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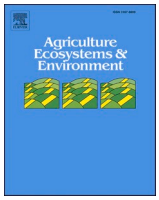

Nematode and protist community responses in soil to land use conversion from conventional to organic farming

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ABSTRACT

Soil organisms at higher trophic positions in the food web, such as nematodes or protists, play a key role in shaping the composition and functioning of soil communities. However, intensive agriculture can put these organisms under pressure and restoring communities of soil organisms will be vital for a more sustainable functioning of agricultural soils. Here, we examine how changing land use from conventional to organic farming influences the composition and diversity of belowground nematodes and protists. We collected soil samples from 68 organic and conventional farmers' fields on clay and sandy soils and used 18S sequencing to determine soil community composition and diversity. In order to test effects of time since conversion from conventional to organic management, we used organically farmed soils that had been converted at time periods varying from 1 to 25 years ago, each being paired with a nearby conventional field in order to account for local environmental differences in climate and soil conditions. The ASV richness and Shannon diversity of protists was lowest in organic fields and while protist community composition differed between organic and conventional fields in clay soils, effects were relatively minor. Similarly, Shannon diversity of nematodes was lower in organic fields at clay soil, but there was no difference in nematode community composition and species richness between conventional and organic fields. Progressing time since conversion to organic management impacted community composition for both nematodes and protists in clay soils and led to an increased protist richness and diversity in both clay and sandy soils. However, we found a similar trend for the conventional fields if we assigned them the age of the paired organic field, suggesting that the observed shifts may not have been driven by time since conversion alone, but also by other hitherto unidentified factors. Although pH, soil organic matter content, and microbial biomass were not related to time since conversion, they could explain functional and taxonomic community composition of both nematodes and protists. Shifts in the relative abundance of feeding/functional groups of protists and nematodes were variable and depended on soil and management type. We conclude that conversion from conventional to organic management influenced protist and nematode diversity and community composition, but effects were relatively minor and dependent on sand versus clay soil. Further studies are needed to determine functional implications of the shifts in protist and nematode community composition for functioning of the organic versus conventionally farmed soils.

1. Introduction

Conventional intensive agriculture makes use of synthetic fertilizers and pesticides to maximize yield, which often lead to the leaching of nutrients and other chemicals to ground- and surface waters, and emissions of greenhouse gasses (Flessa et al., 2002; Pandey et al., 2018;

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Petersen et al., 2006). These losses contribute to a decline of above-ground and below-ground biodiversity in both agricultural and the surrounding natural ecosystems (Hallmann et al., 2017; Harvey et al., 2020; Tsiafouli et al., 2015). Organic farming has been proposed as a more sustainable alternative to conventional farming, because it does not depend on inputs of synthetic fertilizers and pesticides (Maeder et al., 2002). Instead, in organic farming nutrient provisioning to crops and disease suppression are more dependent on the activity of soil organisms that make nutrients available from organic fertilizers, and on natural suppressiveness of soil-borne crop diseases (Arden-Clarke and Hodges, 1988; Pandey et al., 2018; Ren et al., 2023). Therefore, it is important to understand how the soil community responds to the conversion from conventional to organic farming. Several studies have examined responses of soil microbes and nematodes to long-term organic management, but relatively few studies have investigated how soil communities change with the duration of organic management in fields that are used by farmers (Briar et al., 2007; Tsiafouli et al., 2007; van Rijssel et al., 2022).

Nematodes and protists are key drivers of soil community composition and functioning (Biswal, 2022; Chandarana and Amaresan, 2022). For example, many nematode and protist taxa consume bacteria and fungi, thereby having a major impact on the abundance and activity of microorganisms and on availability of nutrients in soils (de Ruiter et al., 1994; Oliverio et al., 2020). It is known that the feeding of higher trophic levels on microorganisms can enhance carbon and nutrient mineralization and suppress the abundance of plant-pathogens (Burki et al., 2021; Geisen et al., 2020; Kane et al., 2023; van den Hoogen et al., 2019). In addition, a number of nematode and protist taxa feed on plants, thereby having negative consequences for crop growth and yield (Ferris, 2010; Griffiths, 1994; Neher, 2001). Several species of nematodes and protists, such as the oomycetes *Pythium spp.* and *Phytophthora spp.*, and species from the plant-parasitic nematode genera *Pratylenchus*, *Meloidogyne*, and *Heterodera/Globodera* are known as parasites of major agricultural crop species (Fry, 2008; Jones et al., 2013). Taken together, understanding community responses of nematodes and protists to agricultural management may provide valuable insights into how soil food webs develop during conversion of land management, and how they potentially impact on soil functioning.

Earlier work is showing that total abundance and alpha diversity of nematodes and protists can be higher in organic than conventional agricultural fields (Lentendu et al., 2014; Quist et al., 2016; van Diepeningen et al., 2006; Yang et al., 2021). However, there are also examples where there are no differences between organic and conventional farming (Guo et al., 2018; Lupatini et al., 2018; Neher, 1999; Zhao et al., 2020). Agricultural land use may also influence community composition of nematodes and protists (Harkes et al., 2019; Lupatini et al., 2018). Nematode feeding types are likely to respond differently to organic management. For example, increased microbial biomass in organic agriculture (Briar et al., 2007; Ferris et al., 1996), would provide more resources for microbial feeders (Biswal, 2022; Liu et al., 2024). Therefore, abundance of bacterivorous and fungivorous nematodes and phagotrophic protists might be higher in organic than in conventional agriculture (Ren et al., 2023; Ugarte et al., 2013; van Diepeningen et al., 2006). At the same time, organic amendments that are more frequently used in organic agriculture generally suppress plant-parasitic nematodes (Akhtar and Malik, 2000; Liu et al., 2016; Rodríguez-Kábana, 1986; Silva et al., 2022; Widmer et al., 2002). In spite of these theoretical considerations on responses of abundance of nematode feeding types to organic farming, various studies report contrasting findings (Briar et al., 2007; Ferris et al., 1996; Ilieva-Makulec et al., 2016; Lupatini et al., 2018; Silva et al., 2022). Diversity of nematode feeding types as response to organic farming is rarely researched. At the same time, while protists represent a large component of the biodiversity in soils, few studies are known analyzing the responses of protist functional group diversity to organic farming (Chandarana and Amaresan, 2022; Geisen et al., 2018a; Oliverio et al., 2020).

Responses of nematode and protist communities to a conversion in agricultural management could increase with time, because the legacies of conventional farming may disappear gradually (Bürgi et al., 2017; Foster et al., 2003). Based on studies of land use conversion towards more natural ecosystems, changes in the soil community composition may easily take ten or more years (Mawarda et al., 2020; Morriën et al., 2017; Wubs et al., 2019). However, other work showed that changes in nematode composition after conversion from conventional to organic management already occurred within four years, but that the direction and magnitude can fluctuate (Briar et al., 2007). Therefore, differences in nematode and protists communities between organic and conventional management will likely become more clear when time since conversion from conventional to organic farming proceeds (Lentendu et al., 2014; Quist et al., 2016). However, we still have a limited understanding of the duration of responses of community composition, diversity and relative abundance of nematodes and protists to changes from conventional to organic farming.

The aim of the present study was to test how diversity, community composition and relative abundance of soil nematodes and protists change during conversion from conventional to organic agriculture. We tested the hypotheses that (1) community composition differs and richness and diversity of nematodes and protists are higher in soils from organic than from conventional fields, (2) differences between organic and conventional fields increase with the duration of organic management, and (3) richness and diversity within nematode feeding types and protist functional groups are higher in organic than in conventional fields, and the differences increase with duration of organic management. Finally, we explored in more detail how the (relative) abundances of microbe-feeding nematodes and protists, as well as of plant-parasites varied between agricultural management types and with soil (a)biotic conditions (i.e., organic matter content, pH and bacterial and fungal biomass).

2. Material & methods

2.1. Field selection and chronosequence set up

We used a space-for-time substitution approach, also known as a chronosequence (Pickett, 1989; Walker et al., 2010; Wardle et al., 2009). Such an approach is complementary to long-term experiments, as it allows sampling fields that have been converted to organic agriculture at different moments in time within the same year. This minimizes impacts of variation in climatic conditions and analytical methods, and avoids sample storage-related biases (Mason-Jones et al., 2020; Rubin et al., 2013; Tzeneva et al., 2009). We established two chronosequences, one on the soil type sand and one on the soil type marine clay. In total the two chronosequences included 34 organic fields (11 on sand and 23 on marine clay), with fields ranging from 1 to 25 years since conversion from conventional to organic management. Each organic field was paired with a nearby conventional field that served as a local control, i.e., similar climate and soil conditions but no management conversion. The fields were distributed across the Netherlands, covering some 140 kilometers of distance between the most remote fields. Fields on marine clay were located in two regions (in and around the Dutch provinces Zeeland and Flevoland) and the fields on sand were located in four regions (in and around the Dutch provinces Gelderland, Overijssel, Drenthe, Limburg). Within pairs, fields were minimally 40 m and maximally 13 kilometers apart (van Rijssel et al., 2022).

