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## ORIGINAL ARTICLE

# Reduced tillage, but not organic matter input, increased nematode diversity and food web stability in European long-term field experiments

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## Abstract

Soil nematode communities and food web indices can inform about the complexity, nutrient flows and decomposition pathways of soil food webs, reflecting soil quality. Relative abundance of nematode feeding and life-history groups are used for calculating food web indices, i.e., maturity index (MI), enrichment index (EI), structure index (SI) and channel index (CI). Molecular methods to study nematode communities potentially offer advantages compared to traditional methods in terms of resolution, throughput, cost and time. In spite of such advantages, molecular data have not often been adopted so far to assess the effects of soil management on nematode communities and to calculate these food web indices. Here, we used high-throughput amplicon sequencing to investigate the effects of tillage (conventional vs. reduced) and organic matter addition (low vs. high) on nematode communities and food web indices in 10 European long-term field experiments and we assessed the relationship between nematode communities and soil parameters. We found that nematode communities were more strongly affected by tillage than by organic matter addition. Compared to conventional tillage, reduced tillage increased nematode diversity (23% higher Shannon diversity index), nematode community stability (12% higher MI), structure (24% higher SI), and the fungal decomposition channel (59% higher CI), and also the number of herbivorous nematodes (70% higher). Total and labile organic carbon, available K and microbial parameters explained nematode community structure. Our findings show that nematode communities are sensitive indicators of soil quality and that molecular profiling of nematode communities has the potential to reveal the effects of soil management on soil quality.

## KEYWORDS

amplicon sequencing, food web indices, long-term field experiments, nematode communities, organic matter addition, tillage

## 1 | INTRODUCTION

The capacity of soils to perform multiple processes defines and determines soil quality (Bünemann et al., 2018). Soil management can negatively affect soil processes exerting threats (e.g., soil erosion, compaction, acidification and organic matter losses) on chemical, physical and biological properties (Toth, Montanarella, & Rusco, 2008). Tillage and fertilization are widespread soil management measures which can have a substantial influence on these soil threats, ultimately affecting soil processes and soil quality.

Soil nematodes are abundant and ubiquitous organisms that have an important role in various processes such as nutrient cycling, decomposition, pest and pathogen population regulation (Ekschmitt et al., 2001; Neher, Weicht, & Barbercheck, 2012). In soils, nematodes are present at all trophic levels, and can therefore be divided into functional groups based on their feeding preferences (Yeates, Bongers, Goede, Freckman, & Georgieva, 1993). Nematodes can also be differentiated according to their life-history strategies reflected in their position on a colonizer-persister (c-p) scale, which goes from group 1 (colonizers = *r* selected species) to group 5 (persisters = *K* selected species; Bongers, 1990). Colonizers thrive in nutrient-rich habitats, are generally bacterivores, tolerant to stress and pollutants, with short generation times, while persisters poorly react to conditions of high food availability, are bigger omnivorous and/or predatory nematodes sensitive to stress, have longer generation times and generally live in temporally stable habitat. Many species have intermediate characteristics. Relative abundance of nematode feeding and life-history groups are used for calculating food web indices, i.e., the maturity index (MI: measure of environmental disturbance), enrichment index (EI: measure of resource availability), structure index (SI: measure of degree of trophic links and capacity to recover from stress) and channel index (CI: indication of predominantly fungal or bacterial decomposition pathway; Bongers, 1990; Ferris, Bongers, & Goude, 2001), which are used to determine soil processes affecting soil quality.

Due to interactions with other soil biota and the influence of chemical and physical abiotic factors (Bongers & Ferris, 1999), changes induced by soil management affect nematode communities (Ferris & Bongers, 2006; Sánchez-Moreno, Nicola, Ferris, & Zalom, 2009). These changes in the nematode community can be due to modifications in food resources such as plant residues, nutrients, and environmental properties such as pH, oxygen content, porosity and temperature (Mekonen, Petros, & Hailemariam, 2017; Yeates & Bongers, 1999). Thus, data on nematode communities integrate information from soil chemical, physical and biological properties (Mekonen et al., 2017; Neher, 2001). This can increase our understanding of the impact of soil management on soil processes and, indeed, on soil quality in general.

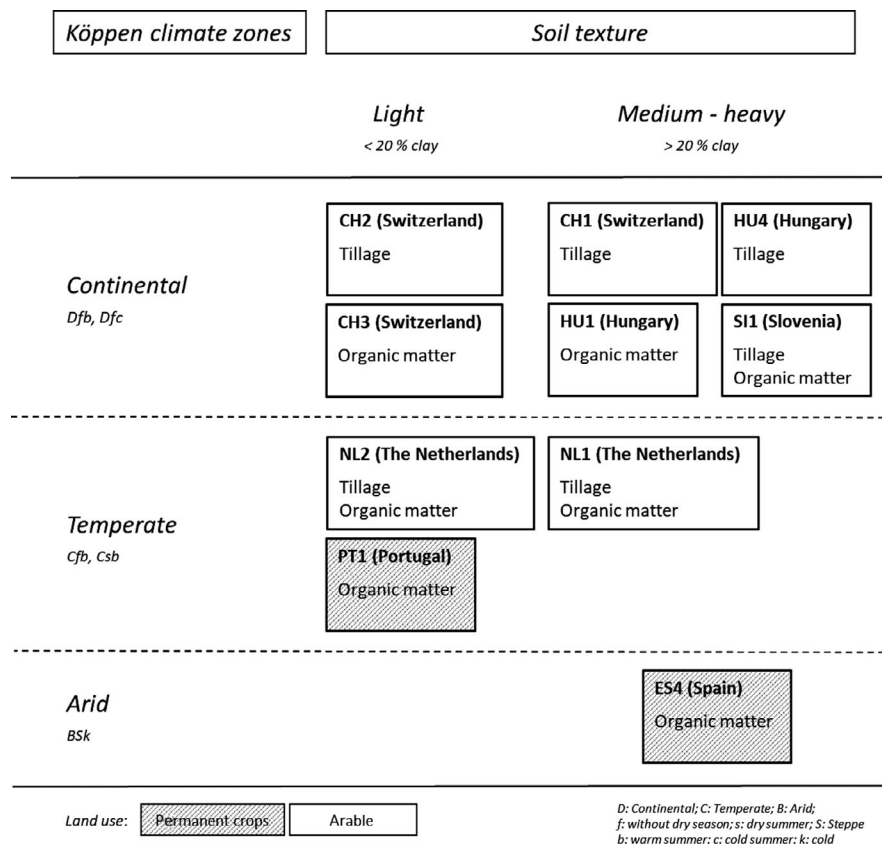
Nematode diversity and specific nematode groups (i.e., based on feeding and/or life-history strategies) or taxa (i.e., family, genus, or species) have been shown to respond differently to soil management such as tillage and fertilization (Moura & Franzener, 2017; Yeates & Bongers, 1999). More in detail, previous studies found higher

nematode diversity and higher percentages of fungal feeders, omnivores and predators (slow-growing nematodes of c-p groups 4 and 5) in less disturbed conditions such as systems under reduced tillage or with perennial crops (Liu et al., 2016; Niles & Freckman, 1998; Yeates & Bongers, 1999). In contrast, fast-growing bacterivorous nematodes (c-p groups 1 and 2) have been associated with eutrophic and mineral fertilized, disturbed systems (Darby, Todd, & Herman, 2013; De Goede, Bongers, & Ettema, 1993; Quist et al., 2016; Zhao & Neher, 2013). Also the application of different organic materials such as manure, compost and cattle slurry has been shown to increase the abundance of bacterivorous nematodes (Forge, Bittman, & Kowalenko, 2005; Leroy, Bommele, Reheul, Moens, & De Neve, 2007), and, in some cases, to decrease the abundance of plant parasitic nematodes (Leroy et al., 2007).

In most publications so far, the response of nematode communities to tillage and fertilization was studied in single field experiments (Ito, Araki, Komatsuzaki, Kaneko, & Ohta, 2015; Quist et al., 2016; Zhao & Neher, 2013), sometimes yielding contradictory results (Ferris et al., 2012; Leroy et al., 2007; Treonis et al., 2018). One factor hampering the study of management effects across multiple study sites is that traditional microscopy is the most common method to study nematodes, which is time-consuming, requires specialists and is expensive (Ritz, Black, Campbell, Harris, & Wood, 2009). Molecular methods to assess nematode absolute abundances (qPCR) and diversity (high-throughput amplicon sequencing, DGGE, T-RFLP) are faster, cheaper, and allow higher throughput than visual methods (Ahmed, Sapp, Prior, Karssen, & Back, 2016; Geisen et al., 2018). Amplicon sequencing may allow identification of taxa that cannot be distinguished morphologically. One limitation of PCR-based molecular methods is that not actual abundances of the specimen but rather their relative number of DNA copies are assessed (Porazinska et al., 2009; Waite et al., 2003). However, there is recent evidence that molecular methods might give similar ecological patterns as traditional methods (Geisen et al., 2018; George & Lindo, 2015; Hamilton, Strickland, Wickings, Bradford, & Fierer, 2009; Porazinska, Sung, Giblin-Davis, & Thomas, 2010; Quist et al., 2016). Hence, amplicon sequencing has high potential to assess soil management effects on nematode communities across multiple field experiments.

