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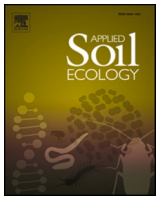

Impacts of long-term organic production on soil fauna in boreal dairy and cereal farming

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ABSTRACT

Intensified arable farming results in fewer functional groups of soil biota with decreased biodiversity. Organic farming with slurry fertilization and long crop rotation cycles favors soil fauna diversity under tropical and temperate conditions. Faunal responses to agricultural practices in northern latitudes may, however, differ from those in southern latitudes due to different soil types, climatic conditions, and intensity of management. We investigated the abundance and diversity of soil fauna communities (nematodes, microarthropods, enchytraeids and earthworms) in a boreal sandy soil in replicated organically and conventionally cultivated field blocks that simulated cultivation in a cereal farm and in a forage producing dairy farm. In addition to traditional methods, we investigated nematode diversity using DNA metabarcoding. The results show that organic production system did not unequivocally result in higher abundance and diversity of soil fauna compared with conventional production systems, neither in the cereal nor the dairy farming type. Instead, abundances of all soil fauna groups were typically higher in the dairy than in the cereal farming type irrespective of production system. This holds up for all functional groups of nematodes except omnivores, all four orders of Acari present, two out of the six families of Collembola, the total number of enchytraeids and density of earthworms. Nematode diversity, measured as average ZOTU number, was in conventionally cultivated cereal farming type an order of magnitude lower than in other production and farming type combinations. Of the various differences between farming types, the crop species was the most important one as it defines the management practice and thus the soil conditions where soil biota lives. Our findings underline that when comparing soil faunal communities under organic and conventional cultivation, the differences between farming types need to be considered in addition to the production system as they can have an overriding importance across the food web.

1. Introduction

The ongoing carbon and biodiversity losses from agricultural soils are closely linked to intensive land use (Díaz et al., 2019; Heikkinen et al., 2013; Heikkinen et al., 2022). Arable management using heavy field machinery, frequent and intensive tillage and little or no crop rotation, together with the use of synthetic fertilizers and chemical plant protection, reduces the abundance and diversity of soil biota (Christel et al., 2021; Tsiafouli et al., 2015). This can harm the functioning of arable systems where soil decomposer communities with high diversity of interacting organisms form the basis of a healthy, functional soil ecosystem (Beare et al., 1992; Mikola et al., 2002). The soil decomposer web implements key soil functions by breaking down dead organic material, mobilizing nutrients for plant uptake, stimulating microbial activity and improving soil structure and aggregation, thereby supporting efficient primary production (Bardgett and van der Putten, 2014; van Groenigen et al., 2014).

While soil bacteria and fungi are largely responsible for organic matter decomposition and nutrient transformation, soil animals, such as nematodes, microarthropods, enchytraeids and earthworms, belonging

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to various trophic and functional groups within the soil food web, have a fundamental role in controlling soil process rates (Mikola and Setälä, 1998; Sulkava et al., 1996; Woods et al., 1982). In general, the intensification of arable land-use results in decreased soil biota diversity in terms of functional groups and species communities (Tsiafouli et al., 2015) but the degree to which this fauna responds to environmental perturbations varies (Christel et al., 2021). For example, plowing can be detrimental to soil macrofauna such as earthworms (Briones and Schmidt, 2017; Wardle, 1995), while soil microfauna, such as nematodes, are far less responsive to soil physical disturbances (Tsiafouli et al., 2015).

The assessment of the abundance, diversity and activity of the soil biota permits evaluation of productivity, sustainability and environmental impact of different farming systems. A highly topical issue is how different regenerative practices, which widely interpreted include also organic farming, succeed in engaging soil faunal communities in transition from less sustainable management. In their research synthesis, Bengtsson et al. (2005) reported positive effects of organic farming on diversity and abundance of soil microarthropods and earthworms. In addition, according to Ilieva-Makulec et al. (2017), autumn nematode abundances in sandy soils were higher in organic farming than in conventional farming, while values measured in clayey soils or in the spring showed a reverse pattern. A recent comprehensive meta-analysis by Christel et al. (2021) assessed the impact of various farming systems on soil biodiversity and functioning. They suggested that various soil biological indicators (abundance, diversity and activity parameters of soil organisms) can be improved, on average, by 70 % under organic farming relative to conventional farming. Organic fertilization and long crop rotation cycles were the most favorable practices for soil fauna, whereas the use of pesticides and soil tillage were the most deleterious (Christel et al., 2021). However, those data did not represent soil fauna in boreal agroecosystems.

Faunal responses to agricultural practices in northern latitudes may differ from those in southern latitudes due to the climatic conditions, such as low temperatures, the short growing season, cold winters with frosts and snow cover (Mela, 1996). In addition, common northern soils (FAO System of Soil Classification) are Podzols, Retisols, Cambisols, Histosols, Cryosols, and Andosols, reflecting the climates under which they evolved and making them unique in terms of utility and adaptation for agriculture (Jenny, 1941). Due to the climatic conditions and soil characteristics, agriculture in boreal region is typically of low-intensity and the number of crops is restricted mainly to annual species (Wiréhn, 2018). Global data syntheses also reveal clear latitudinal gradients in the characteristics of soil faunal communities (Phillips et al., 2019; van Den Hoogen et al., 2019).

To our knowledge, there are only a few studies on the influence of organic production on biota in boreal agroecosystems, and only two of them focus on the impacts on soil fauna. Palojärvi et al. (2002) reported no consistent differences in earthworm, enchytraeid, nematode or microarthropod abundances between adjacent organic and conventionally cropped fields. In an experimental field study, Nuutinen and Haukka (1990) did not find clear differences in earthworm species composition in organic and conventional cropping systems, although the highest abundances of earthworms were detected in organically cultivated vetch ley. Both these studies reported high variation in observed soil fauna groups between the sampling years and experimental fields.

