Soil samples were passed through a 1 cm sieve to remove root debris and stones, thoroughly mixed, and 250 mL aliquots were taken for nematode extraction following the centrifugal flotation method as described in Hooper *et al.* (2005) using MgSO<sub>4</sub> at 1.15-specific density. Nematodes collected after the final centrifugation step on a 20 µm sieve were transferred into a glass beaker and filled up to 10 mL with tap water. The suspension was thoroughly mixed by agitating with air and 1 mL was transferred into a nematode counting slide. Plant-parasitic nematodes were identified and counted at genus level using an inverse microscope at 63× magnification. For better comparison with similar studies, nematode densities were finally expressed as number of specimen per 100 mL soil. For species identification, a composite sample was prepared for each experimental site and sampling date. Nematodes were killed with gentle heat, fixed in a triethanolamine formalin (TAF) solution containing 7 mL formalin (40% formaldehyde), 2 mL triethanolamine and 91 mL distilled water (Courtney *et al.*, 1955). Fixed nematode specimen was then processed to anhydrous glycerol over a period of 12 days using the slow evaporation technique at 39 ± 1°C (Hooper *et al.*, 2005). According to this method, species identification was done after transferring female nematodes into anhydrous glycerol on permanent slide mounts. Specimens were examined under a Leitz Diaplan compound microscope (Leitz, Wetzlar, Germany) equipped with differential interference contrast at 630–1000× magnification.

### Statistical analysis and data processing

The statistical analyses were performed with R, version 3.2.2 (R Core Team, 2013). Prior to analysis, residuals of final nematode data were visually tested with qq-plots for homogeneity of variances and normal distribution. Data

were square root-transformed to improve the homogeneity of variances. The frequent nematode genera were analysed per MEE based on 2-factorial (tillage and subsidiary crops; Sweden) and three-factorial (tillage, subsidiary crops, and fertiliser/compost; Switzerland, Germany, Italy) designs. Frequent genera were defined by densities above 100 nematodes per 100 mL soil for at least one site at the beginning of both MEEs. The R package 'nlme' (Pinheiro *et al.*, 2016) was used to build linear mixed effect models fitted by restricted maximum likelihood ratios taking into account block designs and site-specific split-plot arrangements. *F*- and *P*-values were obtained from Wald-tests, which appeared to be equally conservative than Kenward–Roger and Satterthwaite approximations for degrees of freedom (Luke, 2017), probably due to the large sample size.

A detrended correspondence analysis (DCA) of the nematode community revealed long gradients on the first axis. According to Dormann & Kühn (2009), long gradients (>4) on the first DCA axis reveal that the unimodal reaction of species is fully covered, while short gradients (<2) solely cover a part (ascending, descending) of the species reaction (linear reactions). For this reason, a constrained correspondence analysis (CCA, unimodal model) was performed with the R package *vegan* (Oksanen *et al.*, 2015). The CCA was performed to identify site-specific differences in the nematode composition at the beginning (Pi) and the end (Pf) of the cropping sequence. Therefore, sites (Switzerland, Germany, Sweden, and Italy) and sampling dates (Pi, Pf) were constraining and MEEs and replicates were conditional variables. Tillage, subsidiary crops and fertiliser were not considered in this analysis. Prior to analysis, data were scaled to meet requirements of the CCA and permutation tests. Significances of the CCA model, factors and axes were calculated using a permutation test with 999 permutations.

### Results

All main crops in the MEEs were grown successfully, although in Germany, the undersown clover species did not establish in both MEEs and thus, were referred to as green fallow (C). Furthermore, the summer vetch failed in MEE 2 due to a common vole (*Microtus arvalis*) epidemic since 2014. In Switzerland, subterranean clover (LM) was resown after wheat harvest to ensure a satisfactory establishment during the fallow period. The winter wheat in Sweden in MEE 1 yielded poorly and was infested with *Matricaria inodora* in all treatments. The oilseed radish in MEE 1 was particularly sparse and one cannot expect it to have much impact on the soil microbial community.