We used five criteria to select pairs of organic and conventional fields to ensure contrasts in management, while minimizing variation in soil conditions and geological history: (1) All organic fields were SKAL certified ("Stichting Keur Alternatief voortgebrachte Landbouwproducten"). This is a Dutch certification for organic agricultural products (Skal Biocontrole, 2023). This is based on European legislation: no use of mineral fertilizers and chemical pesticides, and minimally 70 % of the fertilizer was organic-certified animal manure, plant

material or compost. The conventional controls were selected to apply common conventional management practices. This included the use of chemical crop protection against pests and pathogens, chemical weed control and/or seed treatments and application of mineral fertilizer. In addition, most conventional fields also applied a part of their fertilizers as manure or pig slurry, which is a common practice in Northwestern Europe and therefore makes farms using these practices a meaningful control, (2) standardized soil type based on the World Reference Base for soil resources (de Bakker, 1989; IUSS Working Group WRB, 2022): sandy soils were Anthrosols with ≤ 17.5 % silt (<50 μm) and an A-horizon of at least 30 cm; clay soils were calcareous fluvisols from marine origin with a lutum content between 17.5 % and 35 %; (3) standardized crop: fields were covered by a monocotylous crop, preferably winter wheat (*Triticum aestivum*), and if not possible, a grass-legume mixture dominated by grasses; (3) crop rotation: each field was part of a wider crop rotation with tuber crops (e.g. potatoes/onions) and (4) soil tillage: inversion tillage has been applied at least once in the last five years (van Rijssel et al., 2022). The ranges of management practices can be found in the supplementary material (Table S1). Overall, the management intensity was generally lower in fields under organic than conventional management (van Rijssel et al., 2025).

2.2. Soil sampling and chemical analyses

All samples were collected between June to August 2017 within six weeks from each other, which was around the time that winter wheat was harvested. Within each field, we collected soil samples from three subplots that were situated minimally 30 m from the field edge and approx. 2 m from tractor tracks. Subplots were minimally 15 m apart. At each sampling subplot, we collected a soil sample of appr. 3 kg from 5 to 15 cm below the soil surface. The 5 cm topsoil was removed because processes in the top soil may be largely driven by variation in daily weather conditions. Soil samples were stored at 4°C. Within a week, soil samples were gently homogenized and a subsample of 10 g was sieved using a 4 mm mesh to remove stones and roots. From this subsample, we stored 1 g of soil in an Eppendorf tube at −80°C for analysis of the community composition of protists. 4 g were stored at 20°C for PLFA analyses and 5 g were dried and used for chemical analyses. Nematodes were extracted in December from the 4°C stored bigger amount of soil (see next section).

We determined soil moisture by weighing the soil samples on the day of sampling and after drying the soil at 105°C for 24 h. The water content was determined by the difference in weight before and after drying and expressed as a fraction of the dry weight. We determined soil organic matter content by loss on ignition. Samples were dried at 105°C and then placed in a muffle furnace for 8 h at 430°C. Soil organic matter content was calculated as the relative weight difference between samples heated at 105°C and 430°C, respectively, expressed as a weight percentage. We measured pH using a Mettler Toledo pH meter (Ohio, USA) after shaking 10 g dry weight equivalent fresh soil in 25 ml of demi water for 2 hrs. at 250 rounds/minute. Chemical analyses were done within two months after soil collection.

We measured phospholipid fatty acids (PLFA) to determine microbial biomass (Frostegård and Bååth, 1996) by extracting 2.0 g of dried soil (after sieving by 2 mm mesh) with a chloroform/methanol/citrate buffer 0.15 M, pH 4.0 (4:8:3 ml). The extract was shaken in the dark at 250 rpm for 1.5 h; after centrifuging for 2 min at 1500 rpm, the extract was transferred to a clean bottle. The soil was extracted again with 6 ml chloroform. After centrifuging the supernatant was added to the first extract. Water was added to the combined extract to separate the different phases and a part of the lipid-containing phase (the chloroform phase) was transferred to a new tube and carefully dried with nitrogen gas. The total lipid extract was dissolved in 1 ml chloroform, and fractionated using SPE (200 mg silica; first the column was flushed with chloroform, then acetone (both discarded) and finally eluted with methanol). Fractions were dried with nitrogen gas. The eluted phospholipids (incl. internal standards) were derivatized into methyl esters using 0.2 M alkaline methanol for 15 min at 30–40 °C. The liquid was neutralized using 2.5 ml 0.1 M acetic acid and extracted twice using 2 ml pentane. The resulting extract was concentrated using nitrogen gas and then dissolved in 400 μl cyclohexane for GCMS analysis. The phospholipids were separated on a GC Trace 1300 and analyzed on a TSQ 8000 mass spectrometer (20 m X 0.15 mm ID, 0.30 μm VF-5MS Agilent; 1 μl injection; helium; full scan 50–300 m/z).

The retention time of the different phospholipids and the mass spectrum were used for determination of the lipids. A compound database and several internal standards were used to determine any shifts in the retention time. The analysis was performed according to NPR-CEN-ISO/TS 29843–1 and CEN ISO/TS 29843–2. Results are expressed in μg phospholipid fatty acids / g dry soil. These measurements are estimates of bacterial and fungal biomass (i.e., g of phospholipid fatty acids per g dry soil), because there is a constant factor used to convert PLFA quantity to microbial biomass (Joergensen, 2022). The markers used for bacteria were: i15:0, ai15:0, i16:0, ai16:0, 16:1w7c, i17:0, ai17:0, cy17:0, 18:1w7c+ 18:1w9t+ 18:1w12t, cy19:0w7c, 10me-16:0, 10me-17:0 and 10me-18:0 + 12me-18:0 (Joergensen, 2022). The marker for fungi was 18:2w6c (Frostegård and Bååth, 1996; Norris et al., 2023; Willers et al., 2015; Zelles, 1999).

2.3. Nematode extraction

Five months after collection and storage at 4 °C, nematodes were extracted with an Oostenbrink elutriator using 200 g of fresh soil. This was relatively long after field collection, but the storage of soil samples at 4^0C is similar to common winter field conditions under which changes in nematode community composition occur at a slow pace (Takemoto et al., 2010). The extracted nematode suspension was placed on two cotton filters for 48 h in a climate chamber at 20^0C. Then, the nematode suspension was poured into glass jars and stored at 4^0C for 24 h before concentrating the suspension, so that the nematodes were contained in smaller volume. We then added 96 % ethanol to create a final 8 ml solution of 70 % ethanol, which was used for counting the total number of nematodes, and subsequently for DNA isolation. Nematodes were counted using an inverse light microscope (200 ×; Olympus CK40) and nematode numbers were expressed per 100 g of dry soil.

2.4. DNA extraction and amplification

Tubes with ethanol containing the nematode solution were split, and half of the sample was put in a 15 ml tube and centrifuged for 10 min at 1667 rpm. After centrifugation, the solution was reduced to 1 ml, pipetted from above to reduce the amount of ethanol. The deposit was mixed, again centrifuged for 5 min at 2307 rpm, the liquid was removed and the sample was dried and afterwards used for DNA extraction. Protists were not extracted from soil, but bulk soil was directly used to extract DNA (from ±0.250 g of soil). For both groups, DNA was extracted using the DNeasy PowerSoil Kit (Masella et al., 2012) (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Community characterization of both nematodes and protists was done by amplification of the most variable part of the 18S rDNA, the V4 region (Pawlowski et al., 2012) using the universal eukaryotic primers 3NDf ("GGCAAGTCTGGTGCCAG") (Cavalier-Smith et al., 2009) together with 1132rmod ("TCCGTCAATTYCTTTAAGT"), adapted from 1132r (Hugerth et al., 2014) as previously described (Geisen et al., 2018b). We added primers containing Illumina adapters to allow demultiplexing of the reads after sequencing.

Polymerase Chain Reactions (PCRs) were performed in reaction mixtures containing 15.6 μl MQ, 0.15 μl (5 U/μL) Fast Start High Fidelity PCR System (Roche), 2.5 μl FastStart High Fidelity Reaction Buffer with 18 mM $MgCl_2$ (10x concentrated), 2.5 μl 2 mM dNTP's, 1 μl 25 mM $MgCl_2$ Stock Solution, 1.25 μl BSA (4 mg/ml) and 0.5 μl (10 mM) of forward and 0.5 μl (10 mM) of reverse primer and 1 μl genomic DNA.

The PCR conditions were as follows: an initial denaturation step of 94 °C for 5 min, 40 cycles of 45 s at 94 °C, 60 s at 54 °C and 90 s at 72 °C, followed by a final extension step for 10 min at 72 °C. We purified the PCR products using Agencourt AMPurebeads (Beckman Coulter, Indianapolis, IN, USA) using a ratio of 1:0.7 of PCR product to bead volume. The purification was carried out according to the manufacturer's protocol and purified products were diluted in 30 µl MQ-water. We then measured the concentrations of the purified PCR products with a Fragment Analyzer (Advanced Analytical, Ankeny, IA, USA) using the standard sensitivity NGS fragment analysis kit (Advanced Analytical). The products were mixed in equal nano g quantities and sent to BGI (Shenzhen, China) for 300 bp paired-end sequencing with Illumina MiSeq.