The goal of the present study was to: (a) Assess the effect of tillage and organic matter addition on nematode qPCR counts, alpha and beta-diversity, and food web indices as measured by amplicon sequencing of the 18S rRNA gene; (b) investigate the relationships between nematode community characteristics and other soil parameters related to soil processes; and (c) identify taxa that could serve as indicator organisms for soil management. We expected that molecular techniques would be sensitive, efficient tool to reveal general patterns of soil management effects on nematode communities in 10 long-term field experiments across Europe. We hypothesized that (a) reduced tillage would increase nematode qPCR counts, alpha diversity, MI, SI and CI, and decrease levels of bacterivorous nematodes with short life-cycles compared to conventional tillage, and that (b) high organic matter addition would increase qPCR counts, alpha diversity, EI, and alter the

**FIGURE 1** Main pedoclimatic characteristics and soil management (tillage, organic matter input, or a combination of the two) of 10 long-term field experiments analysed in the current study. CH1, Frick trial; CH2, Aesch trial; CH3, DOK trial; ES4, Pago trial; HU1, Keszthely trial; HU4, Keszthely trial; NL1, Basis trial; NL2, De Peel trial; PT1, Vitichar trial; SL1 Tillorg trial. For detailed information about the experiments see Table S1



nematode communities towards higher populations of bacterivorous nematodes compared to low organic matter input. We also hypothesized that (c) the positive effect of reduced tillage and organic matter addition on total and labile organic matter, available nutrients, water stable aggregates, and microbial biomass and activity would result in a positive relationship between these soil parameters and the nematode communities and that (d) nematode taxa with long life cycles and sensitive to management (such as predatory and omnivorous nematodes in c-p groups 4 and 5) would be more associated with less disturbed systems, and as such would be sensitive indicator taxa for soil disturbance.

## 2 | MATERIALS AND METHODS

### 2.1 | Long-term field experiments and management

We selected 10 European long-term field experiments with either arable or permanent crops and a minimum duration of 5 years and a maximum duration of 44 years (Figure 1, Table S1). Throughout the paper we will refer to these long-term field experiments as “LTEs”.

This selection covered five different European climatic zones (Köppen, 1918; Figure 1, Table S1) and six soil textural classes (Table S1; WRB, 2014).

Each LTE had unique management characteristics and a different experimental design, with three or four replicates per treatment (Table S1). However, LTEs were comparable because the main soil management types were tillage (T) and organic matter addition (OM) as described in Bongiorno, Bünemann, et al.

(2019). The contrast in tillage was classified as conventional tillage (ploughing at 20–25 cm depth, CT) versus reduced tillage (no-tillage or noninversion tillage at 0–10 cm with different light machinery, RT). The contrast in organic matter addition was classified as low organic matter addition (LOW, no organic matter additions or only mineral fertilization) versus high organic matter addition (HIGH, organic matter additions without or with mineral fertilizer). At some LTEs, both treatment factors (i.e., tillage and organic matter addition) were applied and at others only one of these was present (Figure 1).

### 2.2 | Sampling procedure and sample handling

A total of 167 soil samples were collected in spring 2016 before any major soil or crop management was started in the LTEs. Each sample consisted of a composite sample of 20 soil cores randomly collected in the central area of the plot, to avoid border effects, and mixed. In the tilled LTEs, samples were taken from two depths: 0–10 cm and 10–20 cm. In the LTEs with organic matter addition as the only management factor (no tillage factor), samples were taken from the 0–20 cm layer because we did not expect to find a stratification effect due to tillage. After soil sampling, 400 g of the samples were air-dried (40°C) for subsequent chemical analysis. Fresh soil samples were sent to Wageningen University (The Netherlands), Research Institute of Organic Agriculture (Frick, Switzerland), University of Trier (Germany) and University Miguel Hernandez (Alicante, Spain), and air-dried samples were sent to University of Ljubljana (Slovenia).

Upon arrival, the samples were sieved at 5 mm and, when fresh, stored at 3°C until further processing.

## 2.3 | Chemical, physical and biological soil properties

The following soil properties were measured for this study: total organic carbon (TOC: %), pH ( $\text{CaCl}_2$ ), total nitrogen (TN: %), cation exchange capacity (CEC: mmol 100 g soil), plant available phosphorus (P: mg/kg soil), plant available potassium (K: mg/kg soil), exchangeable magnesium, calcium, and sodium ( $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ; mg/kg soil), water-stable aggregates (WSA: mg/kg soil), water holding capacity (WHC: %), bulk density (BD:  $\text{g/cm}^3$ ), percentages of silt, clay, and sand, microbial biomass carbon (MBC: mg/kg soil), microbial biomass nitrogen (MBN: mg/kg soil), soil respiration (SR:  $\mu\text{g CO}_2\text{-C hr}^{-1}\text{ g}^{-1}$  soil), number and biomass of earthworms (number and  $\text{g/m}^2$ ), decomposition through tea bag index (% mass loss) and soil suppressiveness to *Pythium ultimum* (%; Bongiorno, Postma, et al., 2019). Microbial quotient (qMic) and metabolic quotient ( $\text{qCO}_2$ ) were calculated as the microbial biomass carbon divided by the total organic carbon, and the soil respiration divided by the microbial biomass carbon, respectively. Besides chemical, physical and biological parameters, five different labile carbon fractions were measured: hydrophilic dissolved organic carbon (Hy-DOC: mg/kg soil), dissolved organic carbon (DOC: mg/kg soil), permanganate oxidizable carbon (POXC: mg/kg soil), hot water extractable carbon (HWEC: mg/kg soil), and particulate organic matter carbon (POMC: mg/kg soil; Bongiorno, Bünemann, et al., 2019). In addition, the specific ultraviolet absorbance of Hy (Hy SUVA:  $\text{L g C}^{-1}\text{ cm}^{-1}$ ) and DOC (DOC SUVA:  $\text{L g C}^{-1}\text{ cm}^{-1}$ ) was measured to assess the recalcitrance of these labile carbon fractions. All analyses were performed within 6 months after sampling and the details of the methodology and locations where the analyses took place are presented in Table S2 (modified from Bongiorno, Postma, et al., 2019).

## 2.4 | Nematode analysis

### 2.4.1 | Nematode extraction, DNA extraction and DNA purification

Within 2 weeks after sampling nematodes were extracted from 100 g field moist subsamples using a modified elutriator (Oostenbrink, 1960). Thereafter nematodes were incubated for 72 hr on a double cotton-wool filter (Hygia milac). A subset of samples from each LTE (a total of 97 samples) was counted microscopically, with 1/10 of each sample counted in duplicate under a dissecting microscope. The number of nematodes was expressed per 100 g of field moist soil. The nematode suspensions were subsequently concentrated and lysed with a lysis buffer containing proteinase K,  $\beta$ -mercaptoethanol and an internal mammalian standard in order to correct for the loss of DNA during lysis and DNA purification (Holterman et al., 2006; Vervoort et al., 2012). Thereafter, DNA extracts were purified using a glass fibre

column-based procedure (Ivanova, Dewaard, & Hebert, 2006) and stored at  $-20^\circ\text{C}$  until further use.

### 2.4.2 | Quantitative PCR (qPCR) analysis of total nematode DNA

The purified DNA extracts were used as templates in qPCR using two primer sets to assess total nematode densities (Quist et al., 2017; Vervoort et al., 2012). The first primer set targeted DNA across the phylum Nematoda and the second targeted the mammalian internal standard. After the qPCR reactions, the  $C_t$ -values obtained were related to the microscopic counts to obtain a calibration curve at the  $^{10}\text{Log}$  scale (see Vervoort et al., 2012). Thereafter,  $C_t$ -values were converted into nematode densities using this linear relationship between the  $C_t$  values and the  $^{10}\text{Log}$  (number of target nematodes; Figure S1a). The maxima of the negative, first mathematic derivative of the melting curves were checked to confirm the correct nature of the amplicons. The internal control was used to monitor and correct for loss of DNA during the sampling handling. Throughout the manuscript qPCR-based quantification of nematode densities is referred to as "nematode qPCR counts".

### 2.4.3 | 18S rRNA gene amplification and sequencing

Nematode DNA was quantified with Nanodrop (NanoDrop 2000 Spectrophotometer, Thermo Fischer Scientific) and subsequently sent on dry ice to GenomeQuebec for 18S rRNA gene amplification and sequencing on the Illumina MiSeq platform. In a first step a targeted PCR amplification with tagged primers for the hypervariable eukaryotic V4 region of the 18S rRNA gene was performed (Table S3). We used the universal eukaryotic primers 3NDf (5'-GGCAAGTCTGGTGCCAG-3') in combination with 1132rmod (5'-TCCGTC AATTCTTAAAGT-3') as used in Geisen et al. (2018). In a next step, Illumina adapters with barcodes sequences were added by PCR to each sample (barcoding step; Table S3). For each sample, the barcoding step was verified with gel electrophoresis. The DNA concentration was quantified with Quant-iT PicoGreen dsDNA Assay kit (Life Technologies) and for each sample, an equal amount of DNA was pooled for a sequencing library. After purification with AMPure beads (Beckman Coulter), the pooled DNA library was quantified using the Quant-iT PicoGreen dsDNA Assay kit (Life Technologies) and the Kapa Illumina GA Library Quantification kit with revised primers (KAPA SYBR FAST qPCR Universal kit, Kapa Biosystems). Average fragment size was determined using a LabChip GX (PerkinElmer) instrument. Sequencing was performed with MiSeq Reagent kit v3 (600 cycles) from Illumina. After sequencing, the sequences were demultiplexed by GenomeQuebec using the Illumina BCL2FASTQ Conversion Software version 2.17.1.14.

### 2.4.4 | Bioinformatic analysis

The amplicon sequencing data was analyzed by the Genetic Diversity Centre (GDC), ETH Zurich, using the HPC Euler of ETH Zurich. The merging efficiency of the forward (R1) and reverse (R2) reads was

relatively low (<11%). For this reason, we restricted the analysis to the forward read only. In a first step, the primer sites were trimmed off the R1 reads and all the reads were trimmed to an equal length of 280 nt using USEARCH (Edgar, 2010). Subsequently, the reads were quality filtered (parameter: GC range 20–80, minimum quality mean 20, no ambiguous nucleotides, and a low complexity filter, dust with threshold 30) using PRINSEQ-LITE (version 0.20.4). About 10% of the total sequencing data was lost during primer trimming (7.5%), trimming (<1%), and quality filtering (2.6%). In a next step, UPARSE (Edgar, 2013) was used to cluster the sequences and create a count table. For the annotation of the OTUs SINTAX (Edgar, 2016) and the protist ribosomal reference database (PR2) were used. The OTUs which could not be assigned to a taxonomic group were verified with manual BLAST searches with NCBI nt based references databases (see Figure S2).