**Table 2** Plant-parasitic nematode species occurring at each site separated by taxa

Site	<i>Helicotylenchus</i>	<i>Paratylenchus</i>	<i>Pratylenchus</i>	<i>Tylenchorhynchus</i> -Group	Others
Switzerland					
MEE 1	<i>H. vulgaris</i>		<i>P. neglectus</i>	<i>T. dubius</i>	
MEE 2	<i>H. vulgaris</i>	<i>Paratylenchus</i> sp.	<i>P. neglectus</i>	<i>Nagelus obscurus</i> <i>Amplimerlinius macrurus</i>	
Sweden					
MEE 1	<i>H. canadensis</i> <i>H. variocaudatus</i>	<i>P. similis</i>	<i>P. crenatus</i> <sup>a</sup> <i>P. neglectus</i>	<i>Geocenamus quadrifer</i>	<i>Heterodera avenae</i> <i>Rotylenchus</i> sp.
MEE 2	<i>H. canadensis</i>	<i>Paratylenchus</i> sp.	<i>P. neglectus</i>	<i>T. dubius</i>	<i>H. avenae</i>
Germany					
MEE 1	<i>H. digonicus</i> <i>H. vulgaris</i>	<i>P. projectus</i>	<i>P. flakkensis</i> <i>P. neglectus</i> <i>P. penetrans</i>	<i>T. dubius</i>	<i>Rotylenchulus borealis</i> <i>Meloidogyne naasi</i> <i>Criconematidae</i>
MEE 2	<i>H. vulgaris</i>	<i>P. bukowinensis</i> <i>P. projectus</i>	<i>P. flakkensis</i> <i>P. neglectus</i> <i>P. penetrans</i>	<i>Amplimerlinius icarus</i> <i>Amplimerlinius macrurus</i> <i>T. dubius</i>	<i>M. naasi</i>
Italy					
MEE 1	<i>H. vulgaris</i>	<i>Paratylenchus</i> sp.	<i>P. neglectus</i>		
MEE 2	<i>H. vulgaris</i>	<i>Paratylenchus</i> sp.	<i>P. neglectus</i>		<i>Xiphinema</i> sp.

<sup>a</sup>Dominant species accounting for >80% of the specimen within this genus.

### Initial nematode densities

Initially, the most prevalent species was *Pratylenchus neglectus* that occurred at all sites in both years (Table 2). The second most common species occurring at three of four sites were *Helicotylenchus vulgaris* (Switzerland, Germany and Italy) and *Tylenchorhynchus dubius* (Switzerland, Sweden and Germany). Several species were site specific, such as *Geocenamus quadrifer* (syn. *Scutylenchus quadrifer*), a species known to be associated with wheat growth inhibition in Poland (Winiszewska et al., 2012) and Spain (Talavera & Jiménez, 1997), *Heterodera avenae* and *Paratylenchus similis* for Sweden, *Amplimerlinius icarus*, *Meloidogyne naasi*, *Paratylenchus projectus*, *Pratylenchus flakkensis*, *P. penetrans* and *Rotylenchulus borealis* for Germany and *Xiphinema* sp. for Italy (Table 2).

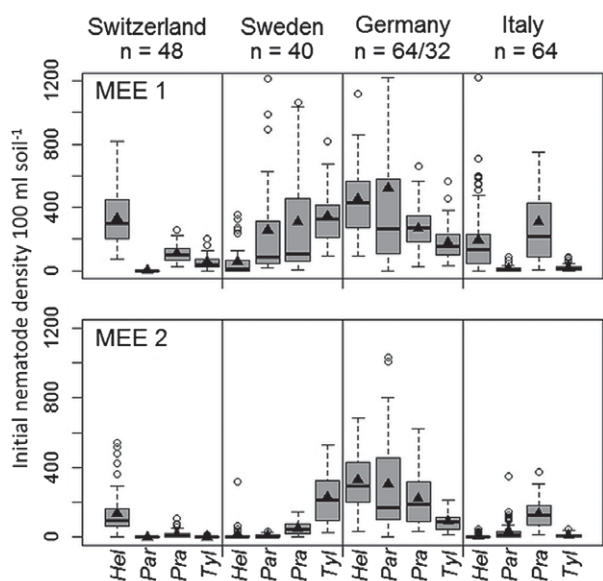
Within sites, species within the genera of *Helicotylenchus*, *Paratylenchus* and *Pratylenchus* found in MEE 1 were also found in MEE 2 with few exceptions. For example, *P. crenatus* was the dominant species in MEE 1 in Sweden, but was not detected in MEE 2. Variability in species spectrum between both MEEs at the same site was also observed for members of the *Tylenchorhynchus*-group in Switzerland and Sweden (Table 2).