2.5. Data analysis

2.5.1. Sequencing data

The 18S rRNA amplicon reads were analyzed using the dada2 pipeline (version 1.18) (Callahan et al., 2016) using the forward reads only (truncLen 240 (maxN=0, maxEE=c(2), truncQ= 2, rm.phix=TRUE). Consensus method was used to remove chimeric sequences. Taxonomy was assigned using the PR2 database (version 4.12) (Guillou et al., 2012; Vaulot et al., 2022).

Sequence data were imported in R (version 4.0.3) (R Core Team, 2023) and we used the "phyloseq" package (version 1.26.1). Low abundant amplicon sequence variants (ASVs) with 3 or fewer reads were removed as they are potentially sequencing artefacts (Auer et al., 2017). We removed ASVs that were not identified as nematodes or protists. For protists we did not include unassigned eukaryota, as well as unassigned Opistokonta, since Opistokonta include protists, but also fungi and animals (Adl et al., 2005). Samples with less than 500 reads in total were removed.

Amplicon sequence variants were merged into feeding types for nematodes and functional groups for protists. Nematode feeding types were based on NINJA ("Nematode INdicator Joint Analysis") (Sieriebriennikov et al., 2014), and include six types: bacterivores, fungivores, omnivores, predators and two plant feeder types. Plant feeders were further split into plant-parasites (ectoparasites, migratory endoparasites, sedentary parasites and semi-endoparasites) and epidermal/root hair feeders. Protist functions were based on literature and clustered to five types: phototrophs, phagotrophs, saprotrophs, parasites and plant pathogens (Geisen, 2016; Geisen et al., 2018a). All Oomycota were considered plant pathogens.

2.6. Statistics

Prior to statistical analyses, we determined the number of reads, nematode counts, richness, Shannon diversity and composition of nematodes and protists at the level of individual samples and then averaged the indices/ordinations at field level. These averages of three samples per field were used in all subsequent analysis.

Amplicon sequence variants (ASV) richness and Shannon diversity were determined as: (1) the observed ASV richness and the Shannon index based on raw counts; (2) the average ASV richness and Shannon diversity of repeatedly −1000 times- rarefied data so that there were 850 reads of nematodes and 500 reads of protists (Cameron et al., 2021) and (3) calculated residuals of the linear model between richness/diversity on ASV level and library size. In this linear model the response variable was either nematode or protist ASV richness or Shannon diversity, whereas the explanatory variable of the model was the library size that was square root transformed (Hiiesalu et al., 2014; Tedersoo et al., 2014). For community composition we derived an indicator by principal coordinate analyses (PCoA), which is based on Bray-Curtis distances. The first two axes are used in subsequent analyses. We first used cumulative sum scaling (CSS) to normalize the data (Paulson et al., 2013). Then we performed PCoA at sample level after which PCoA ordinations were averaged at field level. Relative eigenvalues were used to assess the amount of variation that was explained by the first two PCoA axes. Permutational ANOVAs were used to test for the effects of soil type and management on community composition using the adonis2 () function (Anderson and Walsh, 2013) and the equal spread of points within groups was assessed by the betadisper() function (Oksanen et al., 2019).

We used analysis of variance (2-way ANOVA) to test how ASV richness, Shannon diversity, composition, read depth, nematode abundance, pH, soil organic matter content, as well as bacterial and fungal biomass were affected by soil type (sand vs. clay), management (organic vs. conventional) and their interaction. Data were analyzed at field level. Average read depth of nematodes and protists per field was log-transformed prior to analysis.

For all subsequent analyses, we split the data set based on soil type, i.e., marine clay versus sand. This meant that we ran each of the following tests separately for the chronosequence on marine clay and on sand. We did this because soil type is known to be a main driver of community composition and diversity. Including both soil types in one analysis could mask other effects (Ilieva-Makulec et al., 2016; van Diepeningen et al., 2006; van Rijssel et al., 2022), such as those of time since conversion to organic agriculture, which was of major interest to us. We therefore treated both chronosequences as two parallel data sets, both testing impacts of organic versus conventional agricultural management and time since conversion to organic agricultural on nematode and protist communities. As we used PCoA ordination axes as explanatory variables per soil type in some of the following analyses, we re-ran the PCoA ordinations for each of the soil types separately.

We checked the appropriateness of using the conventional fields as a paired control for the organic fields by giving each conventional field the same 'age' as the nearby organic field. We assumed that when there would be a relationship with time for the organic field, but not for the conventional fields. Also, we tested the relations between duration of organic management and pH, soil organic matter content, bacterial and fungal biomass, and nematode abundance. For this analysis, we included management using linear models to determine possible dependency of time since conversion from conventional to organic farming on management.

We tested whether ASV richness, Shannon diversity and community composition were affected by type of management, time since conversion from conventional to organic management, pH and soil organic matter (SOM) as explanatory variables using multiple linear regression models. For these analyses we tested whether including region as a random factor would improve the model based on comparing the Akaike information indices (Akaike, 1974). If so, we included region and then used linear mixed model. We selected for the best model using backward selection, starting with all three-way interactions involved. We used sequential sum of squares since management should be considered first, because duration of (organic) management can only be assessed properly on the residual variation after considering management. Furthermore, we were interested whether pH, SOM and their interactions added extra explained variation on top of management and time since conversion to organic management.

For each nematode feeding type and protist functional group we determined ASV richness, Shannon diversity and relative abundance. For protists we only have relative abundance based on the molecular data. For nematodes we also multiplied the relative abundance by the total counts to get an indication of absolute numbers per feeding type, which has shown correlation to abundance in earlier studies (Geisen et al., 2018b; Schenk et al., 2019). We used linear models to test the relationship between ASV richness, Shannon diversity and relative abundance of nematode feeding types and protist functional groups and management, pH, soil organic matter content, bacterial and fungal biomass. pH, soil organic matter content, bacterial and fungal biomass were standardized by standard scaling before analysis, which is a z-score transformation. In case the residuals of the model were not normally

distributed, which was assessed by a Shapiro-Wilk test, data was either log- or square root transformed prior to analysis. Region was added as random factor for Shannon diversity of phototrophic protists on clay, ASV richness and Shannon diversity of plant-parasitic nematodes on clay. We selected the best model using backward selection; we started with a model with management and all interactions between management and pH, soil organic matter content, bacterial and fungal biomass as response variables.

For plant-parasites and plant pathogens we analyzed how relative abundances at the genus level were affected by conventional versus organic management and the duration of organic management. We only analyzed genera with less than 40 % zeros using a negative binomial generalized linear model with management and conversion time as fixed factors.

All statistical analyses were performed in R, version (version 4.3.1) (R Core Team, 2023). Linear (mixed) models were performed using the package "nlme", using sequential type of squares (Pinheiro et al., 2023). PCoA and associated statistics were performed using the "vegan" package (Oksanen et al., 2019). Figures were made with "ggplot2" (Wickham, 2016).

3. Results

3.1. Sequencing information

Sequencing depth per sample was on average 6384 reads ± 436 for nematodes (max 57737; min 889) and 2964 reads ± 153 for protists (max 15597, min 515). We found 384 nematode taxa within the classes of Enoplea and Chromadorea, consisting of 71 genera and 118 species. Nematode sequencing depth was greater in sand (8742 ± 1440) than in clay (5528 ± 396; $F_{1,64} = 6.47$, $p = 0.013$), but was neither affected by management type ($F_{1,64} = 0.73$, $p = 0.396$), nor by the interaction between soil type and management. We found 2178 protist taxa that belonged to 500 species in 390 genera and 11 supergroups. Protist sequencing depth was lower in organic fields on clay (1935 reads ± 217) than in conventional fields on clay and lower than in all fields on sandy soils (conventional clay: 3578 reads ± 400; sand: 3311 reads ± 351; $F_{1,64} = 4.283$, $p = 0.043$).

(a) biotic characteristics of soil types and conventional vs organic management

pH was highest and soil organic matter (SOM) lowest in clay (Table S2, Table S3). Analyses per soil type including time since conversion did not reveal significant differences in pH and SOM content between conventional and organic fields (Table S4). There was no effect of time since conversion from conventional to organic farming (Table S4).

Bacterial and fungal biomass were higher in clay than in sand (Table S2, Table S3). For the analysis including duration of farming, we found that bacterial and fungal biomass were higher in organic than in conventional fields (Table S3). However, there was no effect of time since conversion to organic farming, neither in fields on clay nor on sand (Table S4). Total abundance of nematodes was higher in sand than in clay (Table S2). There was no effect of management on nematode abundance (Table S3). There was also no effect of time since conversion to organic farming in any of the soil types (Table S4).