The high taxonomic and functional diversity of soil fauna poses notable challenges for the study of arable soil communities and their responses to different managements. While in some faunal groups the identification of species and their assignment to ecologically relevant categories may be relatively easily established based on macroscopical characteristics, as for instance in earthworms (Bottinelli et al., 2020), in other groups this is much more challenging. For example, nematodes exemplify a diverse and abundant soil microfauna group. They occupy all major trophic levels of the soil food web and are classified into five trophic groups: bacterivores, fungivores, herbivores, omnivores and predators. Because of their essential roles in processing organic nutrients and controlling soil microorganism populations (Crowther et al., 2011; Ferris, 2010), nematodes are widely used as soil indicators (Cardoso et al., 2013). Traditional identification of soil nematodes by microscopy is laborious and expensive and needs a large volume of starting material and a high level of expertise. In this study we utilised both traditional microscopy and DNA-based methods to identify nematodes, to get a more comprehensive and un-biased overview of the focal nematode communities. Most often nematodes are first isolated from other organisms in a large volume sample and only then is DNA extracted from the biomass (Ahmed et al., 2019; Waeyenberge et al., 2019). There have been, however, some attempts to extract nematode DNA directly from environmental samples (e.g., Sapkota and Nicolaisen, 2015; Sikder et al., 2020). Indeed, DNA-based metabarcoding methods are emerging as a promising cost-effective alternative for identification of small fauna, such as nematodes (Oliverio et al., 2018). In addition, it is often possible to achieve higher taxonomic resolution via DNA methodology, compared to morphological approaches.

In this study, we investigated the abundance and diversity of the soil faunal community in organic and conventionally cultivated field soils in a field experiment that simulated cultivation in a cereal farm and a forage-producing dairy farm. It is noteworthy that the division of production systems into conventional and organic management is not an all-embracing division. Management practices vary substantially also between, for example, cereal and forage/dairy farms. In cereal farms, intensive tilling, plant protection products and fertilization are used more frequently than in forage/dairy farms, which may resemble – irrespective of their organic vs. conventional status – organic management due to ley rotation and application of animal manures. For soil fauna, farming type may therefore be more important than the production system, but we are not aware that this would have been addressed in earlier studies. Along with the traditional methods, we investigated nematode diversity using molecular approach on a small volume of soil using two different primer sets targeting the nematode-specific 18S rRNA region. We hypothesized that (1) organic management enhances soil fauna abundance and biodiversity relative to conventional management, especially in cereal farming, (2) forage (dairy) systems with higher cattle slurry application produce higher soil fauna abundance than cereal farming systems with synthetic fertilizers and/or low manure application, and that (3) the DNA-metabarcoding method is a promising complementary tool for identification, especially of small bacterivore and fungivore nematodes.

2. Material and methods

2.1. Experimental site

The experimental field (2.6 ha) was located on sandy soil in Toholampi; Ostrobothnia, western Finland (63.49′N, 24.09′E). The field was originally established for erosion and nutrient leaching studies in 1998 (Turtola and Kemppainen, 1998). In 2001, four different crop rotation treatments (plot size 100 × 16 m) with four replicates were established in the field according to a randomized complete block design. The main aim was to compare conventional and organic production systems between cereal and dairy farming types operating a four-year crop rotation. The four treatments were: 1) Organic cereal crop rotation (OCer), 2) Conventional cereal crop rotation (CCer), 3) Organic forage crop rotation at dairy farm (ODairy) and 4) Conventional forage crop rotation at dairy farm (CDairy) (Supplementary Fig. S1 and Fig. S2). In the organic treatments, the farming practices were designed to meet the EU requirements for organic farming in terms of crop rotation, fertilization and plant protection (2018/848/EC). Thus, the fertilization was based on biological nitrogen fixation (BNF) of legumes and use of cattle slurry; plant protection was conducted proactively using crop rotation and tillage. Tilling practices in differentially treated plots varied, being most intensive in CCer followed by OCer (Supplementary Table S1). Slope of

the experimental plots is on average 0.54 % (0.30–0.74 %) and slope across all plots on average 1.1 %. Plots are isolated from each other and from the surroundings with 0.3 m high ridges and with 1.5 m deep plastic sheetings (for more details see Turtola and Kemppainen, 1998; Peltoniemi et al., 2021).

CCer was fertilized using synthetic fertilizers according to the Agri-Environmental Program in Finland (AEP), being approximately 86 kg ha^{-1} per year total nitrogen (Tot-N). At OCer, manure was provided by a dairy farm in return for silage. During 2001–2020, OCer received 50 kg Tot-N ha^{-1} per year in cattle slurry, applied in the first and last year of the four-year crop rotation (the most recent application prior sampling in spring 2017). CDairy was fertilized with slurry using an application rate of 110 kg Tot- N ha^{-1} per year. Annual fertilization was complemented with synthetic fertilizers according to AEP and was about 61 Tot-N kg ha^{-1} per year. In terms of fertilization, ODairy was self-sufficient so that the crop rotation fodder to feed the cows and the slurry produced by them was used as organic fertilizer. Cattle slurry application rate in ODairy during 2001–2020 was, on average, 85 kg Tot-N ha^{-1} per year. Fertilization practices during the experimental years (2001−2020) are presented in Supplementary Tables S1 and S2.

Within the four-year crop rotations, the crop sequence was as follows: 1) CCer: barley (*Hordeum vulgare* L.) for two successive years, rye (*Secale cereale* L.) and oats (*Avena sativa* L.), 2) OCer: barley with undersown ley seed, ley, rye and oats 3) CDairy: barley with undersown ley seed, ley, ley and barley and 4) ODairy: barley with undersown ley seed, ley, ley, and mixture of oats and common vetch (*Vicia sativa* L). In conventional treatments, ley was a mixture of timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.), while in organic treatments a mixture of timothy and red clover (*Trifolium pratense* L.) was used. In the autumn of 2018 crop rotations were modified. Two-year-old leys were prolonged to three-year-old leys in dairy farms crop rotations and rye was excluded from cereal farms crop rotations. Rye was replaced by ley in OCer and oats in CCer rotation. Thus, the main sampling year 2020 of the present study cultivated plants were oats for CCer and OCer, leys for CDairy and ODairy (Supplementary Table S1).

Soil carbon content, pH and other soil characteristics of the treatments (0–20 cm layer) were previously reported by Peltoniemi et al. (2021). In general, no clear differences existed in soil pH between the treatments. Instead, soil C was higher in systems receiving manure than in CCer receiving synthetic fertilizers, being 3.95 % in CCer without manure application, 4.34 % in OCer receiving manure twice during a four-year cycle, 4.62 % in ODairy with annual manure application and 5.11 % CDairy with the highest manure application. In addition, field plots under the conventional systems (CCer, CDairy) received plant protection agents for weed control (Supplementary Table S1 and S3).