The most frequent nematode genera found at the beginning of the experiments were *Helicotylenchus*, *Pratylenchus* and *Tylenchorhynchus* in all sites, and *Paratylenchus* in all sites but Switzerland (Fig. 1). All other genera occurred in densities below 20 nematodes per 100 mL soil and were not further included in the detailed analysis. Initial species densities in Switzerland and Sweden were generally below 100 nematodes per 100 mL

soil (median) in both MEEs except for *Helicotylenchus* in Switzerland that occurred in densities of 100–300 nematodes per 100 mL soil and *Tylenchorhynchus* in Sweden that was present in densities above 200 nematodes per 100 mL soil (Fig. 1). However, some extreme values were observed in Sweden for *Paratylenchus* and *Pratylenchus* causing deviations between medians and means. At the German site, initial plant-parasitic nematode densities were higher than at the other sites. In particular, *Helicotylenchus*, *Paratylenchus* and *Pratylenchus* reached densities of 100–400 nematodes per 100 mL soil (medians). In Italy, high initial densities of *Helicotylenchus* and *Pratylenchus* occurred in MEE 1 (approximately 200 and 300 nematodes per 100 mL soil, respectively), whereas in MEE 2, nematode densities were low except for *Pratylenchus* with 133 nematodes per 100 mL soil (Fig. 1).

### Nematode dynamics

The total density of plant-parasitic nematodes increased 2.7- and 1.6-fold in MEE 1 and MEE 2, respectively, in Switzerland and 1.1-fold in MEE 2 in Sweden. In contrast, populations decreased by 26–28% in Italy, 38–63% in Germany and 69% in MEE 1 in Sweden (data not shown). The dynamics of *Helicotylenchus*, *Paratylenchus*, *Pratylenchus* and *Tylenchorhynchus* were generally low within sites, resulting in similar initial (Pi) and final (Pf) nematode compositions (Fig. 2). However, treatments and crops grown in the crop sequence studied in the experiments had major impacts on final densities of *Helicotylenchus* and *Pratylenchus* (Figs 3, 4). There were no statistically significant interactions ( $P > 0.05$ ) between tillage system and

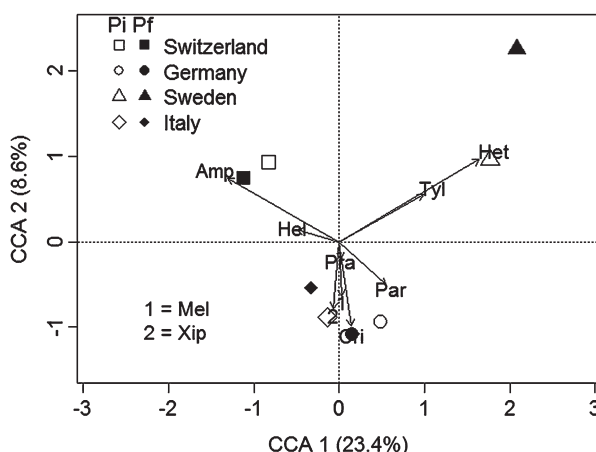


**Figure 1** Medians, means (triangles), upper and lower quartiles (whiskers) and outliers (circles) of initial (Pi) population densities of *Helicotylenchus* (*Hel*), *Paratylenchus* (*Par*), *Pratylenchus* (*Pra*) and *Tylenchorhynchus* spp. (*Tyl*) in the two multi-environment experiments (MEEs) at each site. Very high values (above 1200 nematodes per 100 mL soil) and sporadically occurring *Heterodera*, *Meloidogyne* and *Criconematidae* are not shown. N was 64 and 32 for MEE 1 and MEE 2, respectively, for Germany.

subsidiary crops in any of the variables measured in any of the experiments and therefore, only main effects are presented.

Final densities of other nematode genera, that is, *Amplimerlinius*, *Criconematidae*, *Meloidogyne*, *Heterodera* and *Xiphinema* were generally below 20 nematodes per 100 mL soil and were therefore considered less important for the detection of biologically interesting patterns of treatments. However, the site-specific occurrence of these nematode genera explained a large proportion of variation between sites in the constrained correspondence analysis (CCA, Fig. 2). The first three ordination axes of the CCA biplot were significant at  $P < 0.01$  (permutation test) explaining 96% of constrained eigenvalues. The first and second axes accounted for 23.4% and 8.6% of the total variance, respectively. The sites and sampling time (Pi, Pf) explained 38.8% of the total variance in nematode genera composition ( $P = 0.001$ , permutation test).