3.2. Time since conversion to organic management

Community composition of nematodes and protists differed between clay and sand (Fig. 1). In the case of clay soil 20 % of the variation in community composition was explained by the PCoA axes (Axis 1: 11.2 %, Axis 2: 8.8 %), while for sand, 23.7 % of the variation was explained by the PCoA axes (Axis 1: 13.9 %, Axis 2: 9.8 %). Within soil types, community composition of nematodes differed between conventional and organic management (clay: perMANOVA: $F_{1127} = 4.4$, $R^2 = 0.034$, $p = 0.001$; sand: perMANOVA: $F_{1,58} = 2.2$, $R^2 = 0.044$, $p = 0.004$). However, management explained only 3–4 % of the variation and dispersion was not equal among management regimes (clay: betadisper: $F_{1126} = 3.9$, $p = 0.049$; sand: betadisper: $F_{1,58} = 5.8$, $p = 0.019$). When including time since conversion to organic management into the analysis, community composition of nematodes did not differ between conventional and organic management irrespective of soil type (Table S6). However, in clay soils nematode community composition shifted along both PCoA axes with time since conversion to organic management (Fig. 2; Table 1). There was no interaction between management type and time since conversion to organic farming,

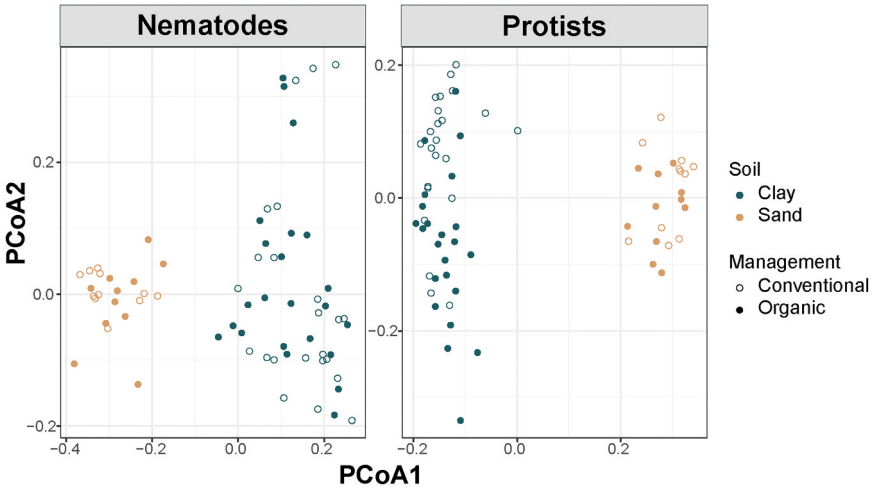


Fig. 1. Biplot plot of community composition of nematodes and protists per soil type and management type. Community composition was determined using a principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity (n = 68). For nematodes, the first two axes of the ordination explained 25.9 % of the total variation in community composition (Axis 1: 19.1 %, Axis 2: 6.9 %). For protists, the first two axes of the ordination explained 18.2 % of the total variation in community composition (Axis 1: 13.6 %, Axis 2: 4.7 %). This is based on the relative eigenvalues of the Bray–Curtis distance matrix. Community composition is different in clay as in sand for both nematodes (perMANOVA: $F_{1186} = 36.93$, $R^2 = 0.17$, $p = 0.001$) and protists (perMANOVA: $F_{1198} = 29.5$, $R^2 = 0.13$, $p = 0.001$). Dispersion among soil types was significantly different for nematodes ($F_{1186} = 7.12$, $p = 0.008$), but not for protists ($F_{1198} = 0.05$, $p = 0.821$). Community composition differed between conventional and organic management for both nematodes (perMANOVA: $F_{1186} = 2.71$, $R^2 = 0.014$, $p = 0.002$) and protists (perMANOVA: $F_{1198} = 3.37$, $R^2 = 0.017$, $p = 0.001$). Dispersion among soil types was significantly different for nematodes ($F_{1186} = 7.12$, $p = 0.008$), but not for protists ($F_{1198} = 0.05$, $p = 0.821$).

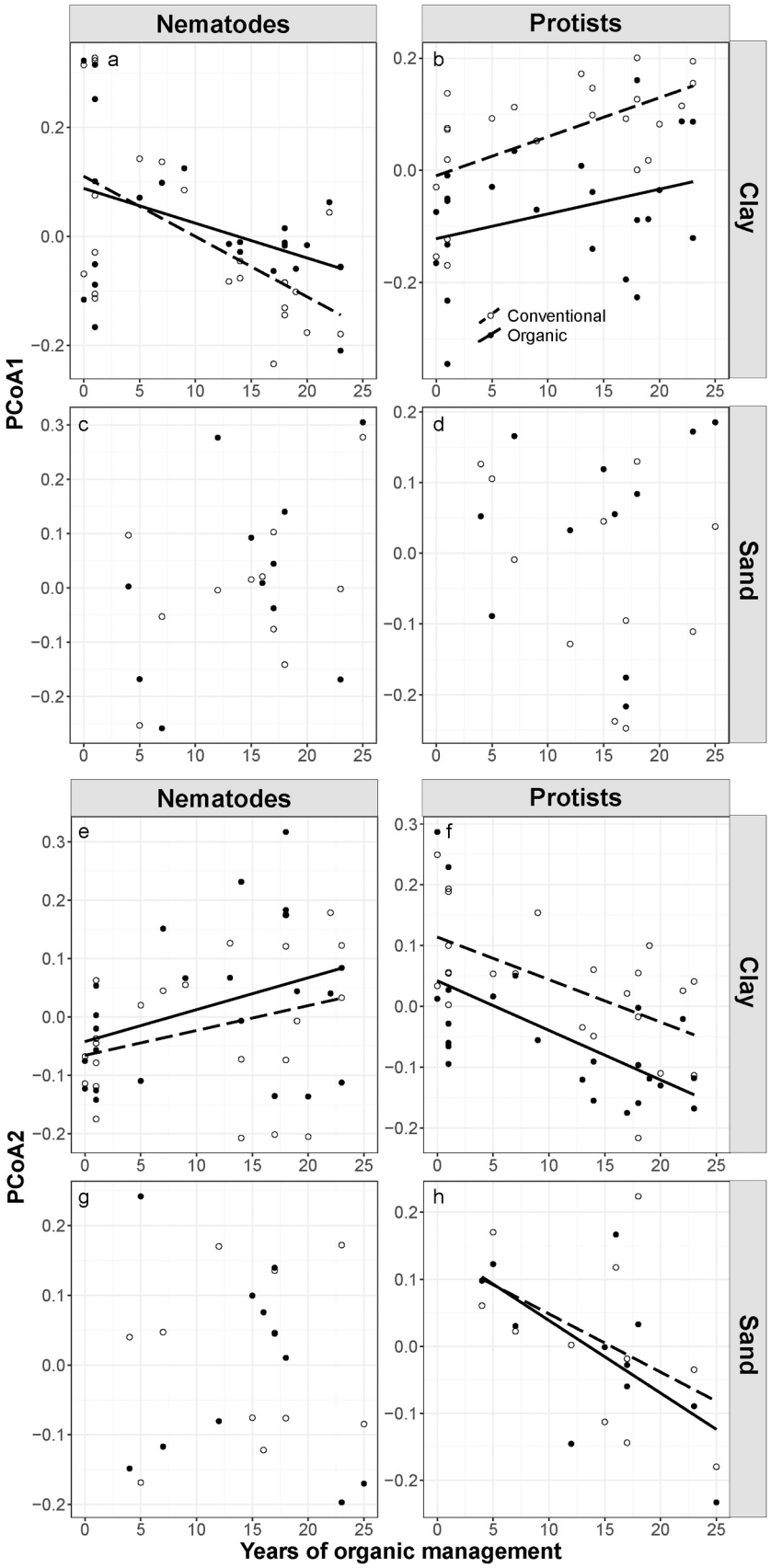


Fig. 2. Effect of duration of organic management from conventional to organic management on ASV composition of nematodes (a,c,e,g, n = 68) and protists (b,d,f,h, n = 68) as represented by PCoA1 (a-d) and PCoA2 (e-h). Conventional fields were assigned the "duration of organic management" from their paired organic neighboring field. Relationships were tested using linear models and are only plotted when significant (Table 1). Note that the scale of the y-axes differs between nematodes and protists, because composition differs strongly between these two groups.

Table 1
Influence of management, pH, SOM content on community composition of nematodes and protists, represented by the first two PCoA axes. These are tested within the soil types clay and sand. Number of observations in clay (n = 46) and in sand (n = 22). Values in boldface represent significant effects with P < 0.05. Numerator degrees of freedom was 1 for all variables; F = F-value, P = P-value. [-] means that the factor was removed from the model after forward selection.