#### 2.4.5 | Nematode alpha diversity, trophic groups and food web indices

Alpha diversity is defined as the diversity of organisms within groups (in our case calculated within plots), while beta diversity is defined as the diversity of organisms between groups (Jost, 2010). Alpha diversity is measured through indices of richness, diversity and evenness (Jost, 2010). Nematode OTU or genus richness was calculated as the sum of the OTUs or genera, respectively. Nematode OTU and genus diversity was calculated as the exponential of the Shannon Index (Magurran, 1988):

$$\exp^H = \exp^{\sum_{i=1}^S -(P_i \cdot \ln P_i)} \quad (1)$$

where  $H$  is the Shannon diversity index,  $P_i$  is the fraction of the entire population made of OTU or genus  $i$ ,  $S$  is the number of OTUs or genera encountered, and  $\Sigma$  is the sum of OTU or genus 1 to OTU or genus  $S$ . Nematode OTU and genus evenness (Sheldon evenness) was calculated as the exponential of the Shannon diversity divided by the number of OTUs or genera (Heip, 1974).

We calculated the percentages of five trophic groups (bacterivorous, fungivorous, herbivorous, predators and omnivorous nematodes), maturity index (MI), enrichment index (EI), structure index (SI), and channel index (CI), according to the classification of nematode OTUs into functional groups, uploading the count table based on OTU observed abundance with taxonomic information obtained after the bioinformatic analysis of the nematode sequencing data in the nematode indicator joint analysis (NINJA) program (Sieriebriennikov, Ferris, & de Goede, 2014; <http://sieriebriennikov.shinyapps.io/ninja/> consulted on 9 January 2019). NINJA was used also to assign nematodes to the colonizer-persister (c-p) scale (from 1 to 5; Bongers, 1990; Ferris et al., 2001). The absolute abundance of trophic groups and c-p groups was calculated by multiplying the total qPCR counts by the trophic and the c-p groups percentages calculated with NINJA.

## 2.5 | Statistical analysis

All statistical calculations were carried out using R version 3.5.1 and RSTUDIO version 1.1.456 (R Development Core Team, 2013; RStudio

Team, 2016). The R script is provided as Appendix S2, and a workflow of the data analysis steps is given in Figure S3. The nematode OTU counts and taxonomy tables were filtered before the analysis to exclude OTUs which were classified as non-nematodes, or whose kingdom or phylum was unassigned. All test results, except for the indicator species analysis, were considered statistically significant at  $p \leq .05$ .

#### 2.5.1 | Nematode qPCR counts, alpha and beta diversity per LTE

Nematode OTU richness and diversity were calculated after rarefaction (500×) to 10,537 seq/sample (the minimum sample sequencing depth; Bodenhause, Horton, & Bergelson, 2013).

A general beta diversity analysis was conducted on the nematode communities of all the sites. For this analysis, we filtered the OTU sequence counts retaining only OTUs with a minimum of five counts in at least eight samples. After normalization using the total sum scaling (TSS) with the decostand (method = "total") function in the VEGAN package (Oksanen et al., 2018), we computed Bray-Curtis dissimilarity matrices on the squared rooted transformed data (Leff et al., 2015). Canonical analysis of proximities (CAP) with vegan function capscale was performed to visualize and test the relationships between the nematode community and the most important soil chemical, physical and biological parameters measured in the LTEs (Anderson & Willis, 2003). The function vif.cca (threshold used  $\text{vif} \leq 10$ ) was used to retain variables which were not highly correlated ( $\rho > 0.80$ ). The effect of the environmental variables on the nematode communities was assessed with permutation analysis (using the anova function in VEGAN by "margin") with  $10^4$  permutations and correlations between the environmental variables and the first two axes of the CAP to assign their relative importance.

#### 2.5.2 | Effects of tillage and organic matter additions on nematode qPCR counts, alpha and beta diversity

To test the effects of tillage and organic matter addition on soil nematode communities, two groups of LTEs were created because we expected stratification effects in LTEs with reduced tillage only, as shown in previous analyses (Bongiorno, Bünemann, et al., 2019). The following two groups were studied separately in the subsequent analyses:

##### Group A

The LTEs in which the layers 0–10 cm and 10–20 cm were sampled separately in space: CH1, CH2, NL1, NL2, SL1, HU4 and ES4. In this group, we assessed the effect of tillage, organic matter addition and soil layer.

##### Group B

The LTEs where the layer 0–20 was sampled: CH3, PT1 and HU1. In this group we only assessed the effect of organic matter addition, since these LTEs were under conventional tillage.



The effect of tillage and/or organic matter addition and, if present, layer on total nematode qPCR counts, OTU and genus richness and diversity, and OTU evenness were assessed in group A and B (using overall models merging the LTEs in the same group) by performing an analysis of variance (standard function anova) on fitted linear mixed effect models. Mixed models were used to take into account the possible correlations introduced by the multisite field experiments and to generalize the effect of the soil management practices across the different LTEs (Bongiorno, Bünemann, et al., 2019). The tillage and/or the soil organic matter addition and, if present, the layer, their two-way and possibly three-way interactions were used as fixed factors. Random effects for LTEs, blocks, main plots and subplots were introduced in the models to represent the experimental designs of the different LTEs. The effect of the pedoclimatic zone was not included in the fixed part of the model because we were interested in management effects across pedoclimatic zones. The model assumptions of normality and homogeneity of variances of the residuals were checked both visually and with the Shapiro-Wilk and Levene's tests (Zuur, 2009). Total nematode qPCR counts and OTU richness, diversity and evenness were square-root-transformed in order to meet the assumption of normality. All tests were considered statistically significant at  $p \leq .05$ . For the linear mixed effects model, the packages nlme, and emmeans were used (Pinheiro, Bates, DebRoy, & Sarkar, 2018). The same linear mixed effect models were used to assess differences in relative and absolute abundances of trophic and c-p groups, and in food web indices between soil management.

We then performed multivariate analysis of nematode communities on Bray-Curtis dissimilarities as outlined by Anderson and Willis (2003) using squared-root TSS normalized data. Using a permutational multivariate analysis of variance (PERMANOVA) with  $10^4$  permutations we tested the effect of tillage and/or organic matter and, if present, the layer on the community dissimilarity. In this analysis, the LTE was specified as random factor in the strata argument which restricts permutations to within LTEs (Anderson, 2001). The function betadisp was used to perform permutational analysis of multivariate dispersion (BETADISP) with  $10^4$  permutations.

We then visualized the effect of soil management with canonical analysis of proximities (CAP) constrained ordination (Anderson & Willis, 2003) using the function capscale in the vegan package with the LTE as a conditional factor in order to control for the effect of the pedoclimatic zone on the nematode communities. Statistical significance of the CAP was assessed using the permutest function in the VEGAN package.

### 2.5.3 | Relationships between nematodes and soil parameters

Partial correlations, correcting for the variation caused by the intrinsic differences between the LTEs (pedoclimatic zones), were used to test the relationships between nematode qPCR counts, OTU richness, diversity and evenness and the soil chemical, physical and

biological parameters. For the correlation analyses the packages CAR, STATS and PPCOR were used (Kim, 2015).

The relationships between nematode communities and environmental variables shaped by the effect of the soil management practices was visualised using canonical analysis of proximities (CAP) and tested using the envfit function in the package VEGAN. The effect of the soil parameters was assessed with permutation analysis with  $10^4$  permutations.

### 2.5.4 | Identification of putative indicator OTUs

Determination of nematode OTUs associated with specific management combinations was done using correlation-based indicator analysis with the function multipatt of the R package indicspecies (De Cáceres, 2016) to calculate the point-biserial correlation coefficient ( $r$ ) of an OTU's positive association to a soil management factor or a combination of factors. The analysis was done with  $10^4$  permutations and considered a more stringent significance level at  $p \leq .01$ , in order to limit the indicator species to a subgroup of highly sensitive OTUs associated with soil management. In the analysis we restricted the permutation within the blocks and within the LTEs to take into account the nested structure of the design.

## 3 | RESULTS

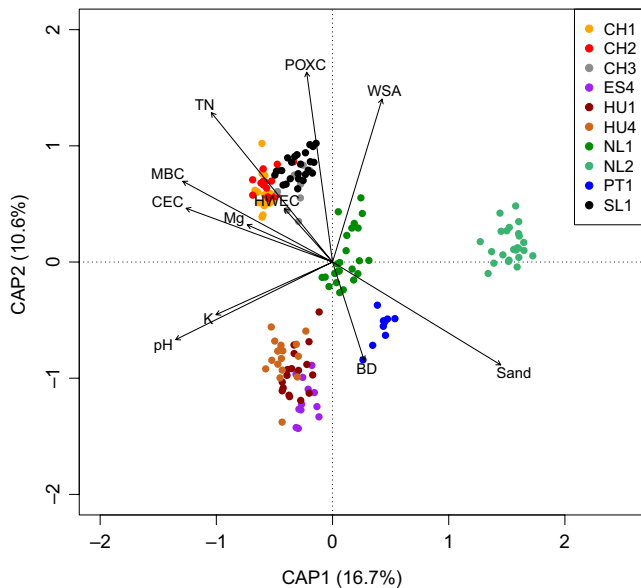
### 3.1 | Nematode beta diversity across the long-term field experiments

In the CAP, the community composition showed a clustering of samples according to the long-term field experiments (LTEs; Figure 2), and PERMANOVA confirmed that the nematode communities were affected by the LTE ( $R^2 = .64$ ;  $p = .001$ ). A total of 50% of variation in the nematode beta diversity among the different LTEs was explained by the constraining variables used in the CAP.