Overall, the CCA discriminated strongest between Switzerland and Sweden on the first axis (Fig. 2). The experimental site in Switzerland was characterised by the highest densities of *Amplimerlinius* and *Helicotylenchus*, which increased during the cropping sequence, regardless of the treatments. *Heterodera* were only found in Sweden, predominantly at the beginning of the experiments (20



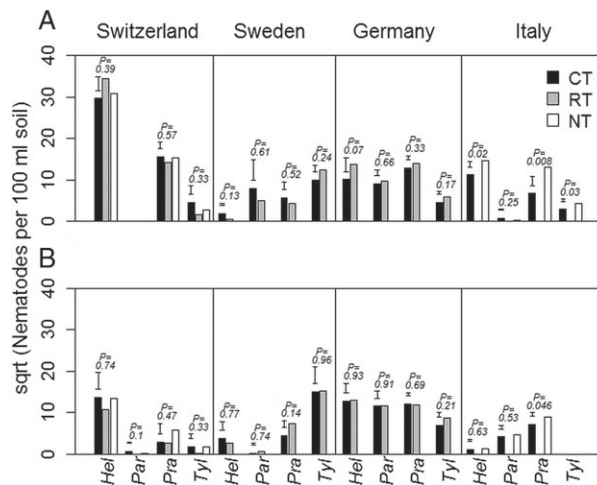
**Figure 2** Constrained correspondence analysis (CCA) biplot showing site effects on nematode composition at the beginning (Pi) and the end (Pf) of the cropping sequence with both experimental years (MEE 1, MEE 2) and replicates as conditional variables. Axis labels showing proportion of constrained total variance. Nematode genera scores were shown with abbreviations and arrows in direction of increasing density (*Amp*, *Amplimerlinius*; *Cri*, *Criconematidae*; *Hel*, *Helicotylenchus*; *Het*, *Heterodera*; *Mel*, *Meloidogyne*; *Par*, *Paratylenchus*; *Pra*, *Pratylenchus*; *Tyl*, *Tylenchorhynchus*; *Xip*, *Xiphinema*).

and 4 nematodes per 100 mL soil in MEE 1 and MEE 2, respectively). Therefore, the CCA clearly put Sweden apart from the other sites in Fig. 2. The CCA discriminated strongly between final and initial population densities in Sweden and to a lesser degree in the other sites. Germany and Italy fell close to each other in the CCA. *Xiphinema* correlated with the site Italy at the beginning of the cropping sequence. The German site was correlated with *Meloidogyne* and *Criconematidae* at the end of the cropping sequence suggesting an increase of these taxa during the experiment. In contrast, *Paratylenchus* correlated with the beginning of the cropping sequence in Germany, suggesting a decrease throughout the experiment. *Pratylenchus* was not correlated to any site, suggesting an equal occurrence (Fig. 2).

**Effect of tillage on plant-parasitic nematodes**

In general, noninversion (RT) and no-tillage (NT) had inconsistent effects on plant-parasitic nematodes compared to conventional tillage (CT). Statistically significant results ( $F_{df1,df2}$ ,  $P < 0.05$ ) were only obtained for the genera *Helicotylenchus* and *Pratylenchus*, which occurred at higher densities under NT than under CT.

In Switzerland, plant-parasitic nematode densities were highly variable and no statistically significant effect of tillage treatment ( $F_{2,6} < 3.5$ ,  $P > 0.099$ ) was observed for any of the genera in both MEEs. In MEE 1, very high densities of *Helicotylenchus* with up to 1241 nematodes



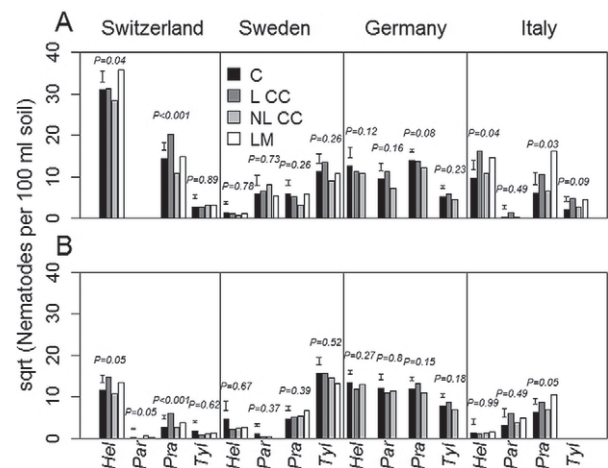
**Figure 3** Effects of tillage (CT: inversion tillage, RT: noninversion tillage, NT: no-tillage) on square root-transformed final densities (means + SEDs) of *Helicotylenchus* (*Hel*), *Paratylenchus* (*Par*), *Pratylenchus* (*Pra*) and *Tylenchorhynchus* (*Tyl*) per 100 mL soil in the first (A) and second (B) multi-environment experiment (MEE) in Switzerland [degrees of freedom denominator (dfd) = 6], Sweden (dfd = 3), Germany (dfd = 3), and Italy (dfd = 15), all separated by vertical lines; actual *P*-values are shown according to Wald-tests.

per 100 mL soil under RT (untransformed densities are shown here in contrast to Fig. 3A) were observed. In MEE 2, nematode densities were much lower than in MEE 1.