	Nematodes		**Protists**	
	F	P	F	P
PCoA1 Clay				
Management	2.06	0.162	28.60	**< 0.001**
Time	20.46	**< 0.001**	13.70	**0.001**
pH.std	14.98	**0.001**	0.15	0.704
SOM.std	6.51	**0.016**	1.29	0.265
Management:Time	0.82	0.372	0.29	0.592
Management:pH.std	0.58	0.452	4.10	0.051
Management:SOM.std	4.29	**0.047**	7.09	**0.012**
Time:pH.std	2.19	0.150	0.09	0.762
Time:SOM.std	7.22	**0.012**	1.43	0.241
pH.std:SOM.std	0.60	0.447	2.88	0.099
Management:Time:pH.std	0.03	0.870	1.28	0.266
Management:Time:SOM.std	0.32	0.573	1.82	0.186
Management:pH.std:SOM.std	1.16	0.290	[-]	[-]
Time:pH.std:SOM.std	0.41	0.529	[-]	[-]
Management:Time:pH.std:SOM.std	1.65	0.209	[-]	[-]
PCoA1 Sand				
Management	0.43	0.538	7.60	**0.033**
Time	13.79	**0.010**	0.57	0.477
pH.std	0.37	0.563	88.39	**< 0.001**
SOM.std	15.24	**0.008**	1.62	0.250
Management:Time	1.77	0.232	1.40	0.281
Management:pH.std	1.78	0.230	0.00	0.995
Management:SOM.std	0.90	0.380	2.19	0.190
Time:pH.std	0.26	0.630	0.99	0.358
Time:SOM.std	2.32	0.179	0.29	0.609
pH.std:SOM.std	11.52	**0.015**	2.22	0.187
Management:Time:pH.std	5.37	0.060	1.93	0.214
Management:Time:SOM.std	0.18	0.690	0.28	0.618
Management:pH.std:SOM.std	7.71	**0.032**	1.27	0.303
Time:pH.std:SOM.std	4.27	0.084	0.02	0.898
Management:Time:pH.std:SOM.std	2.73	0.150	0.72	0.430
PCoA2 Clay				
Management	1.10	0.301	15.94	**0.000**
Time	5.64	**0.023**	20.06	**0.000**
pH.std	1.54	0.223	0.015	0.902
SOM.std	3.95	0.055	0.40	0.530
Management:Time	[-]	[-]	[-]	[-]
Management:pH.std	0.34	0.564	0.66	0.420
Management:SOM.std	0.99	0.326	0.011	0.915
Time:pH.std	[-]	[-]	[-]	[-]
Time:SOM.std	0.55	0.465	3.40	0.074
pH.std:SOM.std	1.20	0.281	0.001	0.974
Management:Time:pH.std	[-]	[-]	[-]	[-]
Management:Time:SOM.std	[-]	[-]	[-]	[-]
Management:pH.std:SOM.std	0.02	0.900	2.94	0.095
Time:pH.std:SOM.std	[-]	[-]	[-]	[-]
Management:Time:pH.std:SOM.std	[-]	[-]	[-]	[-]
PCoA2 Sand				
Management	0.11	0.756	0.60	0.459
Time	0.17	0.698	26.39	**0.001**
pH.std	8.84	**0.025**	0.19	0.676
SOM.std	0.029	0.870	28.20	**0.001**
Management:Time	0.22	0.657	1.97	0.198
Management:pH.std	0.82	0.401	6.58	**0.033**
Management:SOM.std	< 0.001	0.995	1.09	0.327
Time:pH.std	1.55	0.259	0.09	0.768
Time:SOM.std	0.30	0.601	8.59	**0.019**
pH.std:SOM.std	1.42	0.279	1.04	0.337
Management:Time:pH.std	0.17	0.690	< 0.001	0.994
Management:Time:SOM.std	0.001	0.977	0.30	0.600
Management:pH.std:SOM.std	0.86	0.389	[-]	[-]
Time:pH.std:SOM.std	0.96	0.364	6.71	**0.032**
Management:Time:pH.std:SOM.std	2.40	0.173	[-]	[-]

indicating that nematode community composition changed with time since conversion irrespective of soils being organically or conventionally managed. In sand, community composition of nematodes was not affected by the time since conversion to organic management. In both clay and sand, community composition of nematodes was related to pH and soil organic matter content (Table 1).

Nematode ASV richness and Shannon diversity were highest in clay soil (Figure S1; Table S6). In clay, nematode Shannon's diversity was lower in organic than in conventional fields, while there was no difference between organic and conventional management in sand (Fig. 3; Figure S1; Table S5, Table S6). ASV richness and Shannon diversity of nematodes were not affected by the duration of organic management in both soil types (Fig. 3, Table S7). In neither clay nor sand, ASV richness and Shannon diversity for nematodes were related to pH and soil organic matter content (Table 2).

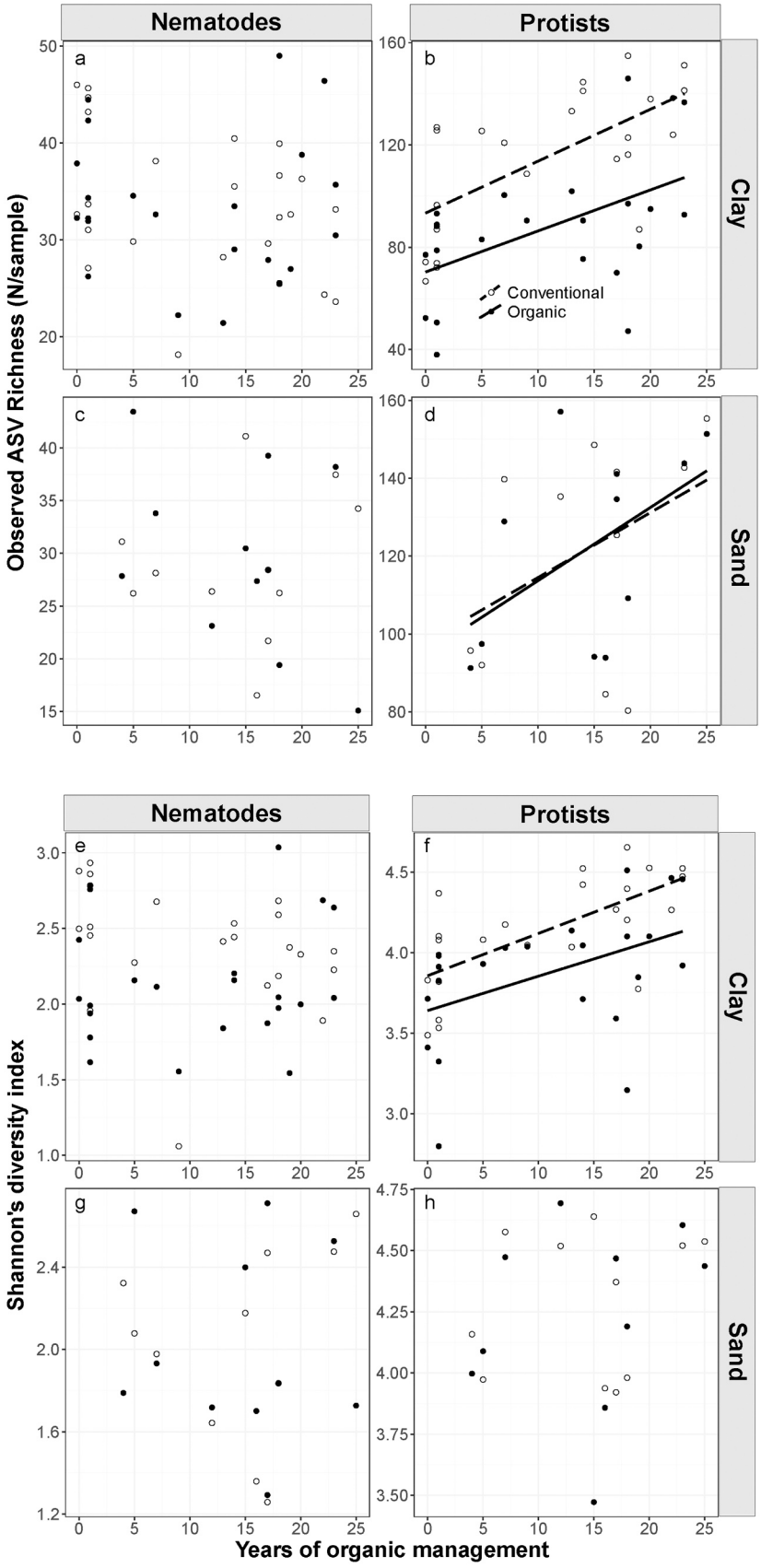
For protists, 12–13 % of the variation in community composition was explained by the first two PCoA axes (clay: Axis 1: 7.3 %, Axis 2: 5.3 %; sand: Axis 1: 7.3 %, Axis 2: 5.8 %). Community composition of protists differed between organic and conventional management of fields on clay (perMANOVA: $F_{1132} = 4.09$, $R^2 = 0.03$, $p = 0.001$), and on sand (perMANOVA: $F_{1,65} = 2.32$, $R^2 = 0.03$, $p = 0.001$), but explained only 3 % of the variation irrespective of soil type. Dispersion among management regimes was different in clay (betadisper: $F_{1132} = 12.2$, $p = 0.001$), but similar in sand (betadisper: $F_{1,65} = 0.8$, $p = 0.356$). Linear models on PCoA ordinations revealed only a difference in community composition between conventionally and organically managed fields of protists in clay, but not in sand (Figs. 1, 2; Table S5). In sand, the initially significant but weak effect of management type (organic vs. conventional) was no longer detectable after accounting for additional factors such as the duration of organic management. For both soil types, community composition of protists changed with the time since conversion to organic management. For clay, shifts in community composition with time since conversion occurred along the first and second PCoA axes, while in sand it only occurred along the second PCoA axis (Fig. 2; Table 1; $F_{1,18} > 5$, $p < 0.030$). The shift in community composition with time since conversion occurred in the same direction for both conventional and organic fields (Table 1, Fig. 2). pH and soil organic matter content affected community composition of protists in both clay and sand (Table 1).