2.2. Sampling of soil fauna

2.2.1. Earthworms

Earthworms were sampled in October 2018 from three sites per experimental plot. The sampling sites were positioned systematically at the plot centerline so that the distance to plot margin was 15 m from the outermost sites. Sampling was done using combined soil-hand sorting and mustard oil (allyl-isothiocyanate, AITC) extraction (ISO 23611-1:2018; Nuutinen, 2019). For hand-sorting, a soil block was taken using a spade from an area of 25 cm × 25 cm and at a depth of 20 cm and the earthworms were hand-sorted from the samples in the field. Chemical extraction was done simultaneously by pouring AITC solution at the bottom of the sampling pit and by collecting the emerging earthworms over a period of 25 min. Because of the low permeability of the soil, usually only a couple of liters of solution was applied. During the sampling, the moisture content of the topsoil (0–15 cm) was 29–34 % (TDR; Trase System, Model no. 6050 × 1 Soil moisture Equipment Corporation, Santa Barbara, California, USA) and the temperature at 0–7 cm depth was +6.0 − +7.5 °C. Earthworms were active in the topsoil and no aestivating individuals were encountered. The specimens were preserved in 4 % formaldehyde in the field and after a 13-week storage at +6 °C the worms were transferred to 70 % ethanol. Earthworm individuals were identified to species or genus level according to Sims and Gerard (1999) and their fresh mass (with gut contents) was determined. In the case of incomplete specimens, only head pieces were used in the estimation of earthworm total density, while all specimens were used in the estimation of fresh mass. Results are expressed per m^2.

2.2.2. Enchytraeids and nematodes

To analyze the treatment effects on soil enchytraeids and nematodes, two randomly placed soil cores (depth 10 cm, diameter 6 cm) were collected from three randomly selected sites in each plot in September in 2020. Thereafter, two soil cores collected from the same site were pooled and carefully mixed to attain three estimates per plot and stored in the fridge (+4 °C) and one soil core per site was used for microarthropod extractions (see Section 2.2.3). After 7–14 days from sampling, enchytraeids were extracted from 80 g and nematodes from 10 g of fresh, non-sieved soil using the wet funnel methods of O'Connor (1955) and Sohlenius (1979), respectively. The number of nematodes was recorded, and their feeding/trophic groups were identified according to Yeates et al. (1993). To further examine the treatment effects on the community composition of nematodes, relative proportions of trophic groups were calculated using a minimum of 50 individuals per sample. In addition, these individuals were identified to genus level. Enchytraeids were counted and classified into size classes (length < 2.0, 2.1–4.0, 4.1–6.0, 6.1–8.0, 8.1–10, 10.1–12 or >12 mm) and their biomass was calculated according to Abrahamsen (1973).

In addition, for the analysis of nematode DNA, six sites were randomly selected in each plot and six cores (depth 10 cm, diameter 1.9 cm) were collected around them. The samples were pooled and mixed to achieve six samples per plot and 50 ml sub-samples were stored frozen (−20 °C) until analysis. Detailed description of the DNA extraction protocol, concentration and purification measurements, library preparation, sequencing and bioinformatics can be found in the Supplementary Text S1. Briefly, nematodes were analyzed from DNA extracted from approximately 250 mg of the 96 sub-samples with the DNeasy PowerSoil Pro Kit (Qiaqen, Germany) according to the protocol of the manufacturer. Library preparations, sequencing and bioinformatics for raw sequence data were conducted in the facilities provided by Bioname company (Turku, Finland; www.bioname.fi). Two different primer sets targeting Nematoda 18S rRNA gene were used: MMS primers, MMSF (GGTGCCAGCAGCCGCGGTA) and MMSR (CTTTAAGTTTCAGCTTTGC) for v6-v8 region (Sikder et al., 2020), and NEM primers, NemF (GGGGAAGTATGGTTGCAAA) and 18Sr2b (TACAAAGGGCAGGGACGTAAT) for v4-v5 region (Porazinska et al., 2009; Sapkota and Nicolaisen, 2015). Library preparation followed Vesterinen et al. (2016) with minor modifications. Sequencing was done on an Illumina MiSeq PE 2 × 300 v3 (Illumina Inc., San Diego, California, USA) by the Turku Centre for Biotechnology, Turku, Finland. Bioinformatics pipeline was followed closely Kaunisto et al. (2020). Reads were clustered into ZOTUs ("ZOTU" = 'zero-radius OTU'). The sequence variants were assigned to taxa using GenBank BLAST tool against the nucleotide nt database (Altschul et al., 1990). Sequence data obtained with both primers were combined and relative abundances of ZOTU reads per sample library were used for analyses. Average relative abundances of ZOTU reads and their taxonomic affiliations per sample library type are shown in Supplementary Table S4. The sequence data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB52179 (https://www.ebi.ac.uk/ena/browser/view/PRJEB52179).

2.2.3. Microarthropods

Microarthropods were extracted simultaneously from the same sites as for enchytraeids and nematodes, but the samples were not homogenized and only the topmost 5 cm layer (including the litter layer) was used. The samples were stored in the fridge at (+4 °C). Seven days after

sampling, microarthropods were extracted from ca. 80 g (fresh) soil, using a fully automated High Gradient extractor by MacFayden (1961) with a 5-day extraction time, having 10 °C increase over every 24 h (during 5 d from 16 °C to 56 °C). Then the faunas were counted and identified as mites (Acari; classified to suborders Oribatida, Mesostigmata, Prostigmata, Astigmata and further to family level) and springtails (Collembola; to suborder or family level; Arthropleona (including the families Hypogastruridae, Poduridae, Onychiuridae), Entomobryomorpha (family: Entomobryidae and Isotomidae) and Symphypleona (family: Sminthuridae).

Nematode, microarthropod and enchytraeid abundances are expressed per g of dry soil. Water content of the soil samples was determined by weighing subsamples before and after drying in an oven (105 °C) for 24 h.

2.3. Statistical analyses

For statistical analyses, mean values (abundance and biomass of enchytraeids, abundance and relative proportions of nematode trophic groups, abundance of microarthropods, total density and biomass of earthworms and density of *Lumbricus rubellus* and *Aporrectodea caliginosa*) per plot were used. For each response variable, the homogeneity of variances and normality of model residuals were tested using Levene's test and Shapiro-Wilk test, respectively. The effects of production system (organic vs. conventional) and farming type (cereal vs. dairy) on the abundance of soil fauna were tested using multivariate ANOVA with treatment as explanatory factor and block as random variable. To meet the assumptions of ANOVA, abundancies of soil nematodes and *L. rubellus* density were lg-transformed. If significant interactions between the production systems and farming type were found, the treatment effects were analyzed separately for each treatment (earthworm abundance, biomass and *A. caliginosa* density). Data for the abundance of soil microarthropods were not normally distributed even after data transformation. Thus, the treatment effects were tested using non-parametric two-way Kruskall-Wallis. The spatial dependencies inside the field were not analyzed. However, due to the randomized complete block design, hydrological isolation of the plots, and 16 m width of individual treatment plots (stripes) it is unlikely that there has been spatial autocorrelation between plots with the same treatment. These statistical analyses were carried out using the *SPSS* statistical package (IBM Corp, 2016).