In Sweden, tillage effects on plant-parasitic nematode densities in both MEEs were low (Fig. 3) and not statistically significant for all genera ( $F_{1,3} < 4.3$ ,  $P > 0.13$ ). The largest average difference was observed for the densities of *Pratylenchus* that were higher in MEE 2 under RT (67 nematodes per 100 mL soil) than under CT (27 nematodes per 100 mL soil). The dominating genus, *Tylenchorhynchus*, with 260 nematodes per 100 mL soil) was not significantly affected by tillage.

Similar to Switzerland and Sweden, no significant effects of any of the nematode genera was observed in Germany. However, densities of *Helicotylenchus* tended to be higher under RT than under CT in MEE 1 (Fig. 3A,  $F_{1,3} = 7.9$ ,  $P = 0.067$ ). In MEE 2, no differences among tillage systems occurred.

In Italy, NT in combination with herbicide application significantly increased densities of *Helicotylenchus* ( $F_{1,15} = 7.1$ ,  $P = 0.02$ ), *Pratylenchus* ( $F_{1,15} = 9.3$ ,  $P = 0.008$ ), and *Tylenchorhynchus* ( $F_{1,15} = 6.0$ ,  $P = 0.03$ ) in MEE 1 (Fig. 3A). In particular, densities of *Pratylenchus* were more than four times higher (270 nematodes per 100 mL soil) under NT compared with CT (60 nematodes per 100 mL soil). In MEE 2, the nematode densities were generally lower than in MEE 1 and only *Pratylenchus* densities were significantly higher ( $F_{1,15} = 4.7$ ,  $P = 0.047$ ) in the NT



**Figure 4** Effects of subsidiary crops (C: green fallow, L CC: leguminous cover crop, NL CC: nonleguminous cover crop, LM: living mulch) on square root-transformed final densities (means + SEDs) of *Helicotylenchus* (*Hel*), *Paratylenchus* (*Par*), *Pratylenchus* (*Pra*) and *Tylenchorhynchus* (*Tyl*) per 100 mL soil in the first (A) and second (B) multi-environment experiment (MEE) in Switzerland [degrees of freedom denominator (dfd) = 33], Sweden (dfd = 21), Germany (dfd = 14), and Italy (dfd = 9), all separated by vertical lines; actual *P*-values are shown according to Wald-tests.

system (94 nematodes per 100 mL soil) compared with CT (61 nematodes per 100 mL soil, Fig. 3B). Both, *Helicotylenchus* and *Paratylenchus* densities were not affected by NT in combination with herbicide application.

#### Effects of subsidiary crops on plant-parasitic nematodes

Overall, leguminous subsidiary crops, either applied as living mulches (LM) or cover crops (CC), increased the densities of *Pratylenchus* and *Helicotylenchus* by the end of the experiment compared to the nonleguminous CC. In both MEEs in Switzerland, subsidiary crops significantly affected densities of *Pratylenchus* [ $F_{3,33} = 10.83$ ,  $P = 0.00004$  (MEE 1),  $F_{3,33} = 8.0$ ,  $P < 0.0004$  (MEE 2)] and *Helicotylenchus* [ $F_{3,33} = 3.0$ ,  $P = 0.04$  (MEE 1),  $F_{3,33} = 2.9$ ,  $P = 0.05$  (MEE 2)]. Hence, leguminous CCs and LM supported population build-up of *Pratylenchus* and *Helicotylenchus* compared with the nonleguminous CC but not necessarily compared to the green fallow control (C) (Fig. 4). Other plant-parasitic nematode genera were not affected by cover crops.

In Sweden, subsidiary crops did not affect plant-parasitic nematode densities significantly in neither of the MEEs (Fig. 4,  $F_{3,21} < 1.5$ ,  $P > 0.26$ ).

Similarly, in Germany effects of subsidiary crops on plant-parasitic nematodes were low. Solely in MEE 1, the densities of *Pratylenchus* were somewhat lower in the nonleguminous CC (152 nematodes per 100 mL



soil) than in the leguminous CC (196 nematodes per 100 mL soil) or the green fallow (205 nematodes per 100 mL soil) ( $F_{2,14} = 3.0$ ,  $P = 0.08$ ). In MEE 2, no significant differences were found between treatments (Fig. 4B,  $F_{2,14} < 2.2$ ,  $P > 0.15$ ).