Protist ASV richness and Shannon diversity were highest in sand (Figure S1; Table S6). For clay soils, ASV richness and Shannon diversity of protists and Shannon diversity of nematodes were higher in conventional than in organic fields, but on sand there were no differences between management types (Figure S1, Table S7). ASV richness and Shannon diversity of protists increased with time since conversion from conventional to organic management in clay soils, however, this pattern also occurred in the conventional fields when they were given the same time as the paired organic fields (Fig. 3, Table S7; lm; $F > 7$, $p < 0.014$). Interestingly, there was no such effect of time in the conventional fields on sandy soils (Fig. 3, Table S7). pH and soil organic matter content did not affect ASV richness and Shannon diversity of protists (Table 2).

Community composition (PCoA ordinations) differed between geographic regions (as represented by Dutch provinces) for both nematodes and protists along PCoA1 and for nematodes also along PCoA2 (Figure S2 Table S5). Although these analyses mainly indicated differences between sand and clay soils, it also showed that within clay there was a difference between the regions around Flevoland and Zeeland along PCoA2 (Figure S2; Tukey test marine clay: $p < 0.001$; Tukey test sand; $p > 0.140$).

3.3. Nematode feeding types and protist functional groups

For both sand and clay, ASV richness and Shannon diversity of nematode feeding types generally did not differ between conventional and organic fields, and results were not affected by the time since conversion to organic management (Table S8). However, Shannon diversity



(caption on next page)

Fig. 3. Effect of duration of organic management from conventional to organic management on observed ASV richness (a-d) and Shannon diversity (e-h) of nematodes (a,c,e,g) and protists (b,d,f,h). Conventional fields were assigned the "duration of organic management" from their paired organic neighboring field. Relationships were tested using linear models and are only plotted when significant (Table S4). Note that the scale of the y-axes differs between groups, because the number of ASVs and Shannon diversity between nematodes and protists differ strongly. Lines are plotted for significant relationships between time and ASV richness (Table 2). Solid lines are organically managed fields, dashed lines are conventionally managed fields.

of fungivorous nematodes in clay was lowest in organic fields (Table S8). Also, in clay, ASV richness and Shannon diversity of epidermal and root hair feeders, as well as predators, were reduced with increasing time since conversion to organic management, and the same effect was observed in conventional fields when those were given the same time as the paired organic fields (Table S8). For sand, ASV richness and Shannon diversity of predators were affected by interaction between time and management, because these values declined with time since organic management in organic fields only (Table S8).

In general, relative abundances of nematode feeding types did not differ between conventional and organic management (Fig. 4, Table S8). There was one exception, as on both soil types relative abundance of bacterivorous nematodes was lowest in organic fields (Fig. 4, Table S8). In clay, the relative abundances of omnivores, epidermal and root hair feeders, and predators decreased with time since conversion, however this time effect also occurred in the paired conventionally managed soils (no significant interaction between time and management; Table S8). In sand there was no relationship between the duration of organic management and the relative abundance of nematode feeding types. Absolute abundances of nematode feeding types were not affected by management type and by the duration of organic management, except for Chromadorea in clay, which is a nematode class that includes a variety of feeding types (Table S9).

pH and SOM content were related to ASV richness, Shannon diversity and the relative abundance of some nematode feeding types, but this often differed between soil types and management. For example, with increasing pH in clay, ASV richness and Shannon diversity of plant-parasitic nematodes increased, while in sand richness and diversity of plant-parasitic nematodes decreased (Table S8). Moreover, there was a positive relationship between pH and ASV richness and Shannon diversity of fungivores, but only in organic fields on sand. SOM content in clay was positively related to ASV richness of bacterivorous nematodes and epidermal and root hair feeders. ASV richness of plant-parasitic nematodes was positively related with SOM content in sand. Finally, ASV richness of fungivorous nematodes in clay was negatively related with SOM content (Table S8).

Bacterial and fungal biomass were related to the ASV richness, Shannon diversity and relative abundance of some nematode feeding types, but this often differed between soil type and management (Fig. 4, Table S8). For example, there was a positive relationship between bacterial biomass and the Shannon diversity of fungivorous nematodes in clay and between bacterial biomass and the ASV richness of fungivorous nematodes in clay soils under organic management (Table S8). In contrast, for several other nematode feeding types we found a negative relationship between bacterial biomass and ASV richness, Shannon diversity and relative abundance, but mostly in sand (Table S8). In clay, ASV richness and Shannon diversity of epidermal and root hair feeders and relative abundance of predatory nematodes were negatively related to bacterial biomass. In sand, there were negative relationships between bacterial biomass and ASV richness of omnivores, relative abundance of plant-parasites, Shannon diversity of predators and ASV richness and Shannon diversity of plant-parasites, with the latter three only occurring in organic fields. There was a positive relationship between fungal biomass and abundance of epidermal and root hair feeders and with ASV richness and Shannon diversity of epidermal and root hair feeders in both soil types (Table S8). In contrast, Shannon diversity of bacterivorous nematodes in clay, ASV richness of bacterivorous nematodes in organic fields on both soil types, and ASV richness and Shannon diversity of predatory nematodes in sand were negatively related to fungal biomass (Table S8). There was also an interaction effects observed between fungal biomass and management on the relative abundance of omnivorous nematodes (Table S8), but visual inspection did not reveal any direction of the relationship.

Relative abundance of phagotrophic protists was lower in organic fields on sand than in conventional fields on sand (Fig. 4; *t*-test; $t = 3.2$, $df = 52.4$, $p = 0.002$). In contrast, for several protist functional groups ASV richness, Shannon diversity and relative abundance increased with the duration of organic management. However, this was observed for both conventional and organic fields (Table S10). Specifically, ASV richness and relative abundance of parasitic protists in sand increased with duration of organic management in both management types (Table S10). Also, in clay, ASV richness and Shannon diversity of phagotrophic, phototrophic and saprotrophic protists increased with duration of organic management in organic and paired conventional fields (Table S10).

pH and SOM content were related to ASV richness, Shannon diversity and the relative abundance of several protist functional groups. In sand, there were positive relationships between pH and the relative abundance of parasitic protists, between pH and the ASV richness and Shannon diversity of saprotrophic protists, and between SOM content and ASV richness and Shannon diversity of parasitic and phagotrophic protists (Table S10). In clay, SOM content was negatively related to Shannon diversity of phototrophic protists and ASV richness and Shannon diversity of saprotrophic protists, especially under conventional management (Table S10). There were no effects of pH and SOM content on ASV richness and Shannon diversity of parasitic protists in clay, and on richness, diversity and relative abundance of phototrophic protists in sand and relative abundance of plant-pathogenic protists in sand.

ASV richness and relative abundance of parasitic and saprotrophic plant-pathogenic protists in clay increased with bacterial biomass (Table S10). In sand, bacterial biomass was positively related to Shannon diversity of plant-pathogenic protists. There were interaction effects between bacterial biomass and management on relative abundance of saprotrophic protists in clay and all characteristics of saprotrophic protists in sand. In organic clay fields, bacterial biomass was positively related to relative abundance of saprotrophic protists. In organic fields on sand, bacterial biomass was negatively related to all three characteristics of saprotrophic protists. Bacterial biomass was negatively related to Shannon diversity of phototrophic and phagotrophic protists on clay and relative abundance of phagotrophic protists in both soil types. ASV richness and Shannon diversity of phagotrophic protists in clay increased with fungal biomass, while Shannon diversity of saprotrophic protists and phagotrophic protists in clay and relative abundance of phagotrophic protists in both soil types decreased with higher fungal biomass (Table S10). There was interaction between fungal biomass and management on ASV richness and Shannon diversity of plant-pathogenic protists in sand and relative abundance of phototrophic protists in clay. In organic fields on sand, fungal biomass was positively related to ASV richness and Shannon diversity of plant-pathogenic protists. In conventional fields on clay, fungal biomass was negatively related to the relative abundance of phototrophic protists (Table S10).

The relative abundance of the protist genus *Pythium* was higher in organic than conventional fields, both in clay (nb glm; $z = -2.17$; $p = 0.030$) and in sand (nb glm: $z = 2.70$, $p = 0.007$). Similarly, the relative abundance of the protist genus *Spongospora* was relatively more abundant in organic fields, however, this was only the case in fields on clay (nb glm, $z = 2.27$, $p = 0.023$). In addition, for *Spongospora* we

Table 2
Influence of management, pH, SOM content and their interactions by soil type, on observed ASV richness and Shannon alpha diversity of nematodes and protists. These are tested within the soil types clay and sand. Number of observations in clay (n = 46) and in sand (n = 22). Values in boldface represent significant effects with P < 0.05. Numerator degrees of freedom was 1 for all variables; F = F-value, P = P-value. [-] means that the factor was removed from the model after forward selection.