In addition, nematode ZOTU numbers (richness) between the farming types were investigated with the function glmer (R package *lmerTest*) producing a generalized linear mixed model that considered the impact of the block design (Kuznetsova et al., 2017). The significance of the linear model was tested with type III analysis of variance with the ANOVA function. Pairwise analyses and significant differences for farming types were tested with the lsmeans function (R package *lsmeans*) (alpha $p < 0.05$) (Length, 2016). To study the effect of farming type on the proportions of sequences affiliated to six different nematode trophic groups (bacterivores, fungivores, herbivores, omnivores, predators, unknown) we used permutational multivariate analysis of variance with the adonis function from the *vegan* package (Oksanen et al., 2020) with treatment as an explanatory factor and block as a random (strata) factor (Anderson, 2001). Data analyses for DNA-based data were conducted in R studio version 7.1.554 (RStudio Team, 2022) and R Stastical Software version 4.2.1. (RCore Team, 2022).

In addition, hierarchical cluster analysis by K-means algorithm (MacQueen, 1967) was conducted using R Statistical Software (version 1.3.959) for the detection of the similarity within the dataset, including nematodes, enchytraeids and microarthropods. Optimal number of clusters in the data was determined using the elbow method. K-means positioned the data by measuring the distance of the sample to the center of the clusters determined with the elbow method.

3. Results

3.1. Earthworms

There was a significant interaction between production system and farming type in earthworm abundance with significantly higher earthworm density and fresh mass in organic than in conventional production systems but only in cereal farming type (Fig. 1a, Supplementary Table S5). In conventional production systems earthworm density and fresh mass were in dairy farming type higher than in cereal type. When species were analyzed separately neither the density of *A. caliginosa* (the clearly dominant species in all treatments) or L. *rubellus* were discernibly affected by production system or farming type (Supplementary Table S5). Two additional species found, *Lumbricus terrestris* and *Aporrectodea rosea*, were both present only in one sample.

3.2. Enchytraieids and nematodes

Enchytraeid abundance and biomass were strongly affected by farming type, their abundance being five times and biomass 2.7 times higher in the dairy than in the cereal farming type, respectively. Mean enchytraeid abundance and biomass did not differ statistically significantly between organic and conventional production systems (Fig. 1b, Supplementary Table S5).

Farming type had a significant effect on total nematode abundance which was 2.7–9.5 times higher in the dairy than in the cereal farming type. When analyzing the trophic groups separately, abundance of bacterivores, fungivores and herbivores were several times higher in dairy than cereal farming types, while no clear effects on the omnivores were detected (Fig. 2, Supplementary Table S6). Only two predatory nematodes were found in the samples. However, there was a significant interaction between production system and farming type for herbivorous nematodes that were more abundant in conventional than organic production systems, but only for the dairy farming type. Production system or farming type had no effect on relative abundances of nematode trophic groups (Supplementary Table S7). There was no significant difference between the farming types or production systems at the nematode genus level in the data based on morphological identification (Supplementary Table S8).

Sequence reads that were affiliated to nematodes were detected from 47 out of 96 samples, and 91 ZOTUs (83.3 % of all the sequence reads) from a total of 120 ZOTUs affiliated to Nematoda (Supplementary Table S4 and S8). Altogether, 87 % of all identified nematode ZOTUs were identified at genus level and 42 % at species level. The rest of the sequence reads belonged to ZOTUs that were affiliated with other phyla: Annelida (8 ZOTUs; 14.25 % of reads), Cercozoa (13 ZOTUs; 1.14 % of reads), Arthropoda (4 ZOTUs; 0.63 % of reads), Endomyxa (3 ZOTUs; 0.65 % of reads) and Apicomplexa (1 ZOTU; 0.03 % of reads). The most dominant nematode ZOTU obtained from all four treatments was affiliated with *Paratylenchus* cf. *neoamblicephalus*. Nematode richness, assessed by ZOTU reads, was significantly lower in CCer rotation compared with other three rotations (OCer, CDairy and ODairy) and higher in OCer rotation compared with ODairy rotation (Table 1). There was no difference in nematode richness between CDairy and ODairy rotations. The proportions of sequences from the nematode trophic groups did not differ between treatments. The result implies that sequences of many trophic groups were not detected in some or only in a few replicate rotation blocks, causing substantial variation in the data. However, sequences of omnivores or predators were not obtained from CCer rotation, and there was an increasing trend in proportion of sequences from the fungivores in OCer rotation (Table 1).

From the 40 identified nematode genera identified with the DNA-method, 13 of those were also detected by microscopy (Supplementary Table S8). Six of them were bacterivores (*Acrobeloides, Alaimus, Cervidellus, Eucephalobus, Plectus, Rhabditis*), four herbivores (*Aphelenchoides*, *Paratylenchus*, *Pratylenchus, Sauertylenchus*), two fungivores