In the MEE 1 in Italy, *Helicotylenchus* ( $F_{3,9} = 4.1$ ,  $P = 0.04$ ) and *Pratylenchus* ( $F_{3,9} = 5.0$ ,  $P = 0.03$ ) were significantly affected by subsidiary crops. Highest densities of *Helicotylenchus* were found in the leguminous CC (296 nematodes per 100 mL soil) and LM (261 nematodes per 100 mL soil) treatments compared with green fallow (116 nematodes per 100 mL soil) or nonleguminous CC (159 nematodes per 100 mL soil, Fig. 4A). Similarly, higher densities of *Pratylenchus* occurred in leguminous CC (170 nematodes per 100 mL soil) and LM (385 nematodes per 100 mL soil) than in nonleguminous or green fallow treatments (54 nematodes per 100 mL soil). In contrast, total nematode densities in MEE 2 were much lower than in MEE 1 and did not exceed 130 nematodes per 100 mL soil. Nevertheless, in MEE 2, subsidiary crops showed tentatively similar patterns for *Pratylenchus* ( $F_{3,9} = 3.8$ ,  $P = 0.052$ ) than observed in MEE 1. Accordingly, leguminous CC (81 nematodes per 100 mL soil) as well as LM (121 nematodes per 100 mL soil) treatments resulted in higher densities of *Pratylenchus* than green fallow (45 nematodes per 100 mL soil) and nonleguminous CC (64 nematodes per 100 mL soil, Fig. 4B).

#### Effects of nitrogen fertiliser and compost on plant-parasitic nematodes

Different nitrogen application rates in Switzerland and Italy (full or half dose;  $P > 0.25$ ) and the use of compost in Germany ( $F_{1,39} < 1.6$ ,  $P > 0.21$ ) had no statistically significant effects on plant-parasitic nematode dynamics in any of the MEEs. Only in MEE 1 in Switzerland, densities of *Pratylenchus* were significantly higher (300 nematodes per 100 mL soil) at full nitrogen dose compared to half dose (220 nematodes per 100 mL soil,  $F_{1,47} = 11.7$ ,  $P = 0.001$ ).

#### Discussion

The densities of plant-parasitic nematodes by the end of the experiments were not higher with noninversion tillage than inversion tillage at three of the four sites. However, in Italy the densities of plant-parasitic nematodes with a broad host spectrum, such as *Pratylenchus* and *Helicotylenchus* were greater under no-tillage than inversion tillage. Furthermore, both genera were more abundant in the vetch (*Vicia* spp.) and clover (*Trifolium* spp.) treatments. Thus, the use of nonleguminous subsidiary crops could be a useful strategy to reduce the density of some species of *Pratylenchus* and *Helicotylenchus*. However,

compost and different application rates of nitrogen did not affect the plant-parasitic nematode community. The effects of tillage, subsidiary crops and compost/fertiliser were evident despite of highly site and year-specific effects in the eight multi-environment experiments (MEEs), conducted in four countries over two successive years.

Site-specific climatic and soil conditions, such as organic matter contents that ranged from 1.2% (Italy) to 4.4% (Sweden) and soil texture (sandy in Italy to loamy in Germany), may have contributed to varying initial plant-parasitic nematode populations as well as to the nematode dynamics during the crop rotations.

#### Initial infestation

Initial densities of plant-parasitic nematodes were generally below the economic threshold level reported for each of the species. The highest nematode densities at experimental start were found in Germany, a site managed organically. This can probably be attributed to the 2 years of grass-clover mixtures (*Trifolium* spp., *Medicago sativa*, *Lolium perenne*, *Dactylis glomerata*, *Festuca pratensis*) in the preceding years. These are excellent hosts for migratory species with a broad host spectrum such as *Helicotylenchus*, *Paratylenchus*, *Pratylenchus* and *Tylenchorhynchus* (Sharma, 1971; Wouts & Yeates, 1994; Knight *et al.*, 1997).

For Sweden, the differences in initial nematode densities were most likely caused by the varying host status of the pre-crops, that is, winter wheat in MEE 1 and summer oilseed rape in MEE 2. Low initial densities of plant-parasitic nematodes in Switzerland and Italy are explained by growing pea (*P. sativum* L.) as preceding crop or applying 14 months of bare fallow before the start of the experiment, respectively. Peas are known to be poor hosts for the plant-parasitic nematodes occurring in Switzerland (Ferris *et al.*, 1993; Taylor *et al.*, 2000; Kruse, 2006; Smiley *et al.*, 2014). In Italy, the bare fallow fostered natural decline of plant-parasitic nematodes due to the absence of any food source (Viaene *et al.*, 2013).