According to ANOVA of the constraining variables, all the soil parameters were significantly related to the nematode beta diversity in the LTEs (Table S4). The soil parameters that were most important in explaining the variation between the different LTEs (i.e., significant relationship and Pearson correlation coefficient ( $r$ ) with the canonical axes greater than +0.50 or smaller than -0.50) were for CAP1: sand content, pH, microbial biomass carbon (MBC), cation exchange capacity (CEC), and total nitrogen (TN); for CAP2: permanganate oxidizable carbon (POXC), water stable aggregates (WSA), and total nitrogen (TN).

### 3.2 | Effect of soil management on total nematode qPCR counts and alpha diversity

In group A (i.e., LTEs with tillage and organic matter addition as treatments, sampled at two soil depths), nematode qPCR counts were higher in the first layer (0–10 cm) than in the second layer (10–20 cm; Table 1). We found higher nematode OTU richness, diversity, and evenness and genus diversity and evenness in reduced tillage compared to conventional tillage across the LTEs of group A. In this



**FIGURE 2** Constrained analysis of proximities (CAP) of the nematode communities in the long-term field experiments and the relation with soil parameters. The first axis, CAP1 explains 16.7% and the second axis explains 10.6% of the variation in the beta diversity between the nematode communities in the different sites. BD, bulk density; CEC, cation exchange capacity; HWEC, hot water extractable carbon; K, available potassium; MBC, microbial biomass carbon; Mg, magnesium; pH, potential of hydrogen; POXC, permanganate oxidizable carbon; Sand, sand; TN, total nitrogen; WSA, water stable aggregates. CH1, Frick trial; CH2, Aesch trial; CH3, DOK trial; ES4, Pago trial; HU1, Keszthely trial; HU4, Keszthely trial; NL1, Basis trial; NL2, De Peel trial; PT1, Vitichar trial; SL1, Tillorg trial [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

analysis, OTU richness and diversity, and genus richness had higher values in the upper than in the lower layer, regardless of the tillage treatment (OTU richness and diversity 11% and 18% higher, respectively, and genus richness 9% higher). OTU and genus diversity and evenness were lower (16% and 22% for the OTU and 28% and 28% for genus, respectively) in the high organic matter addition plots.

In group B (i.e., LTEs with organic matter addition only, sampled between 0–20 cm soil depth), we found no significant effects of organic matter addition on total nematode qPCR counts, OTU and genus richness and diversity (Table 1).

### 3.3 | Effect of soil management on beta diversity

PERMANOVA of group A revealed that the largest proportion of the variation in nematode beta diversity was explained by the LTEs ( $R^2 = .628$ ,  $p = .0001$ ). Despite this, tillage ( $R^2 = .012$ ,  $p = .0001$ ), organic matter addition ( $R^2 = .006$ ,  $p = .006$ ), layer ( $R^2 = .014$ ,  $p = .0001$ ) and the interaction between tillage and layer ( $R^2 = .006$ ,  $p = .002$ ) had significant effects on the nematode beta diversity (Figure 3a, Table S5). The significant interaction between tillage and layer indicates that under reduced tillage a significant effect of the layer was found, but not under conventional tillage.

The CAP model of group A explained in total 8% of the variation in beta diversity related to soil management (tillage, organic matter addition), and the first two axes explained 2.6% and 2.3% of variation, respectively. CAP1 axis separated the samples belonging to the lower layer of reduced tillage from the rest, while CAP2 axis, from top to bottom, separated the different tillage treatments.

In group B, PERMANOVA did not reveal effects of organic matter addition ( $R^2 = .013$ ,  $p = .186$ ) on the nematode beta diversity (Table S5).

The dispersion tests were not significant, suggesting that differences between management were driven primarily by true biological differences and not by an artefact of the differences of the within-group dispersion (Table S6).

### 3.4 | Effect of soil management on nematode trophic groups and food web indices

Bacterivorous nematodes were the most abundant trophic group, followed by herbivorous, fungivorous, omnivorous and predatory nematodes (Table 2, Table S7). For group A, we found a stratification effect of reduced tillage on relative abundance of bacterivorous nematodes, with lower values in the lower than in the upper layer (24% lower,  $p = .0005$ ; Figure S4). The proportion of herbivorous nematodes was higher in the lower layer of reduced tillage (44%) compared to the upper layer of reduced tillage (19%) and both layers of conventional tillage (16% and 19% for higher and lower layer, respectively;  $p = .0004$ ; Figure S4). Its absolute abundance was 70% higher in reduced tillage compared to conventional tilled treatment ( $p = .007$ ), both in the 0–10 and 10–20 cm soil layer and regardless of organic matter. There was a 44% higher proportion of fungivorous nematodes in the upper layer of reduced tillage combined with low organic matter addition compared to the lower layer of the same treatment ( $p = .009$ ). No effect of soil management was found for relative abundances of omnivorous and predatory nematodes, but the relative abundance of omnivorous nematodes was 68% higher in the upper than in the lower layer across tillage and organic matter treatments.

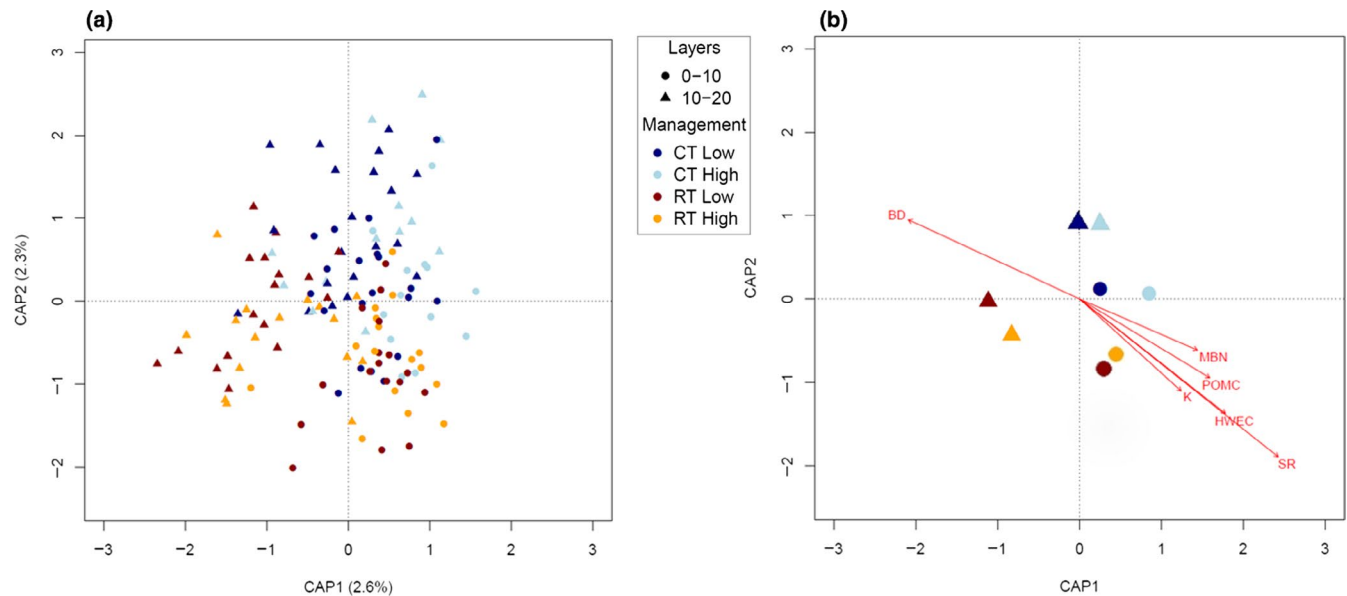
The food web indices, MI, SI and CI were significantly higher in plots where reduced tillage was applied (MI = 1.8, SI = 37.0, CI = 8.0) than in conventional tillage plots (MI = 1.6, SI = 29.8, CI = 5.0), while the EI was significantly higher under conventional tillage (EI = 81.1) than under reduced tillage (EI = 75.1; Table 3, Figure 4). We found significantly higher values of MI in the upper (MI = 1.7) than in the lower layer (MI = 1.6), and significantly higher values of EI in the lower (EI = 79.4) than in the upper layer (EI = 76.8; Table 3). Accordingly, we found a 13% higher proportion of c-p 1 (colonizers) and a 32% lower proportion of c-p 4 (persisters) in the lower layer than in the upper layer (Table S8), but in terms of absolute abundance the c-p 1 nematodes were 29% higher in the upper layer (2,286 nematodes 100 g field moist soil<sup>-1</sup>) compared to the lower one (1,812 nematodes 100 g field moist soil<sup>-1</sup>; Table S9).