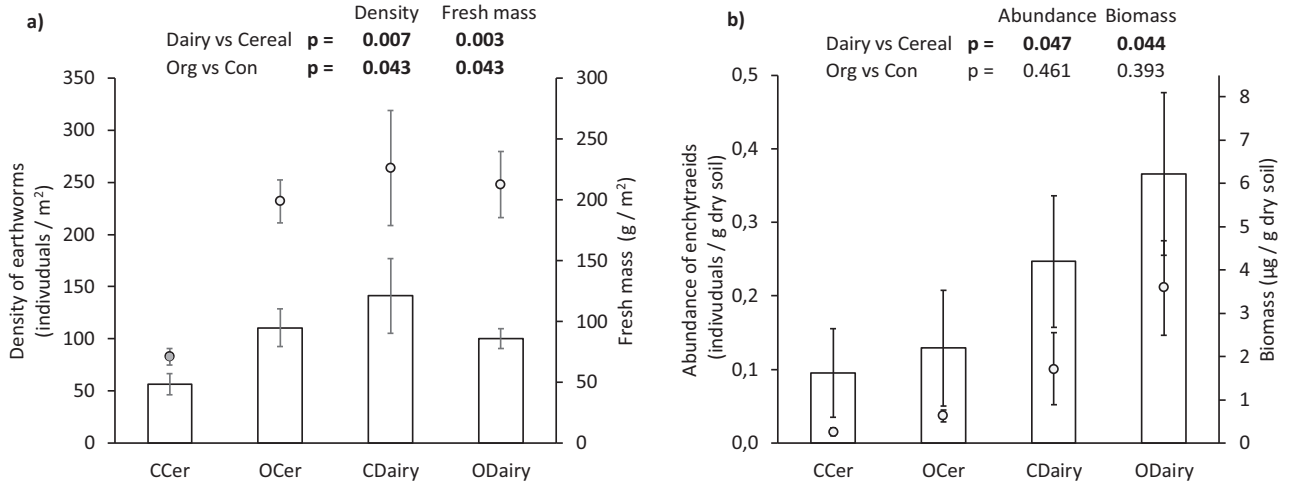

Fig. 1. a) Mean (±se) density (circles) and fresh mass (bars) of earthworms and b) abundance (circle) and biomass (bars) of enchytraeids in the in the field plots with four different crop rotation treatments: 1) Conventional cereal farm (CCer), 2) Organic cereal farm (OCer), 3) Conventional dairy farm (CDairy) and 4) Organic dairy farm (ODairy) ($n = 4$). Statistical significance (p-value: multivariate ANOVA) of production system and farming type on response variables is marked above figures. For further statistical comparison of treatments and their interactions, see Supplementary Table S5.

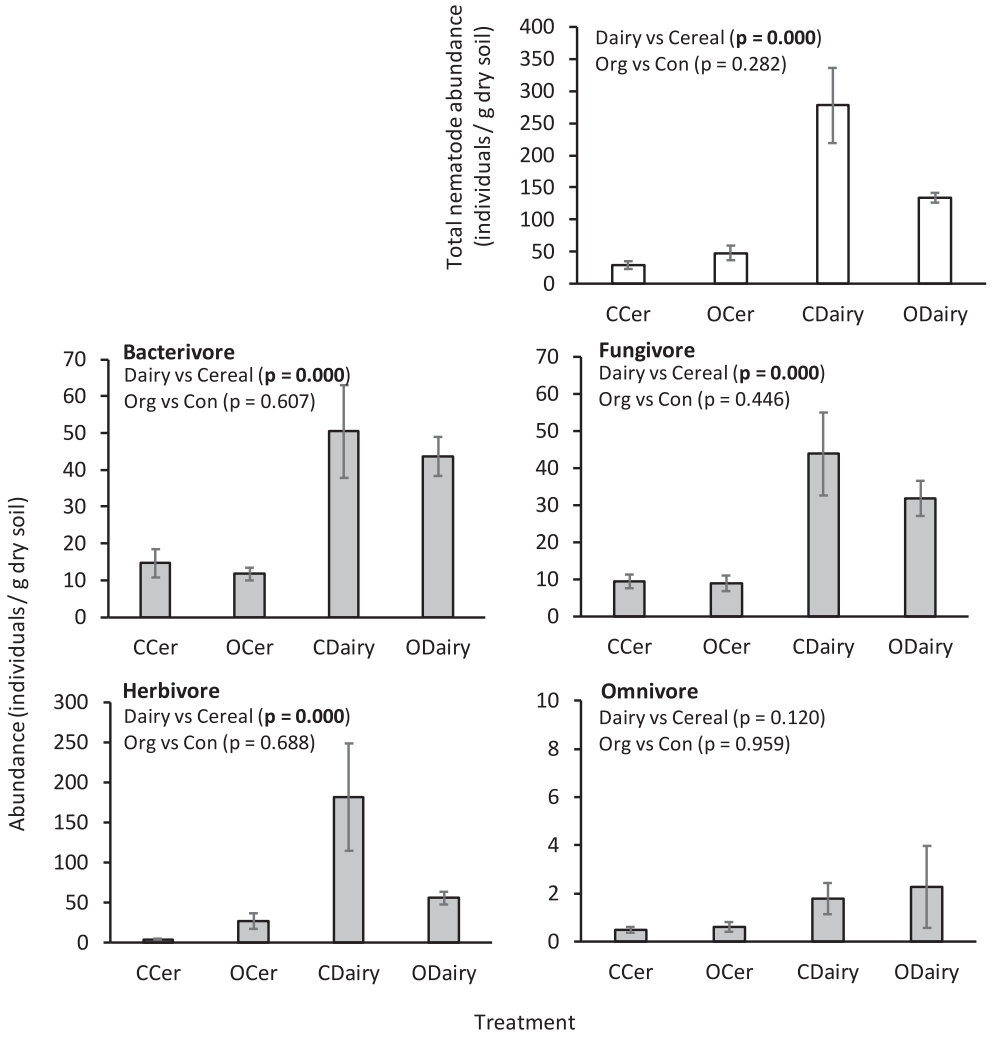

Fig. 2. Total abundance (the uppermost panel) and abundance of nematode trophic groups (mean + s.e., $n = 4$) identified by microscopy in the field plots with four different crop rotation treatments 1) Organic crop rotation producing cereals (OCer), 2) Conventional cereal crop rotation (CCer), 3) Organic crop rotation of the milk production farm (ODairy) and 4) Conventional forage crop rotation of a dairy farm (CDairy) in 2020. Statistical comparison of treatments, i. e. production systems (Organic vs. Conventional) and farming type (Dairy vs. Cereal) see Supplementary Table S6.

Table 1
The average nematode ZOTU numbers (richness) (mean + s.e.), and the proportions (%) of different nematode trophic groups in the field plots with four different crop rotations, organic cereal farm (OCer, $n = 9$), conventional cereal farm (CCer $n = 12$), organic dairy farm (ODairy, n = 12) and 4) conventional dairy farm (CDairy, $n = 14$) in 2020. Letters show significant differences between rotations.

Rotation	ZOTU#	Bacterivore	Fungivore	Herbivore	Omnivore	Predator	Unknown
CCer	1.4 (0.2)a	23 (18)	1 (1.5)	27 (20)	0	0	49 (32)
OCer	16 (6)b	11 (7)	10 (6)	69 (40)	1 (0.7)	0.2 (0.1)	7 (4)
CDairy	14 (7)bc	23 (14)	3 (2)	63 (36)	2 (1.2)	0.3 (0.2)	9 (5)
ODairy	13 (5)c	22 (13)	5 (3)	51 (30)	8 (5)	0.1 (0.1)	13 (8)

(*Aphelenchus, Ditylenchus*), and one omnivore (*Aporcelaimellus*). Some of these genera were identified at species level, such as *Acrobeloides apiculatus* and *A. buetschii*, *Aphelenchus avenae*, *Eucephalobus striatus* and *Pratylenchus pratensis*. However, most nematode genera detected with the DNA method were not identified microscopically and, in contrast, 11 genera that were not identified by DNA methods were observed microscopically, including four omnivores and one predator. A genus identified as *Tylenchus* by microscopy was identified as *Malenchus* and *Filenchus* by the DNA method. These genera belong to the family Tylenchida and the reason for this discrepancy likely stems from the identification keys used.