The number of plant-parasitic nematode genera found at each site varied between three (Switzerland, Italy) and six (Sweden, Germany). Among all identified genera, *Pratylenchus* was the only one recorded from all sites, independent of the pre-crops. This can be explained by the broad host range of this genus (Castillo & Vovlas, 2007). However, the high densities of *Pratylenchus* after 14 months of bare fallow in summer-arid Italy were unexpected. Barley that was grown before the bare fallow period is a good host for *P. neglectus* (Taylor *et al.*, 2000). It apparently left over high densities of *P. neglectus* that could have survived the bare fallow under arid conditions in an anhydrobiotic resting stage (Glazer & Orion, 1983; Talavera & Vanstone, 2001). Another explanation could

be that weeds have contributed to *Pratylenchus* spp. survival. Although measures were applied to keep the fallow bare, ryegrass (*Lolium* spp.) survived in the fallow before MEE 1 in Italy and could have served as host for *Helicotylenchus* and *Pratylenchus*.

#### Final infestation

In general, plant-parasitic nematode genera that were found initially were also detected at the end of the experiment. Final population densities of plant-parasitic nematodes were higher than initial densities in Switzerland, but were equal or lower in Sweden, Germany, and Italy. The increase in nematode densities in Switzerland can be best explained by low initial densities followed by good hosts (wheat-maize). The reductions in Germany (MEE 1 + 2) and Sweden (MEE 1) were most likely due to high initial densities followed by less suitable hosts to the initially dominant species within the crop rotation. Somewhat unexpected were the low final infestations in Italy (MEE 1 + 2) following good host plants (legumes, tomato).

#### Tillage effects

The effect of tillage intensity on plant-parasitic nematode dynamics is discussed quite ambiguous in the literature. For example, an increase of *Pratylenchus* under long-term no-tillage conditions compared with 15 cm deep rotary tilling is reported from Japan (Okada & Harada, 2007). Similarly, in a study conducted in Iowa State, USA, *Helicotylenchus* and *Pratylenchus* tended to increase under no-tillage (Thomas, 1978). Furthermore, Thompson *et al.* (2008) reported from Australia that the root lesion nematode *Pratylenchus thornei* was more abundant under non-inversion and no-tillage compared to frequently tilled soils. In contrast, *Pratylenchus* and *Helicotylenchus* densities were not different among conventional ploughing (0–20 cm), minimum tillage (7 cm) and no-tillage, in a study conducted in the UK (Griffiths *et al.*, 2012) and Westphal *et al.* (2009) even reported that reducing tillage intensity reduced population densities of the specialised soybean cyst nematode *Heterodera glycines*. An explanation for those controversial observations could be variable weed infestation levels. In general, conservation tillage results in higher-weed pressure than conventional tillage (OSCAR, 2016) supporting a higher density of plant-parasitic nematodes. However, if weeds are thoroughly controlled in a minimum tillage system by herbicides or other means, plant-parasitic nematode densities can be lower than in a ploughed system without weed control as shown by Corbett & Webb (1970) for *Pratylenchus* in wheat. This underlines the importance of proper weed management for preventing plant-parasitic nematode build-up (Hallmann & Kiewnick, 2015).

#### Crop rotation effects

Plant-parasitic nematodes require a suitable host plant to feed on and propagate. Thus, the host status of a crop is the most important determinant of seasonal and annual changes in their population structure, density and distribution (Nusbaum & Ferris, 1973). As crops differ in their host status for certain nematode species, crop rotation plays a key role in managing plant-parasitic nematodes. However, the use of crop rotation for nematode management might be of limited value when several damaging species of nematodes are present or for species with broad host ranges (Barker & Koenning, 1998).

In the wheat–maize rotations, plant-parasitic nematode densities tended to increase. However, the constrained correspondence analysis showed that nematode spectra and dynamics were quite different between Switzerland and Sweden. For example, in Switzerland populations of *P. neglectus* and *H. vulgaris* increased over time, whereas in Sweden populations of *P. crenatus* and *H. variocaudatus* declined. On the other hand, *T. dubius* decreased in Switzerland, but increased in Sweden in MEE 2. Most likely this can be explained by site- and species-specific effects, such as different pre-crops resulting in different nematode spectra and therefore different initial infestation levels, climatic distinctions, soil discrepancies (e.g. organic matter) and differences in cultivars grown (Nusbaum & Ferris, 1973). The host status is species and cultivar specific and thus, contributes a great deal to the often observed variability in nematode reactions towards a certain crop species (Nicol & Rivoal, 2008).