**TABLE 1** Results of the mixed linear models testing the effect of soil management on total nematode qPCR counts, OTU richness, diversity, and evenness, and genus richness, diversity and evenness

Group A		qPCR counts	OTU richness (total OTU number)	OTU diversity (exp <sup>H</sup> )	OTU evenness (exp <sup>H</sup> / OTU number)	Genus richness (total genus number)	Genus diversity (exp <sup>H</sup> )	Genus evenness (exp <sup>H</sup> /genus number)
0–10 cm								
CT–LOW	CT–LOW	6,373 (4,428–8,671)	112 (101–122)	15.8 (12.6–19.4)	0.14 (0.11–0.17)	41 (38–45)	8.3 (6.9–9.6)	0.20 (0.17–0.24)
	RT–LOW	6,640 (4,596–9,059)	118 (107–130)	19.2 (15.6–23.3)	0.16 (0.13–0.20)	42 (38–45)	9.8 (8.4–11.2)	0.24 (0.20–0.28)
	CT–HIGH	6,725 (4,574–9,289)	117 (105–129)	13.7 (10.5–17.2)	0.11 (0.09–0.15)	42 (38–45)	6.3 (4.9–7.8)	0.15 (0.11–0.19)
	RT–HIGH	6,999 (4,870–9,512)	124 (112–136)	16.8 (13.4–20.6)	0.14 (0.11–0.17)	42 (38–46)	7.9 (6.5–9.3)	0.19 (0.15–0.23)
10–20 cm								
CT–LOW	CT–LOW	4,832 (3,162–6,856)	100 (90–110)	13.4 (10.5–16.7)	0.13 (0.11–0.16)	39 (36–43)	7.5 (6.2–8.8)	0.19 (0.16–0.23)
	RT–LOW	5,065 (3,304–7,201)	106 (96–118)	16.6 (13.2–20.3)	0.16 (0.12–0.19)	40 (36–43)	9.1 (7.7–10.5)	0.23 (0.19–0.27)
	CT–HIGH	5,139 (3,285–7,407)	105 (94–116)	11.4 (8.5–14.7)	0.11 (0.08–0.14)	40 (36–43)	5.6 (4.1–7.1)	0.15 (0.11–0.19)
	RT–HIGH	5,379 (3,537–7,606)	112 (101–123)	14.3 (11.2–17.9)	0.13 (0.10–0.16)	40 (36–44)	7.2 (5.8–8.6)	0.18 (0.14–0.22)
Tillage (T)								
F	F	0.22	6.56	10.26	6.45	0.39	7.31	5.89
	p	.64	.02	.004	.02	.54	.01	.02
OM								
F	F	0.18	2.70	3.78	8.69	0.21	12.49	11.05
	p	.67	.11	.05	.007	.65	.002	.003
Layer (L)								
F	F	8.40	38.73	7.75	0.75	10.60	2.47	0.53
	p	.005	<.0001	.007	.40	.002	.12	.47
Group B								
LOW–CT	LOW–CT	4,353 (617–11,473)	110 (83–140)	17.6 (10.7–26.3)	0.16 (0.10–0.25)	41 (36–46)	9.0 (5.0–14.0)	0.22 (0.12–0.35)
	HIGH–CT	5,898 (1,393–13,521)	117 (91–147)	16.6 (10.9–23.4)	0.14 (0.08–0.21)	43 (38–47)	8.9 (5.4–13.2)	0.21 (0.12–0.32)
OM								
F	F	3.65	2.29	0.22	1.29	2.47	0.01	0.17
	p	.08	.16	.65	.28	.14	.94	.69

Note: We tested: for group A (CH1, CH2, NL1, NL2, SL1, HU4 and ES4) the effect of tillage, organic matter addition and layer, and for group B (CH3, PT1 and CH3) only the effect of organic matter addition. For each group, in the upper part of the table the estimated means and 95% confidence intervals (in parentheses) are reported. In the lower part of the table, *F* statistics and *p*-values (values  $\leq .05$  in bold) for the main factors and their interactions are reported. The interactions are not reported because they were all not significant. Abbreviations: CT, conventional tillage; exp<sup>H</sup>, exponential of the Shannon diversity index; OM, organic matter; RT, reduced tillage.



**FIGURE 3** Constrained analysis of principal coordinates (CAP) showing in (a) the effect of management and layer on the nematode beta diversity in group A (CH1, CH2, NL1, NL2, SL1, ES4 and HU4). The CAP model explained in total 8% of the variation in beta diversity related to soil management (tillage, organic matter addition), and the first two axes explained 2.6% and 2.3% of the total variation, respectively. (b) Shows the relationship between the nematode communities (displayed as centroids) and the soil parameters. Only the significant variables at  $p < .01$  are shown. The long-term field experiment (LTE) was used as a random effect (conditioned), and the blocking structure plus tillage, organic matter addition and layer were used as fixed effects. The different colours show the soil management and the different shapes show the different layers [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

For the LTEs belonging to group B, the proportion of bacterivorous nematodes was significantly increased with high compared to low organic matter addition, while herbivorous nematodes showed the opposite pattern (Table 2). However, in absolute abundance the herbivorous nematodes did not differ between the two treatments (Table S7). We found no effect of organic matter addition on most food web indices. Only the CI was significantly higher in the low than in the high organic matter treatment (Table 3).

### 3.5 | Relationships between soil parameters and nematode communities

Partial correlations between total nematode qPCR counts and soil chemical, physical, and biological parameters are reported in Table 4. In group A, qPCR counts were positively correlated with many chemical (TN, TOC, available K, Mg), physical (WSA) and biological (SR, MBC, MBN, qMic, soil suppressiveness) parameters, and with four of the labile carbon fractions (Hy-DOC, POXC, HWEC, and POMC). Negative correlations were found with the soil C to N ratio, BD, tea bag decomposition, and Hy- and DOC SUVA (Table 4).

Correlations between OTU richness and soil parameters were similar to those of nematode qPCR counts and soil parameters, although the correlation coefficients were weaker for all the variables except K (Table 4). In contrast, correlations between OTU diversity or evenness and soil parameters were fewer, and, with the exception of CEC, explained less or the same amount of the variance (Table 4). For group

B we found very few and not very strong significant relationships between soil parameters and nematode communities (Table 4).

TOC, available K, BD, MBC, MBN, SR, HWEC, POXC, and POMC were significantly associated with nematode community composition (Table S10). Of these variables, only the ones with a significance level  $<0.01$  are reported in Figure 4b (BD, available K, MBN, POMC, HWEC, SR). With the exception of BD, these parameters, plus TN and Mg, were positively correlated with CAP1 and negatively correlated with CAP2 (Table S11), being higher in the upper compared to lower layers (Figure 3b). The contrary was true for the BD, which was higher in the lower layer, in particular under reduced tillage (Figure 3b, Table S11). In addition, qMic and DOC SUVA were positively and negatively related, respectively, only with CAP1, CEC and WSA were negatively correlated only with CAP2, and C to N ratio was positively correlated only with CAP2 (Table S11).

### 3.6 | Indicator OTUs for tillage and organic matter addition

Out of 349 OTUs finally used for analysis, 12 OTUs were significantly associated with specific management combinations in the upper layer, and 10 OTUs were significantly associated with the lower layer (group A only, as no differences in nematode communities were found in group B, Table 5). The indicator OTUs were herbivorous (OTUs assigned as *Pratylenchus*, *Neopsilenchus*, *Merlinidae*), fungivorous (OTUs assigned as *Aphelenchoides*, *Nothotylenchus*) and bacterivorous (OTUs assigned as *Acrobeloides*, *Panagrolaimus*,

**TABLE 2** Results of the mixed linear models testing the effect of soil management on the percentage of nematode trophic groups (bacterivores, fungivores, herbivores, omnivores and predators)

	Bacterivores	Fungivores	Herbivores	Omnivores	Predators
	Relative abundance (%)				
Group A					
0–10 cm					
CT–LOW	52 (35–68)	12 (6–22) <sup>b, c</sup>	17 (6–39)	1.3 (0.3–4.6)	0.6 (0.2–2.4)
RT–LOW	53 (35–70)	13 (6–25) <sup>c</sup>	18 (7–41)	2.2 (0.5–8.3)	0.9 (0.2–3.50)
CT–HIGH	65 (46–80)	9 (4–18) <sup>a,b,c</sup>	16 (5–38)	1.1 (0.2–4.8)	0.4 (0.1–1.5)
RT–HIGH	56 (38–73)	7 (3–15) <sup>a,b,c</sup>	20 (7–44)	1.4 (0.3–5.5)	0.8 (0.2–3.0)
10–20 cm					
CT–LOW	58 (40–73)	10 (5–19) <sup>a,b,c</sup>	21 (8–45)	0.7 (0.2–2.9)	0.9 (0.2–3.1)
RT–LOW	40 (25–58)	7 (3–14) <sup>a</sup>	45 (21–72)	0.5 (0.1–2.3)	0.8 (0.2–3.0)
CT–HIGH	67 (49–81)	6 (3–13) <sup>a,b</sup>	17 (6–40)	0.3 (0.1–1.5)	0.4 (0.1–1.6)
RT–HIGH	43 (26–61)	8 (4–17) <sup>a,b,c</sup>	43 (19–70)	0.4 (0.1–1.6)	0.6 (0.1–2.1)
Tillage					
<i>F</i>	12.2	0.97	20.15	0.09	1.52
<i>p</i>	.002	.33	.0001	.76	.23
OM					
<i>F</i>	3.7	5.98	0.20	1.27	3.45
<i>p</i>	.067	.02	.65	.27	.07
Layer					
<i>F</i>	3.64	10.27	27.43	25.35	0.02
<i>p</i>	.06	.002	<.0001	<.0001	.88
T × OM					
<i>F</i>	2.14	0.83	0.52	0.01	1.01
<i>p</i>	.15	.37	.47	.92	.32
T × L					
<i>F</i>	13.55	0.17	14.49	1.82	1.60
<i>p</i>	.0005	.68	.0004	.18	.21
OM × L					
<i>F</i>	0.13	3.92	0.39	0.43	0.25
<i>p</i>	.71	.05	.53	.51	.62
T × OM × L					
<i>F</i>	0.06	7.22	0.006	0.90	0.005
<i>p</i>	.79	.009	.94	.35	.94
Group B					
LOW–CT	47 (11–86)	9 (4–21)	29 (6–72)	0.7 (0.01–33)	1.7 (0.06–32)
HIGH–CT	62 (20–92)	11 (4–26)	18 (6–72)	0.9 (0.01–0.36)	1.8 (0.07–32)
OM					
<i>F</i>	9.82	1.55	6.65	0.33	0.05
<i>p</i>	.009	.24	.02	.58	.82

Note: We assessed for group A (CH1, CH2, NL1, NL2, SL1, HU4 and ES4) the effect of tillage, organic matter addition and layer, and for group B (CH3, PT1 and CH3) the effect of organic matter addition. For each group, in the upper part of the table the estimated means and 95% confidence intervals (in parentheses) are reported. In the lower part of the table, *F* statistics and *p*-values (values ≤ .05 in bold) for the main factors and their interactions are reported. Different superscript letters (a, b, c) following means (to be read per column) show treatments which are significantly different (*p* ≤ .05) according to Tukey post-hoc tests for the three way interactions.