3.3. Microarthropods

All investigated mite suborders (Mesostigmata, Oribatida, Astigmata and Prostigmata) were more abundant in dairy than cereal farming types (Fig. 3, Table 2), resulting in 5.5–7.0 times higher total abundance of mites in dairy than cereal farming type. Similarly, the total number of springtails was 9.2–17.5 higher in dairy than cereal farming types, mostly due to the high abundancies of individuals belonging to families of Isotomidae and Onychiuridae. No differences in the abundance or number of suborders/families of mites or springtails were found between conventional and organic production systems (Fig. 3, Table 2).

Also, the number of microarthropod families was >40 % higher under dairy than cereal farming types (Supplementary Table S9). In terms of numbers of Acari families per farming type, ODairy was associated with, on average, 11.5 microarthropod species (mites and springtails) per sample, in contrast to 12.3 in CDairy. Corresponding values in OCer and CCer were 6.8 and 5.3 species per sample, i.e. no differences existed in the numbers of microarthropod families per sample between organic and conventional production systems ($p > 0.05$). Markedly more Oribatid families (10) were present in dairy than cereal farming types (2) (Supplementary Table S9).

The relative proportion of Acari suborders varied between treatments. In the cereal farming types, the relative proportion of Astigmatid mites was much higher (52–85 %) compared with dairy farming (13 %–25 %) ($p < 0.05$), whereas dairy farming types were dominated by Mesostigmatid mites (Supplementary Fig. S3). There were also differences between organic and conventional production systems: the relative proportion of Prostigmatids was higher in organic than conventional plots and in cereal production Astigmatids were lower in organic than in conventional plots (Supplementary Fig. S3, Supplementary Table S10). The mite/collembolan ratio was higher (3.5) in conventional cereal treatment (CCer) compared with OCer (1.4), CDairy (1.8), and ODairy (2.2).

3.4. Communities

The K-means analysis based on combined nematode, microarthropod and enchytraeid data grouped the experimental plots into four clusters. Cluster 1 consists of two OCer plots originating from the two individual samples with predatory nematodes. Cluster 2 contains nine CDairy and three ODairy plots with high diversity and large numbers of herbivorous nematodes; no cereal plots are present in this cluster. Cluster 3 contains nine ODairy and two CDairy plots with high diversity, but less dense population of herbivorous nematodes; there were no cereal plots in this cluster. Cluster 4 comprises 22 plots, of which 21 were cereal and had low mite abundance. ODairy (plot 11), also with a very low number of mites, was included in this cluster (Fig. 4).

4. Discussion

Our first hypothesis was disproved as the organic production system did not unequivocally result in higher abundance and diversity of soil fauna compared with the conventional management systems, neither in the cereal nor dairy farming type. We further hypothesized that dairy farming involving manure application would result in markedly higher soil fauna abundance compared with cereal farming. This hypothesis was supported: abundancies of all investigated soil fauna groups (nematodes, enchytraeids, earthworms and microarthropods) were higher in dairy than in cereal farming types. In addition, the DNA-metabarcoding methods allowed identification of small bacterivore and fungivore nematodes but were not optimal for larger omnivores and predators, as was expected in the third hypothesis.

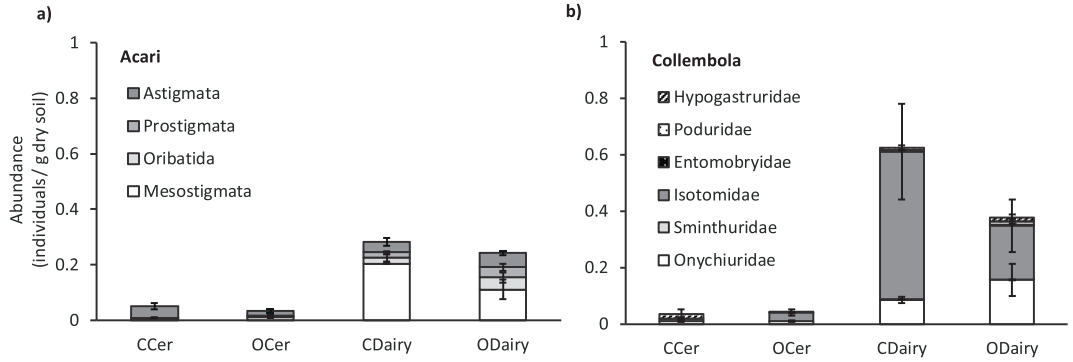

Fig. 3. Total abundance of A) mites and B) springtails trophic groups (mean + s.e., n = 4) in the field plots with four different crop rotation treatments: 1) Organic crop rotation producing cereals (OCer), 2) Conventional cereal crop rotation (CCer), 3) Organic crop rotation of a dairy farm (ODairy) and 4) Conventional forage crop rotation of a dairy farm (CDairy) in 2020. Statistical comparison of the treatments (i.e. production systems Organic vs. Conventional), see Table 2.

Table 2
F, H and P statistics of two-way Kruskal-Wallis of the effects of treatments: 1) production systems (Organic vs. Conventional) and 2) farming type (Dairy vs. Cereal) on soil acari and collembola abundance.