This may further explain the higher densities of *Helicotylenchus* and *Pratylenchus* after leguminous than after nonleguminous subsidiary crops, especially in Switzerland and Italy. Both genera have a broad host range (Castillo & Vovlas, 2007) and are enhanced by the leguminous subsidiary crops used in this study (Townshend & Potter, 1976; Taylor *et al.*, 2000; Berry *et al.*, 2011; Visser & Molendijk, 2015). In contrast, nonleguminous subsidiary crops, such as *R. sativus* and *A. strigosa*, are nonhosts of *Pratylenchus* (Hirling, 1977; Visser & Molendijk, 2015). This could explain the lower densities of this genus in the nonleguminous CC treatments compared to the leguminous CC in Switzerland and Italy in both MEEs (Fig. 4).

Many nematode species reproduce best on sand and sandy-loam soils (Norton, 1979) most likely due to optimal particle sizes for nematode movement and water: oxygen ratio (Hallmann & Kiewnick, 2015). In addition, low soil organic matter contents are generally associated with higher densities of plant-parasitic nematodes due to the lack of antagonists (Sikora, 1992). For these reasons, the effects of subsidiary crops (and also tillage) were most

apparent and consistent in Italy and Switzerland, sites with the highest sand and low organic matter contents.

### Compost and fertiliser effects

The lack of effects of 5 t ha<sup>-1</sup> and per year compost application in Germany on the observed plant-parasitic nematode species could be due to the low dosage of application. However, the application of 269 t ha<sup>-1</sup> fresh yard waste compost (>10-fold the amount used in this study) also did not affect *Meloidogyne* and *Pratylenchus* spp. (McSorley & Gallaher, 1997). Although immediate effects of compost products appeared to be generally too low for nematode suppression (Akhtar & Malik, 2000), the long-term use of such organic amendments may increase the soil microflora (Griffiths *et al.*, 2010) and probably microbial antagonists of nematodes over time and therefore, need further investigation.

Similarly, nitrogen dose did not affect plant-parasitic nematode species, except for higher densities of *P. neglectus* that were found after application of the full nitrogen dose in MEE 1 in Switzerland. Similarly, 5 years' repeated application of full and half nitrogen dose in field experiments did not affect *Helicotylenchus*, *Pratylenchus* and *Tylenchorhynchus* densities in the UK (Boag *et al.*, 1998). However, nitrogen effects on plant-parasitic nematodes may also be source and dose dependent. For example, nitrogen applied in high doses as ammonia suppressed nematode populations in soil (Akhtar & Malik, 2000), while Elmer & LaMondia (1999) found higher root-lesion nematode densities in strawberry roots after ammonia than nitrate fertilisation. Higher nematode densities following nitrogen fertilisation were also observed by Okada & Harada (2007) in field experiments with soybean in Japan where the authors compared the effect of fertiliser application with no fertiliser application. Thus, plants not receiving fertiliser probably failed to provide sufficient nutrients for nematode propagation, which could explain the lower densities of plant-parasitic nematodes without fertiliser in their study. In our study, the low nitrogen level still provided enough nitrogen for adequate crop growth, while the nitrogen concentration in the high nitrogen level was probably too low to suppress plant-parasitic nematodes.

### Conclusion

We conclude that the combination of noninversion or no-tillage with specific subsidiary crops can be used to influence plant-parasitic nematode densities and the species composition to a certain extent. However, site-specific effects need to be taken into account. Thus, the effects were most evident for light soils with low organic matter contents. Nitrogen rates and compost

application did not affect plant-parasitic nematode compositions under our circumstances.

In general, effects of tillage intensity on plant-parasitic nematodes were marginal, although the genera *Helicotylenchus* and *Pratylenchus* were promoted by no-tillage on one site with sandy soils and low humus contents. Similarly, leguminous subsidiary crops supported higher densities of both species than the nonleguminous subsidiary crops *R. sativus* and *A. strigosa*. Thus, it is possible to counteract potential problems occurring with no-tillage with respect to nematode infestations by making use of the right cover crops. However, longer-term results with a variety of subsidiary crops are needed to provide farmers with appropriate and widely applicable recommendations suitable to their particular site-specific conditions.

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