Abbreviations: CT, conventional tillage; L, layer; OM, organic matter; RT, reduced tillage; T, tillage.

**TABLE 3** Results of the mixed linear model testing the effect of soil management on the maturity index, enrichment index, structure index and channel index

	Maturity index	Enrichment index	Structure index	Channel index
Group A				
0–10 cm				
CT–LOW	1.64 (1.44–1.85)	79.4 (65.9–92.9)	32.9 (15.6–50.4)	6.5 (2.0–21.3)
RT–LOW	1.84 (1.63–2.04)	73.4 (59.9–86.9)	40.3 (22.8–57.8)	10.3 (3.1–33.8)
CT–HIGH	1.56 (1.35–1.77)	80.1 (66.4–93.7)	29.2 (11.5–47.0)	4.9 (1.5–16.4)
RT–HIGH	1.75 (1.54–1.96)	74.1 (60.5–87.6)	36.5 (18.9–54.1)	7.8 (2.4–25.7)
10–20 cm				
CT–LOW	1.56 (1.36–1.76)	82.1 (68.6–95.5)	30.2 (12.8–47.6)	5.2 (1.6–16.9)
RT–LOW	1.75 (1.54–1.96)	76.1 (62.5–89.6)	37.5 (19.9–55.0)	8.1 (2.5–26.7)
CT–HIGH	1.48 (1.26–1.69)	82.7 (69.1–96.3)	26.4 (8.7–44.2)	3.9 (1.2–12.9)
RT–HIGH	1.67 (1.46–1.88)	76.7 (63.1–90.2)	33.7 (16.1–51.3)	6.1 (1.9–20.3)
Tillage				
<i>F</i>	13.13	12.56	8.16	8.28
<i>p</i>	<b>.001</b>	<b>.001</b>	<b>.008</b>	<b>.008</b>
OM				
<i>F</i>	2.40	0.12	1.64	2.65
<i>p</i>	0.13	0.72	0.21	0.11
Layer				
<i>F</i>	4.92	4.45	1.56	3.58
<i>p</i>	<b>.03</b>	<b>.04</b>	.22	.06
Group B				
LOW–CT	2.1 (1.1–3.1)	67.2 (42.9–91.5)	49.0 (–0.24.8–122.9)	20.8 (–1.2–42.9)
HIGH–CT	1.9 (1.0–2.9)	74.4 (51.6–97.2)	47.5 (–25.9–121.0)	11.8 (–9.3–33.0)
OM				
<i>F</i>	1.85	3.10	0.17	8.8
<i>p</i>	.20	.10	.69	<b>.01</b>

Note: We assessed for group A (CH1, CH2, NL1, NL2, SL1, HU4 and ES4) the effect of tillage, organic matter addition and layer, and for group B (CH3, PT1 and CH3) the effect of organic matter addition. In the table *F* statistics and *p*-values (significance at  $p \leq .05$  in bold) for the main factors are reported. The interactions are not reported because they were all not significant.

Abbreviations: CT, conventional tillage; OM, organic matter; RT, reduced tillage.

*Rhabditis*). Indicator OTUs belonged mainly to c-p groups 1 and 2 and were all present in relative abundance <0.1%, apart from OTU\_2 (OTU assigned as *Rhabditis*) which was an indicator OTU for conventional tillage in the lower layer. This OTU comprised more than 20% of the relative abundance of all nematode reads.

## 4 | DISCUSSION

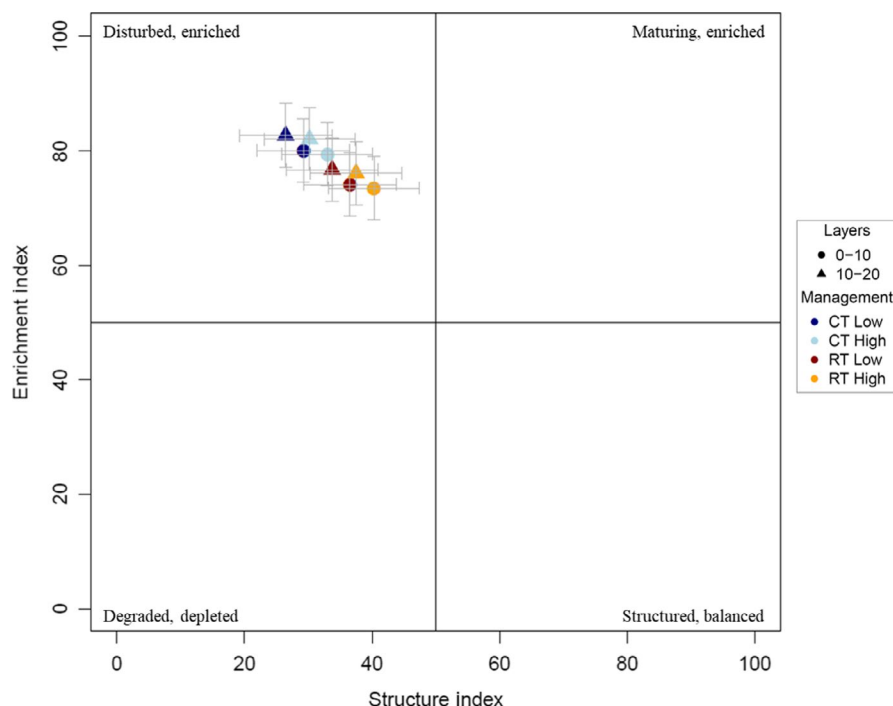
### 4.1 | Largest proportion of variation in nematode communities is explained by site

Measured abiotic and biotic (MBC) differences between the LTEs explained most of the variation in nematode communities, in line with results from Neher, Peck, Rawlings, and Campbell (1995) and

Thomson et al. (2015). This result is plausible, since the LTEs were selected to maximize intersite variation and to test if, in spite of large differences in sites across pedoclimatic conditions, effects of agricultural management were yet significant. Indeed, nematode communities were significantly related to all other measured soil parameters when LTE was not used as a random factor.

### 4.2 | Reduced tillage increases nematode alpha diversity and alters beta diversity compared to conventional tillage

In accordance with our first hypothesis, nematode OTU richness and, to a larger extent, OTU (and genus) diversity and evenness were increased in reduced compared to conventional tillage across



**FIGURE 4** Enrichment (y axis)—structure (x axis) diagram for the long-term field experiments (LTEs) of group A (CH1, CH2, NL1, NL2, SL1, ES4, HU4). The points and the triangles represent the estimated means from the linear effect mixed models for the respective combination of factors (tillage, organic matter addition) for the first layer and the second layer, respectively. The bars represent the estimated standard errors for the group averages. In the corner of each of the four quadrants we report information relative to structure of the food web and nutrient enrichment, respectively, according to Ferris et al. (2001) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

the LTEs of group A, i.e., in LTEs where the 0–10 and 10–20 cm layers were sampled (LTEs: CH1, CH2, SL1, NL1, NL2, ES4, HU4). Previous studies reported positive effects of reduced tillage on nematode abundance, richness, and diversity (Fu, Coleman, Hendrix, & Crossley, 2000; Okada & Harada, 2007; Zhang et al., 2015). Reduced soil disturbance (here very shallow or noninversion cultivation in the 0–10 cm layer) can exert a positive effect on nematodes through the increase of total organic carbon, soil aggregation and microbial biomass, and a lower physical pressure (Kladivko, 2001). The lower nematode qPCR counts, richness and diversity in the lower soil layer under reduced tillage, where disturbance is lower, could be due to decreased resources present in this layer. Under reduced tillage, soil parameters related to soil organic matter and nutrients have lower values below the plough layer (Franzluebbers, 2002), which can be explained by the retention of crop residues on the soil surface, and the lack of mechanical mixing of soil layers.

In group A, reduced tillage led to a shift in nematode community structures, in agreement with previous studies (Brmež, Ivezić, & Raspudić, 2006; Griffiths, Daniell, Donn, & Neilson, 2012; Okada & Harada, 2007). In this group of LTEs, nematode beta diversity was affected by the organic matter additions, and OTU diversity was lower in the plots with high organic matter additions, which might suggest positive effects of the organic matter added on a few opportunistic nematodes. However, in disagreement with our second hypothesis, we did not find an effect of organic matter additions on nematodes qPCR counts, and alpha and beta diversity in group B, i.e., in LTEs where the 0–20 cm layer was sampled as a whole (LTEs: CH3, PT1, HU1). Also in the literature contradictory results were found, reporting negative (Wang, McSorley, & Gallaher, 2004), neutral (Ito et al., 2015; Li et al., 2018; Quist et al., 2016) and positive effects of organic matter on nematode

numbers (Nahar et al., 2006; Sánchez-Moreno et al., 2009; Ugarte, Zaborski, & Wander, 2013), richness (Sánchez-Moreno et al., 2009) and alpha diversity (van Diepeningen, de Vos, Korthals, & van Bruggen, 2006; Okada & Harada, 2007) in systems where organic matter was added.