	Nematodes		Protists	
	F	P	**F**	P
Observed ASV richness Clay				
Management	0.19	0.667	19.87	**< 0.001**
Time	1.74	0.197	24.84	**< 0.001**
pH.std	0.51	0.479	0.29	0.592
SOM.std	0.42	0.522	0.03	0.867
Management:Time	0.89	0.353	[-]	[-]
Management:pH.std	0.00	0.974	1.60	0.214
Management:SOM.std	0.13	0.720	[-]	[-]
Time:pH.std	[-]	[-]	1.79	0.189
Time:SOM.std	0.25	0.621	4.63	**0.038**
pH.std:SOM.std	0.09	0.769	3.47	0.071
Management:Time:pH.std	3.07	0.089	[-]	[-]
Management:Time:SOM.std	1.78	0.191	[-]	[-]
Management:pH.std:SOM.std	[-]	[-]	[-]	[-]
Time:pH.std:SOM.std	3.32	0.08	[-]	[-]
Management:Time:pH.std:SOM.std	[-]	[-]	[-]	[-]
Observed ASV richness Sand				
Management	0.062	0.812	0.0002	0.989
Time	0.28	0.618	5.24	0.056
pH.std	0.005	0.945	0.010	0.924
SOM.std	0.93	0.373	4.03	0.085
Management:Time	1.49	0.268	0.21	0.658
Management:pH.std	2.62	0.157	0.29	0.607
Management:SOM.std	1.54	0.261	0.027	0.875
Time:pH.std	1.82	0.226	0.91	0.373
Time:SOM.std	0.82	0.400	1.53	0.257
pH.std:SOM.std	0.67	0.445	1.22	0.306
Management:Time:pH.std	0.60	0.469	0.012	0.916
Management:Time:SOM.std	0.008	0.933	0.15	0.715
Management:pH.std:SOM.std	3.19	0.125	0.35	0.572
Time:pH.std:SOM.std	0.19	0.682	4.16	0.081
Management:Time:pH.std:SOM.std	1.04	0.348	[-]	[-]
Shannon diversity Clay				
Management	4.35	**0.045**	9.41	**0.004**
Time	0.53	0.470	21.25	**< 0.001**
pH.std	0.26	0.611	0.08	0.783
SOM.std	0.04	0.840	0.00	0.962
Management:Time	1.70	0.201	[-]	[-]
Management:pH.std	0.57	0.455	1.52	0.226
Management:SOM.std	0.02	0.887	3.45	0.072
Time:pH.std	0.36	0.554	[-]	[-]
Time:SOM.std	0.42	0.524	1.77	0.191
pH.std:SOM.std	0.00	0.978	2.71	0.109
Management:Time:pH.std	2.61	0.116	[-]	[-]
Management:Time:SOM.std	2.60	0.116	[-]	[-]
Management:pH.std:SOM.std	[-]	[-]	3.76	0.060
Time:pH.std:SOM.std	[-]	[-]	[-]	[-]
Management:Time:pH.std:SOM.std	[-]	[-]	[-]	[-]
Shannon diversity Sand				
Management	0.00	0.984	0.05	0.838
Time	0.05	0.827	0.77	0.410
pH.std	0.44	0.524	0.00	0.949
SOM.std	0.59	0.459	0.29	0.607
Management:Time	0.18	0.678	0.07	0.794
Management:pH.std	[-]	[-]	0.02	0.888
Management:SOM.std	1.15	0.309	0.18	0.686
Time:pH.std	0.71	0.418	0.20	0.668
Time:SOM.std	0.01	0.939	0.35	0.570
pH.std:SOM.std	0.12	0.736	0.36	0.568
Management:Time:pH.std	[-]	[-]	0.10	0.759
Management:Time:SOM.std	0.65	0.440	0.15	0.711
Management:pH.std:SOM.std	[-]	[-]	2.02	0.198
Time:pH.std:SOM.std	1.68	0.225	3.41	0.107
Management:Time:pH.std:SOM.std	[-]	[-]	[-]	[-]

found an increase in relative abundance with time since conversion in organic fields on sand (nb glm; time: z = 2.96, p = 0.003; interaction: z = -2.32, p = 0.021). In sandy soils, a protist genus of the order *Peronosporales* had highest relative abundance in conventional fields (nb glm: z = -2.22, p = 0.026). In clay, none of the plant-pathogenic genera showed differences in relative abundance between conventional and organic management. Relative abundances of the protist genus *Polymyxa* and the nematode genus *Pratylenchus* were not significantly affected by management.

4. Discussion

In this study we used a chronosequence approach to investigate how the community composition, diversity and relative abundance of functional groups and feeding types of nematodes and protists changed by land use transition to organic farming. We found that both protist and nematode communities in fields on clay shifted following change from conventional to organic land management. However, when we assigned conventional fields with the same age of the organic fields to which they were paired with, we found a similar temporal change of soil properties, while this was not expected to happen. This time effect in conventional fields shows that proper controls are essential in chronosequences (Morriën et al., 2017; van Rijssel et al., 2022; Walker et al., 2010), when interpreting results as a temporal change following land management conversion from conventional to organic.

Unlike many earlier studies, we have used a DNA-based instead of a morphological technique to identify nematodes. Although such techniques are still under development (Giachello et al., 2023; Mau et al., 2024), it has been shown that they reveal similar ecological patterns as the ones we know from morphological methods (Griffiths et al., 2018; Kitagami et al., 2022; Porazinska et al., 2009; Treonis et al., 2018). It is however still challenging to directly compare findings using traditional and molecular methods, because of biases in primers for detecting certain species and because separate amplicon sequence variants (ASVs) can be assigned to one species (Du et al., 2020; Geisen et al., 2018b; Waeyenberge et al., 2019). Some of these ASVs may represent different species that are hard to distinguish morphologically (Rivas et al., 2024). In our study, the use of a primer with a high taxonomic coverage used on extracted nematodes from a large volume of soil should provide a representative estimate of the nematode community (Ficetola et al., 2024; Geisen et al., 2018b).

In contrast to our first hypothesis, we found limited responses of nematode community composition and diversity to agricultural management. Only, Shannon diversity of nematodes in clay was lower in organic than in conventionally managed fields. This is opposite to earlier work showing higher diversity in soils under organic than conventional farming (Quist et al., 2016; van Diepeningen et al., 2006; Yang et al., 2021). Nematodes are commonly used as bioindicators due to their sensitivity to changes in environmental properties (Biswal, 2022; Du Preez et al., 2022; Ferris et al., 2012; Neher, 2001; Yeates, 2003), so that our results suggest that there are limitations to these indicator values. However, our results support earlier work that found limited impacts of organic management on nematode communities, so that it is possible that these farming practices only have mild impacts on nematode communities (Guo et al., 2018; Lupatini et al., 2018; Neher, 1999; Zhao et al., 2020). One of the reasons might be that most conventional fields also receive animal manure as organic fertilizer, because of its availability in the Netherlands (Bos et al., 2014; De Wit and Verhoog, 2007). In addition, individual management practices, such as crop rotation, ley cropping or tillage, may be more important than organic versus conventional management as drivers of the diversity and community composition of nematodes in agricultural soils (Bongiorno et al., 2019a; Puissant et al., 2021; Santos et al., 2020).

In line with our first hypothesis, richness, diversity and community composition of protist communities were different between conventional and organic management, although this was only observed in