	Variable	Effect	df	F	H	Sig	Treatment effect
Acari	Mesostigmata	Dairy vs Cereal	1	45.176	11.294	**<0.01**	**Cer < Dairy**
		Organic vs Conventional	1	0.000	0.000	>0.05	Con = Org
		Dairy vs Cereal x Organic vs Conventional	1	2.824	0.706	>0.05	
	Oribatida	Dairy vs Cereal	1	54.751	8.040	**<0.01**	**Cer < Dairy**
		Organic vs Conventional	1	0.207	0.031	>0.05	Con = Org
		Dairy vs Cereal x Organic vs Conventional	1	3.013	0.442	>0.05	
	Prostigmata	Dairy vs Cereal	1	52.563	10.260	**<0.01**	**Cer < Dairy**
		Organic vs Conventional	1	0.004	0.001	>0.05	Con = Org
		Dairy vs Cereal x Organic vs Conventional	1	3.394	0.662	>0.05	
	Astigmata	Dairy vs Cereal	1	45.176	11.294	**<0.01**	**Cer < Dairy**
		Organic vs Conventional	1	0.000	0.000	>0.05	Con = Org
		Dairy vs Cereal x Organic vs Conventional	1	2.824	0.706	>0.05	
	Total	Dairy vs Cereal	1	22.668	22.667	**<0.01**	**Cer < Dairy**
		Organic vs Conventional	1	0.000	0.000	>0.05	Con = Org
		Dairy vs Cereal x Organic vs Conventional	1	3.000	3.000	>0.05	
Collembola	Onyciuridae	Dairy vs Cereal	1	40.689	11.294	**<0.01**	**Cer < Dairy**
		Organic vs Conventional	1	0.993	0.276	>0.05	Con = Org
		Dairy vs Cereal x Organic vs Conventional	1	0.358	0.099	>0.05	
	Sminthuridae	Dairy vs Cereal	1	1.919	0.065	>0.05	Cer = Dairy
		Organic vs Conventional	1	0.081	0.003	>0.05	Con = Org
		Dairy vs Cereal x Organic vs Conventional	1	0.081	0.003	>0.05	
	Isotomidae	Dairy vs Cereal	1	57.421	11.294	**<0.01**	**Cer < Dairy**
		Organic vs Conventional	1	0.056	0.011	>0.05	Con = Org
		Dairy vs Cereal x Organic vs Conventional	1	6.785	1.335	>0.05	
	Entomobryidae	Dairy vs Cereal	1	1.000	0.010	>0.05	Cer = Dairy
		Organic vs Conventional	1	1.000	0.010	>0.05	Con = Org
		Dairy vs Cereal x Organic vs Conventional	1	1.000	0.010	>0.05	
	Poduridae	Dairy vs Cereal	1	9.005	1.148	>0.05	Cer = Dairy
		Organic vs Conventional	1	0.031	0.004	>0.05	Con = Org
		Dairy vs Cereal x Organic vs Conventional	1	0.031	0.004	>0.05	
	Hypogarstruidae	Dairy vs Cereal	1	0.045	0.010	>0.05	Cer = Dairy
		Organic vs Conventional	1	0.000	0.000	>0.05	Con = Org
		Dairy vs Cereal x Organic vs Conventional	1	0.436	0.096	>0.05	
	Total	Dairy vs Cereal	1	45.176	22.667	**<0.01**	**Cer < Dairy**
		Organic vs Conventional	1	0.000	0.000	>0.05	Con = Org
		Dairy vs Cereal x Organic vs Conventional	1	2.824	0.706	>0.05	

Statistically significant treatment effect ($p < 0.05$) marked in bold.

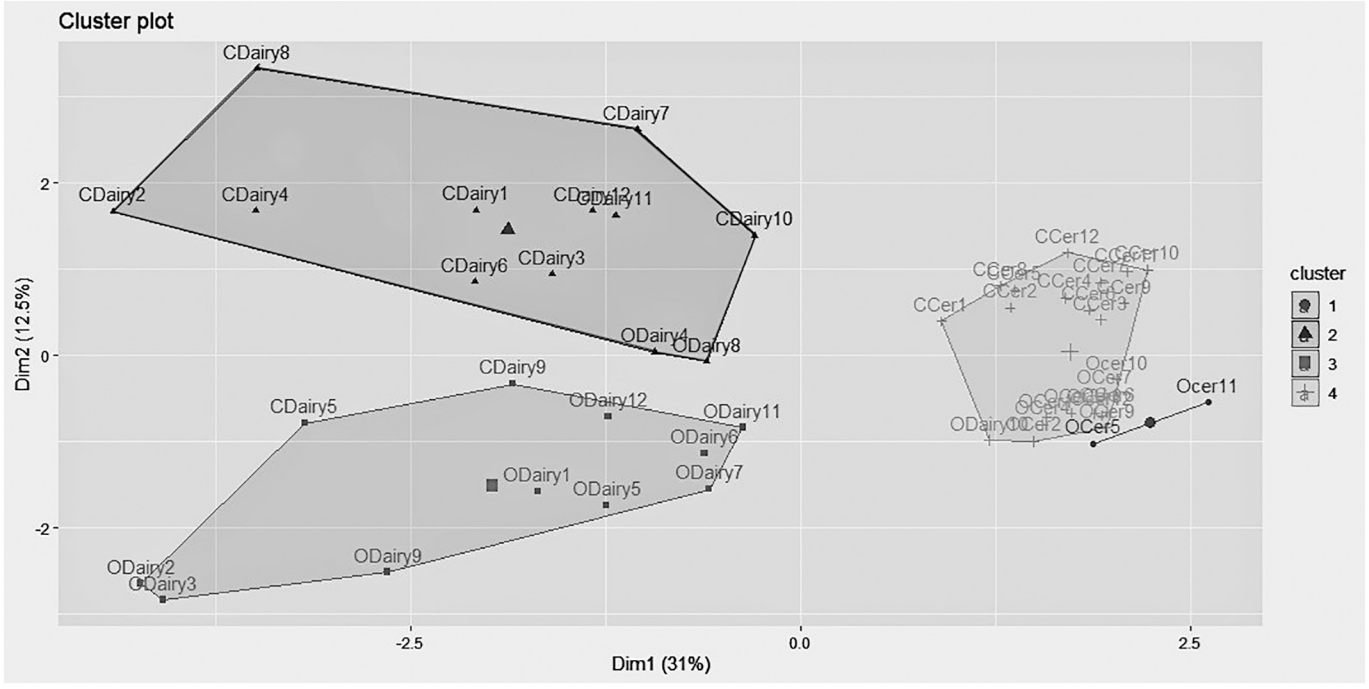

Fig. 4. K clusters graphically illustrate the results of the K-means clustering performed by the R packages.

Organic production requires a functional soil decomposer web to maintain soil fertility (Birkhofer et al., 2008). Management practices typical to organic production systems, such as the use of organic fertilizers and a diverse crop rotation, have shown beneficial impacts on soil organisms in temperate, subtropical and tropical climate zones (Christel et al., 2021; Mantoni et al., 2021; Quist et al., 2016). Therefore, we expected that they would likewise increase soil faunal abundance in boreal conditions. However, of all the soil faunal groups studied, only the total density and fresh mass of earthworms were in cereal farming higher in organic than in conventional production systems. This finding is parallel with earlier findings from warmer climates, according to which increased soil organic matter content associated with organic production increases earthworm abundances, likely via improved food availability (Birkhofer et al., 2008; Crittenden and de Goede, 2016).