Organic matter is a food source for microorganisms which in turn are a food source for bacterivorous, fungivorous and omnivorous nematodes; therefore, organic matter, similarly to reduced tillage, can change soil properties favourable to nematodes (food availability, but also water retention and soil aggregation; Bongers & Ferris, 1999). In the LTEs of group B, we found higher concentrations of total (TOC) and labile (POXC) organic matter ( $p = .03$  and  $p < .0001$ , respectively) in the high compared to the low organic matter input treatments, but we did not find differences in microbial biomass, cation exchange capacity and water stable aggregates ( $p = .06$ ,  $p = .12$  and  $p = .51$ , respectively). Our contradicting results on the effect of organic matter additions on nematodes could be related to the different types of organic matter used in our LTEs (e.g., compost, biochar, farmyard manure, etc). The composition and the amount of organic matter applied to the soil is an important factor for its effect on nematodes (Ito et al., 2015; Li et al., 2018; Liu et al., 2016). Also, it is possible that the conventional tillage applied to the LTEs of group B neutralized the effect of organic matter additions (Briar, Grewal, Somasekhar, Stinner, & Miller, 2007). This weak effect of organic matter addition supports previous studies that suggested that tillage has a stronger effect on nematode communities than organic matter addition or other agricultural practices such as organic versus conventional management, irrigation, and cover crops (Ito et al., 2015; Neher, 1999; du Preez, Daneel, Wepener, & Fourie, 2018; Zhong, Zeng, & Jin, 2017).

**TABLE 4** Partial correlation coefficients between total nematode qPCR counts, OU richness, diversity, and evenness and chemical, physical and biological indicators for the samples belonging to group A ( $n = 132$ ) and group B ( $n = 35$ )

	Group A				Group B			
	qPCR counts	OTU richness (total OTUs number)	OTU diversity ( $\exp^H$ )	OTU evenness ( $\exp^H$ /OTU number)	qPCR counts	OTU richness (total OTUs number)	OTU diversity ( $\exp^H$ )	OTU evenness ( $\exp^H$ /OTU number)
Chemical parameters								
TOC	0.31**	0.36***	0.14	-0.0002	0.12	0.003	0.15	0.14
pH	-0.02	0.06	-0.02	-0.04	0.37	0.06	-0.05	-0.07
TN	0.34***	0.34***	0.18*	0.04	0.02	-0.22	0.05	0.15
C/N	-0.35**	-0.28*	-0.25*	-0.15	0.12	0.29	0.17	0.06
CEC	0.10	0.14	0.33***	0.30**	-0.14	-0.39*	0.19	0.37
Ca	-0.02	0.04	-0.09	-0.10	0.03	0.07	0.29	0.28
Mg	0.13	0.18*	0.24*	0.18*	-0.15	-0.27	0.04	0.15
K	0.21*	0.39***	0.25*	0.14	0.25	0.17	0.11	0.03
Na	-0.19*	-0.20*	-0.10	-0.04	-0.05	0.10	0.11	0.06
P	0.14	0.25*	0.08	-0.01	-0.10	0.21	0.15	0.07
Physical parameters								
WSA	0.24*	0.30***	0.17*	0.06	0.10	-0.14	-0.24	-0.17
WHC	0.06	0.04	0.03	0.01	0.007	-0.03	0.10	0.11
BD	-0.38***	-0.38***	-0.17	-0.03	-0.20	-0.06	0.15	0.17
Sand	0.04	-0.009	-0.08	-0.08	-0.11	0.48*	0.28	0.07
Silt	0.07	0.10	-0.06	-0.11	0.27	0.23	0.02	-0.08
Clay	-0.05	-0.20*	-0.04	0.03	-0.51*	-0.37	0.19	0.36
Biological parameters								
MBC	0.43***	0.41***	0.16	0.0007	-0.08	-0.23	-0.06	0.04
MBN	0.44***	0.21*	0.05	-0.04	-0.24	0.13	0.19	0.14
SR	0.45***	0.33***	0.24*	0.09	0.10	-0.05	-0.15	-0.12
qMic	0.22*	0.22*	0.09	0.009	0.009	-0.22	-0.15	-0.05
qCO <sub>2</sub>	-0.02	-0.02	0.13	0.19*	0.20*	0.21	-0.02	-0.11
Earthworm number	-0.10	-0.09	-0.17	-0.02	0.08	-0.16	-0.10	-0.03
Earthworm biomass	0.05	-0.04	-0.12	-0.05	0.09	-0.24	-0.16	-0.06
Tea bag decomposition	-0.49*	-0.31*	-0.35*	-0.27*	0.002	0.22	-0.12	-0.20
Soil suppressiveness	0.37*	0.20	0.13	0.07	-0.16	0.09	0.0008	-0.04
Labile carbon fractions								
Hy SUVA	-0.20*	0.06	0.07	0.05	-0.10	0.17	-0.11	-0.19
DOC SUVA	-0.26*	-0.06	0.07	0.10	0.009	-0.02	0.03	0.04
Hy-DOC	0.27*	0.14	-0.06	-0.13	0.08	-0.02	0.05	0.06
DOC	0.06	0.09	0.08	0.03	0.10	-0.13	-0.09	-0.03
HWEC	0.48***	0.35***	0.19*	0.05	0.06	-0.16	0.08	0.14
POXC	0.46***	0.36***	0.18*	0.04	0.17	0.003	0.10	0.09
POMC	0.49***	0.46***	0.12	-0.07	0.24	0.16	0.08	0.01

Abbreviations: BD, bulk density; C/N, carbon to nitrogen ratio; CEC, cation exchange capacity; DOC SUVA, specific ultraviolet absorbance of dissolved organic carbon; DOC, dissolved organic carbon; HWEC, hot water extractable carbon; Hy SUVA, specific ultraviolet absorbance of hydrophilic carbon; Hy, hydrophilic carbon; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; POMC, particulate organic matter carbon; POXC, permanganate oxidizable carbon; qCO<sub>2</sub>, metabolic quotient; qMic, microbial quotient; TOC, total organic carbon; TON, total nitrogen; WHC, water holding capacity; WSA, water stable aggregates.

\* $p \leq .05$ .

\*\* $p \leq .001$ .

\*\*\* $p \leq .0001$ .



**TABLE 5** Indicator species for the combination of tillage, organic matter addition and layer for group A (CH1, CH2, NL1, NL2, SL1, ES4 and HU4)

	OTU	Genus	Family	Feeding group	c-p	Correlation	p-Value	Relative abundance (%)							
								CT-Low		CT-High		RT-Low		RT-High	
								N = 20	N = 13	N = 17	N = 16				
Layer 0–10 cm															
CT–LOW	OTU_329	Aphelenchoidea	Aphelenchoididae	F	2	0.34	0.007	0.68 (12)	0.06 (6)	0.01 (6)	0.142 (9)				
	OTU_436	NA	Tylenchidae	H (1e)	NA	0.38	0.005	0.0001 (2)	0.02 (4)	0.0003 (1)	0 (0)				
CT–HIGH	OTU_75	Aphelenchoidea	Aphelenchoididae	F	2	0.26	0.007	0.35 (15)	0.68 (10)	0.15 (15)	0.035 (9)				
	OTU_310	Dicelis	Drilonematidae	B	2	0.39	0.006	0.02 (8)	0.004 (9)	0.02 (14)	0.01 (9)				
	OTU_44	Oscheius	Rhabditidae	B	1	0.39	0.0001	0.07 (14)	0.21 (13)	0.71 (15)	0.25 (14)				
	OTU_84	Aphelenchoidea	Aphelenchoididae	F	2	0.37	0.004	0.07 (9)	0.04 (10)	0.28 (11)	0.05 (6)				
RT–HIGH	OTU_120	Pratylenchus	Pratylenchidae	H (1b)	3	0.39	0.003	0.002 (2)	0.0001 (1)	0.0001 (1)	0.20 (6)				
	OTU_48	NA	NA	NA	NA	0.33	0.0007	0.25 (11)	0.0005 (3)	0.003 (7)	1.41 (12)				
	OTU_79	Acroboloides	Cephalobidae	B	2	0.35	0.005	0.113 (9)	0.047 (3)	0.01 (6)	1.29 (7)				
CT–HIGH, RT–LOW, RT–HIGH	OTU_256	NA	NA	NA	NA	0.32	0.004	0.002 (2)	0.01 (5)	0.02 (10)	0.03 (10)				
	OTU_82	Aphelenchoidea	Aphelenchoididae	F	2	0.43	0.0008	0.06 (16)	0.16 (12)	0.25 (17)	0.23 (16)				
	OTU_728	Oscheius	Rhabditidae	B	1	0.33	0.002	0.02 (11)	0.12 (9)	0.21 (14)	0.15 (9)				
Layer 10–20 cm															
CT–LOW	OTU_329	Aphelenchoidea	Aphelenchoididae	F	2	0.31	0.005	0.71 (11)	0.02 (7)	0.03 (8)	0.06 (7)				
CT–HIGH	OTU_75	Aphelenchoidea	Aphelenchoididae	F	2	0.37	0.0007	0.38 (12)	1.06 (10)	0.04 (8)	0.02 (12)				
RT–HIGH	OTU_218	Nothotylenchus	Anguinidae	F	2	0.24	0.006	0.01 (5)	0.004 (4)	0.01 (3)	0.08 (6)				
	OTU_257	Panagrolaimus	Panagrolaimidae	B	1	0.37	0.009	0.00 (2)	0.0002 (1)	0.0003 (2)	0.01 (6)				
	OTU_30	Pratylenchus	Pratylenchidae	H (1b)	3	0.38	0.003	0.04 (12)	0.003 (9)	0.007 (11)	4.26 (15)				
High	OTU_712	Neopsilenchus	Tylenchidae	H (1e)	2	0.51	0.005	0.06 (13)	0.43 (12)	0.07 (12)	0.82 (13)				
RT	OTU_15	NA	Merlinidae	H (1d)	NA	0.25	0.008	0.70 (20)	0.07 (13)	2.91 (17)	1.04 (16)				
	OTU_70	NA	Tylenchidae	H (1e)	2	0.28	0.001	0.14 (11)	0.04 (7)	0.47 (14)	0.48 (10)				
CT	OTU_2	Rhabditis	Rhabditidae	B	1	0.40	0.002	24.82 (20)	30.31 (13)	14.14 (17)	7.85 (16)				
	OTU_39	NA	NA	NA	NA	0.32	0.008	0.88 (17)	2.73 (10)	0.02 (13)	0.11 (11)				

*Note:* In the table we report the OTU number, taxonomic information at the level of nematode family and genus, feeding group, c-p colonizer-persister class, the correlation coefficient and the *p*-value from the analysis, and the relative abundances of the taxa for the combinations of soil management (tillage and organic matter addition). Above the columns with the OTU relative abundance the number of samples belonging to each group is indicated, and in parentheses the number of samples in which that specific OTU was found is given. The analysis has been done for the two layers separately, and only OTUs that had a level of significance  $p \leq .01$  are reported.