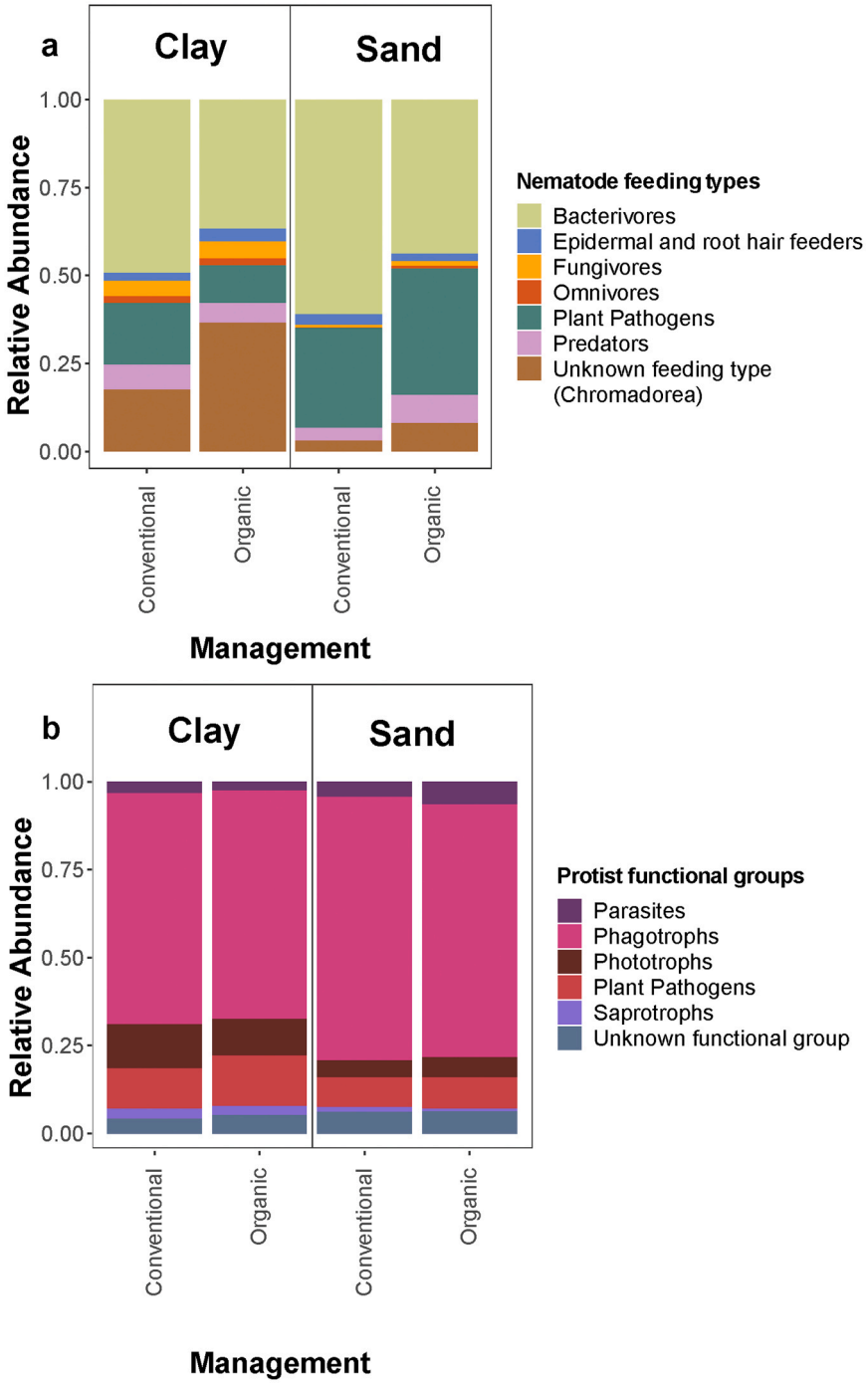


Fig. 4. Relative abundance of nematode feeding types (a) and protist functional groups (b), under conventional and organic management in clay and sand.

clay. This result partly aligned with earlier work reporting changes in protist community composition between organic and conventional management, but no differences in diversity (Guo et al., 2018; Harkes et al., 2019; Lentendu et al., 2014; Lupatini et al., 2018; Puissant et al., 2021). Protist communities were more responsive than nematode communities, which is in line with earlier work where protists were most sensitive to fertilizer management (Zhao et al., 2020, 2019). It is possible that the relatively long storage time of the soil samples before nematode extraction had an effect on the live nematodes in the soil samples. Therefore, comparing within protists and within nematodes might be less biassed than comparing the compositional responses to land use and conversion among both taxonomic groups.

We found that community composition of nematode and protists in both soil types and protist richness in clay changed with time since conversion in organic fields. This was in support of our hypothesis and of earlier work in long-term field experiments showing that differences in community composition of nematodes and protists between conventional and organic fields increasingly diverged with duration of organic management (Briar et al., 2007; Lentendu et al., 2014). However, in our study we found a similar trend for the conventional fields when these were assigned the same 'time since conversion' as the paired organic field. This suggests that time since conversion in our chronosequence may have been co-influenced by other variables driving changes in soil community composition than the ones we have measured (van Rijssel et al., 2022). Although we could not fully untangle the variables underlying the time gradient other than time, further work on the soil

samples suggests that the concentration of iron- and aluminium oxides may be correlated to time since conversion to organic agriculture (Koorneef et al., 2025). We propose that both time series and chronosequences may provide interesting complementary results that ultimately may help elucidating temporal changes in soils under conversion management. Our results point out that such future studies on chronosequences need to particularly account for proper controls in time series impacted by the study design, sampling strategy and identification method (Banerjee and van der Heijden, 2022; Melakeberhan et al., 2021; Su et al., 2024).

In contrast to our hypothesis, we found that diversity of nematode feeding types generally did not respond to organic farming and that the richness of all functional groups of protists was even lower in organic than in conventional fields. The only exception was the diversity of hyphal- and plant-parasitic nematodes, which were increased by organic farming. We found fewer bacterivorous nematodes in organic fields, which is in line with some (Berkelmans et al., 2003), but not all earlier studies (Guo et al., 2021; Tsiafouli et al., 2007; Yang et al., 2021). While bacterivorous nematodes can be enhanced by organic matter additions (Liu et al., 2016; Porazinska et al., 1999), the conventional fields in our study also predominantly received nutrients in the form of manure, or slurry (Bos et al., 2014; De Wit and Verhoog, 2007). This pattern of nutrient supply by manure or slurry in both organic and conventional Dutch farming systems may have led to greater numbers of bacterial-feeding nematodes in conventional fields. For protists there is limited work to compare our results to. Two studies showed that increased land use intensity could reduce the diversity of saprotrophs and plant pathogens (Aslani et al., 2024; Santos et al., 2020). This contrasts our findings, if we consider organic agriculture to be less intensive than conventional agriculture (Armengot et al., 2011).

We expected that plant-parasitic nematodes and plant-pathogenic protists would be relatively less abundant in organic than in conventional fields. For some species (e.g. *Peronosporales*) we indeed found a lower relative abundance in organic fields, however, for others (e.g., *Pythium* and *Spongospora*) we found the opposite. Most previous work on nematodes also showed variable responses of plant feeders to organic management (Ilieva-Makulec et al., 2016; Silva et al., 2022). It could be that some plant-parasitic nematodes show opposite responses, or that they respond to specific management practices and not per se to organic versus conventional management. For most plant-pathogenic protists, responses to management practices are still poorly known (Dumack and Bonkowski, 2021). Only for *Pythium* organic soils are known to be more suppressive than conventional ones, especially in nutrient-limiting circumstances (Bongiorno et al., 2019b; Kurm et al., 2023; Tamm et al., 2010). Future studies may need to study protists responses in more detail, focusing on specific management practices, such as tillage, fertilization or crop rotation in addition to conventional versus organic management. This may help to understand under which conditions nematode and protist plant-pathogens can be suppressed by farming practices.

In line with earlier work, we found that soil organic matter content was positively related to the richness of multiple nematode feeding types and protist functional groups and, for example, the relative abundance of parasitic protists (Dupont et al., 2016; Quist et al., 2019; Xue et al., 2023). Bacterial and fungal biomass were differently related to some nematode feeding types and protist functional groups. Clear trophic relations, such as a positive correlation between bacterial biomass with bacterivores and phagotrophs or fungal biomass with fungivores could not be established, despite observed higher bacterial and fungal biomass under organic management than under conventional management. These results oppose earlier work showing that fungal and bacterial biomass are known as important drivers of nematode and protist community composition (Bongiorno et al., 2019a; Köninger et al., 2023; Quist et al., 2016; Santos et al., 2020; Xue et al., 2023), possibly because such dynamic relationships are hard to identify in one-time sampling campaigns (Kendall, 2001; Liu et al., 2024; Seppey et al., 2017).

In conclusion, we found effects of organic farming on nematode and protist communities, but not all these effects occurred in both soil types. More significant results were observed in clay soils and for protist communities. Although community composition generally shifted in response to conventional versus organic management, the explained variation was relatively minor. Effects of time since conversion on protist and nematode community composition occurred in both conventionally and organic managed fields, indicating that other factors than time contributed to driving changes in protist and nematode community composition. For instance, another study on the same soil samples showed that variation in the concentration of non-crystalline iron-(hydr)oxides and silt and clay was correlated with time since conversion in both organic and conventional fields in the clay and sand chronosequences, respectively (Koorneef et al., 2025). However, it is unclear how those soil properties may have influenced the observed responses in nematode and protist community compositions. Further studies are needed in order to determine how conversion from conventional to organic farming influences soil health, for which information on nematode feeding types and functional groups could be used for as indicator values (Biswal, 2022; Martin et al., 2022; Melakeberhan et al., 2021; Neher, 2001). Furthermore, it is key to know how farm management, especially which context-dependent combination of practices is fruitful for an well-functioning and healthy soil food web, including larger soil fauna (Biswal, 2022; Chassain et al., 2024; Cozim-Melges et al., 2025).

Author contributions

GJK, SG, WvdP, RdG and CV developed the study concept and design; SQvR, GJK, TB and SG collected the samples and analysed soil properties; TB completed the DNA preparation; FtH ran the pipeline for the sequencing data. SvR performed the data analysis with input of CV, GJK, SG, RdG and WvdP; and SvR, CV and WvdP drafted the manuscript with input of all authors. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Stefan Geisen: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Petra C.J. van Vliet:** Writing – review & editing, Resources, Methodology, Funding acquisition, Data curation. **Freddy C. ten Hooven:** Writing – review & editing, Formal analysis, Data curation. **J.M.T. (Tanja) Bakx-Schotman:** Writing – original draft, Methodology, Formal analysis. **Guusje J. Koorneef:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Wim H. van der Putten:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Van Rijssel Sophie Quirina:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **G.F. (Ciska) Veen:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Formal analysis, Conceptualization. **Ron G.M. de Goede:** Writing – review & editing, Validation, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: W.H. van der Putten reports financial support was provided by Dutch Research Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109854.

Data availability

Metadata are available at zenodo: https://doi.org/10.5281/zenodo.15845683. R script for both DADA2 as well as downstream statistical analyses can be found at Github: https://github.com/SophievanRijssel/18SVitalSoils. Sequencing data have been submitted to the European Nucleotide Archive (accession number: PRJEB84927).

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