Feeding group: B, bacterivorous nematode; F, fungivorous nematode; H, herbivorous nematode; 1b, herbivorous migratory endoparasitic nematodes; 1d, herbivorous ectoparasitic nematodes; 1e, herbivorous epidermal and root hair feeders; NA, not assigned to taxon.

Abbreviations: CT, conventional tillage; RT, reduced tillage.

### 4.3 | Reduced tillage increases stability and structure of the nematode community compared to conventional tillage

Agricultural management did not have strong effects on the relative abundance of the trophic groups, but it affected the food web indices, indicating effects on rates rather than on structural changes in the food web. This observation supports the suggestion by Neher (1999) that food web indices are less variable and more likely to detect effects of management practices on soil processes than measures based on individual trophic groups.

In accordance with our first hypothesis and in line with previous reports (Habig & Swanepoel, 2015; Zhang et al., 2015; Zhong et al., 2017), reduced tillage resulted in a less disturbed environment than conventional tillage, increasing the stability and the number of food web interactions of the nematode communities (higher MI and SI) in the LTEs of group A. Despite the decreasing level of disturbance in the lower soil layer of reduced tillage, a lower MI and reduced proportions of omnivorous and stress-tolerant c-p 4 nematodes compared to the upper layer seems to indicate a more stressed environment where opportunistic nematodes can prevail. In our study, reduced tillage increased the channel index (CI), i.e., among the opportunistic microbivorous nematodes there was an increase in the proportion of fungal feeders, confirming previous findings (Minoshima et al., 2007; Okada & Harada, 2007; Sánchez-Moreno, Minoshima, Ferris, & Jackson, 2006). Reduced tillage is known to favour the fungal decomposition pathway (Six, Frey, Thiet, & Batten, 2006), due to less or no disruption of the hyphal network (Minoshima et al., 2007). Since lower values of CI are associated with faster rates of decomposition and nutrient turnover, our results suggest that changes in nematode communities under reduced tillage may contribute to the increased capability of the system to retain nutrients and store carbon (Griffiths et al., 2012). The higher relative and absolute abundance of herbivorous nematodes in reduced tillage compared to conventional tillage is in line with previous studies (Brmež et al., 2006; Freckman & Ettema, 1993; Fu et al., 2000; Treonis et al., 2010, 2018), and can be explained by a higher incidence of roots in the field, stimulating this nematode group (Minton, 1986; You et al., 2017). Our results indicate a possible trade-off in reduced tillage systems in terms of soil processes, and that in these types of systems care must be taken regarding the assessment and control of herbivorous nematodes. However, the higher alpha diversity, MI and SI found in reduced tillage could indicate that the activity of herbivorous populations might be controlled by a more stable and structured food web.

In agreement with our second hypothesis, high organic matter addition plots resulted in higher percentages of bacterivorous nematodes than low organic matter addition plots, and they showed a statistically lower CI and a tendency towards lower SI, MI, and higher EI. High EI (Berkelmans, Ferris, Tenuta, & van Bruggen, 2003; Forge et al., 2005; Sánchez-Moreno et al., 2009), low MI (Forge et al., 2005; Neher & Olson, 1999; Wang, McSorley, Marshall, & Gallaher, 2006) and low SI (Pan et al., 2015; Villenave

et al., 2010) have been previously reported in systems with organic matter addition. Such changes in MI and CI can be explained by an increase in opportunistic bacterivores (Ferris & Bongers, 2006), and a stimulation of the bacterivore decomposition channel (Pan et al., 2010; Wang et al., 2004). Altogether, these results indicate higher nutrient cycling, N mineralization and fertility in soils with high organic matter additions (Ferris & Matute, 2003). By contrast, the addition of organic matter decreased the proportion of herbivorous nematodes, but this did not coincide with an absolute decrease as this relative decrease resulted from the absolute increase of bacterivorous nematodes.

### 4.4 | Nematode communities are mainly related to soil organic carbon and biological parameters

Total and labile organic carbon and microbial parameters were most strongly and positively related to nematode qPCR counts and richness, partly confirming our third hypothesis. Abundance (Sánchez-Moreno et al., 2006), richness (van Diepeningen et al., 2006), but also diversity (Zhong et al., 2017) of soil nematodes have previously been positively linked with the levels of total and labile organic carbon fractions. Higher total and labile carbon are linked to higher microbial biomass, soil respiration, water retention, soil structure and lower bulk density (Bongiorno, Bünemann, et al., 2019). Increased levels in these soil parameters can optimize the habitat conditions for nematodes, and facilitate their movement through the soil pore water (Nielsen et al., 2014).

Some of the properties that correlated most with nematode qPCR counts and richness (total organic and labile carbon, available K, bulk density, microbial biomass and activity) proved important in explaining differences between nematode communities caused by reduced versus conventional tillage. This suggests that reduced tillage affects nematode communities through its positive effects on these soil properties, either directly through absence of soil inversion, i.e. lower soil disturbance, or indirectly through retention of crop residues at the soil surface, which can increase water retention and infiltration, soil organic carbon, and organism biomass and activity (Mloza-Banda, Makwiza, & Mloza-Banda, 2016; Ranaivoson et al., 2017).

### 4.5 | Only *r* selected taxa were found to be indicator OTUs for tillage and organic matter addition

Indicator OTU analysis based on group A revealed OTUs that were significantly associated with tillage and organic matter management. Most of the indicator OTUs had a very low relative abundance. These taxa belonged mainly to the c-p 2 group, and to bacterivorous, fungivorous and herbivorous nematode trophic groups. Therefore, contrary to our fourth hypothesis none of the predatory and omnivorous nematodes, or nematodes belonging to c-p groups 4 and 5 were detected as indicator taxa. This can be due to the fact that in these intensively managed European arable systems, relative and absolute abundances of highly sensitive nematode taxa were underrepresented and too variable (i.e., not present in all samples).

#### 4.6 | Advantages and limitations of studying nematode communities with amplicon sequencing

Our molecular analyses revealed that, despite the big influence of the pedoclimatic characteristics, agricultural soil management resulted in changes in nematode communities and nematode food web structure in line with previous findings from microscopic analysis and general knowledge of agricultural systems. In addition, nematode molecular analyses provided advantages in terms of costs and number of samples analyzed at the same time, and did not require expert skills for morphological characterisation.

A limitation of current amplicon sequences approaches is that previous studies found that the relative read abundance obtained do not perfectly match absolute abundance data determined microscopically. Possibly, the number of ribosomal DNA copies differ depending on the taxon, the organism's body size, the developmental stage, and PCR primer bias (Darby et al., 2013; Geisen et al., 2018). This has to be considered and standardized in future efforts to allow direct comparisons between morphological and molecular approaches in determining nematode communities.

In our study, a relatively large group of OTUs could not be classified at all. This underlines the problems in reliably assigning OTUs to their correct taxonomic group. Such taxa could belong to not yet studied nematode species, but most likely could indicate lack of information in the data bases. In addition, our methodology used to assign taxonomy, using only forward reads, could have had negative consequences for annotation (resolution power) and error correction which can be applied during read merging.

All in all, future studies should work towards an optimization of molecular methods for assessing relative and total nematode abundance, nematode taxonomy and the definition of standardized protocols and the amelioration of data bases in order to guarantee a more confident application of nematode communities studied with molecular methods in soil quality assessments.

In conclusion, molecular nematode community analyses effectively differentiate soil management across 10 different European long-term field experiments. In particular, reduced tillage had a stronger effect on nematode communities than organic matter addition, increasing nematode taxon richness, diversity and evenness. Reduced tillage also affected the nematode food web indices, stimulating more mature and fungal-based nematode communities, indicating a more stable food web with higher nutrient retention capability, but also increasing the number of herbivorous nematodes. These results are in line with previous findings based on microscopic analysis and general knowledge on nematode community dynamics in agricultural systems.

The relationships found between soil nematode communities and total and labile organic carbon, total nitrogen, available K, and microbial biomass and activity, underline the relationship between nematode communities and biological soil quality achieved by reduced tillage, and indicate that nematode communities are equally sensitive indicators of soil quality as these parameters.

Our findings indicate that molecular methods are promising in the assessment of biological soil quality based on nematode community

structure and indices, especially if future research will work toward an optimization and standardization of the methods.

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#### AUTHOR CONTRIBUTIONS

G.B., R.G.M.G., E.K.B., L.B., and P.M. agreed on the study design with options given by the iSQAPER consortium. C.W.Q., and S.G. contributed analytical tools and primers. G.B., and C.W.Q. collected the data. J.-C.W. performed the bioinformatic analysis. G.B., N.B., and R.G.M.G. analyzed and interpreted the data. G.B. wrote the manuscript and N.B., C.W.Q., S.G., J.-C.W., R.G.M.G., E.K.B., L.B., and P.M. reviewed it.

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#### DATA AVAILABILITY STATEMENT

Sequences reads have been submitted to the European Nucleotide Archive (ENA) and are available under accession number ERP114920. The script used for data analyses is provided as part of the Appendix S2. The data on soil chemical, physical and biological indicators others than nematodes that support part of the findings of this study are available from iSQAPER work package 3.3. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the authors with the permission of iSQAPER work package 3.3 consortium, and they will be submitted in a public database after their publication in a separate paper. All the other data have been published in the DRYAD database (<https://doi.org/10.5061/dryad.q21d0b9>; Bongiorno et al., 2016a, 2016b).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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