Earthworm abundance in agricultural systems relates strongly to the degree of physical soil disturbance, with less intense tillage usually promoting growth of populations (Briones and Schmidt, 2017). Also in our study, the density of earthworms - and also enchytraeids - was the lowest in CCer with the most frequent tillage. However, no differences between the other three treatments were found despite their differences in tilling practices. Previously, Domínguez and Bedano (2016) compared the abundance of earthworms and enchytraeids of two organic, chisel ploughed fields with two conventional fields under no-till and chisel plowing. Earthworm densities were generally low but higher in organic fields compared with ploughed conventional fields. The pattern was the same for enchytraeids. In their study, earthworm abundance was exceptionally at the lowest under no-till whereas enchytraeid abundances were there at the highest. It was suggested that negative effects of plant protection products on earthworms under no-till had released enchytraeids from resource competition with earthworms and resulted in their population growth. In our study, no obvious indications for similar interactions between earthworms and enchytraeids were seen as, for example, abundance of both enchytraeids and earthworms were the lowest in conventional cereal systems. In our case the abundances of earthworms and enchytraeids were both more likely driven by tillage intensity, vegetal richness, diversity and availability of organic matter resources.

Interestingly, nematodes (microscopical identification) did not benefit from organic production, neither in cereal nor dairy farming types. The same pattern was observed for nematodes in molecular-based community analysis as the five different trophic groups did not differ between treatments. However, nematode richness measured as ZOTU numbers was higher in organic production type in the cereal farming compared with respective conventional production type. There was an indication of higher abundance of sequences affiliated to fungivorous nematodes in cereal rotation under organic production. Earlier investigations from the same site showed increased fungal abundance (ITS2 copy numbers) in the same (Peltoniemi et al., 2021) and together the findings suggests that fungi provided, at least in autumn, a good nutrient source for fungivorous nematodes. There are a few studies on the influence of the two production systems on the abundance of nematodes and their trophic groups. Birkhofer et al. (2008) observed a 33 % increase in herbivorous nematodes and a 43 % decrease in the abundance of fungivorous nematodes under organic production. According to Ilieva-Makulec et al. (2017), nematode abundances in sandy soils in the autumn were higher in organic production than in conventional production type, while values measured in clayey soils or in the spring showed a reverse pattern. The varying results can be attributed to variation in climatic conditions, soil characteristics, such as organic matter content, moisture conditions and soil type (Christel et al., 2021 and references therein).

As far as we know our study is one of the first attempts to identify nematodes from DNA extracted directly from environmental samples of boreal agricultural soil. The differences in nematode richness given by the microscopy and DNA methods may derive mainly from small nematodes such as bacterivores, the proportion of which may be overestimated by DNA methods. Furthermore, the very small volume of soil used for DNA extraction likely underestimates the presence of larger omnivorous and predatory nematodes (Dopheide et al., 2019). In addition, body size is also known to affect largely on spatial distribution of nematodes (Luan et al., 2020; Wang et al., 2022). The lack of comprehensive databases for nematodes may also have left some sequences without a proper taxonomic tag. However, molecular methods allowed identification of more nematode genera, and even species, compared with microscopy.

Soil microarthropods have been shown to react to various cultivation practices and have been regarded as plausible environmental indicators in agro-ecosystems (Mantoni et al., 2021; Menta et al., 2008; Tabaglio et al., 2009). Studies on microarthropod abundances in various production systems are scarce, but the few existing ones report higher abundance and diversity of microarthropods in organic than in conventional fields (Christel et al., 2021). In our study, no substantial differences existed between organic and conventional production systems in terms of microarthropods. However, the mite/collembola ratio (one of the indicators) was higher in conventional systems, but only in cereal farms. This finding likely relates to the higher physical soil disturbance in conventionally managed cereal soils due to more frequent tillage. The result contrasted with that of Mazzoncini et al. (2010), who reported a lower mite/collembolan ratio in an organic production system. However, in their study, the organic treatments were disturbed by frequent soil tillage for mechanical weed control.

We expected greater differences in soil fauna abundance and diversity between organic and conventional production systems in boreal agricultural soil given that Peltoniemi et al. (2021) earlier reported that, in the same experiment, the organic system was associated with higher microbial activity, biomass and richness and numbers of soil and plant health promoting microbes, compared with conventional systems. This was especially so in the cereal farming type. We therefore assumed that, irrespective of farming type, especially the higher biomass of soil microbes due to organic production would be reflected in soil fauna that feed on microbes (Lauber et al., 2013; Swift et al., 1979). As Peltoniemi et al. (2021) analyzed microbial characteristics of the plots two years before our study, linking microbial results to those of soil fauna is not straightforward. This is especially so for smaller soil fauna that react faster to varying stages of crop rotation and other environmental attributes (Tsiafouli et al., 2015). In terms of diversity patterns, the methodological differences in our analyses and those of Peltoniemi et al. (2021), and the levels of taxonomic resolution, also likely affected the results. Nevertheless, the results, suggest that the response of microbial and faunal soil communities to farming practices can vary widely.

It is known that the absence of pesticide application in organic production can promote soil fauna (e.g. Birkhofer et al., 2008). A characteristic feature of conventional agricultural production in Finland is low pesticide application rate compared with more southern regions of Europe (Wossink and Feitshans, 2016). In addition, in Finland, soil organic matter (SOM) content is typically higher than in southern Europe (de Brogniez et al., 2014). Thus, organic production induced increase in SOM may be relatively smaller in Nordic than in southern European soils. Together these local characteristics of agriculture may contribute to the relatively high similarity of faunal communities between the two production systems in our study.

As assumed in our second hypothesis, several times higher abundance (all fauna groups) and diversity of soil fauna (especially in oribatid mite families) was detected in dairy than cereal farming type soil in both organic and conventional systems. The more frequent tillage in the cereal crops may explain, at least partly, the lower soil fauna abundance and diversity in conventional cereal farming, a phenomenon which has also been observed in other studies (Christel et al., 2021) and is particularly well known in the case of earthworms (Briones and Schmidt, 2017) and oribatid mites (Bosch-Serra et al., 2014). In our study, the crops in the sampling year (2020) were oats in the conventional and organic cereal farming types and leys in the dairy farming

types. One year before the sampling, the conventional cereal farming types grew oats while organic cereal farming types and both dairy farming types grew leys. Consequently, the more diverse crop selection and continuous plant cover for the dairy farming types may also have promoted richer below-ground fauna, as reported previously by Menta (2012). Indeed, forage plant rotation has been shown to conserve biodiversity (Pellegrino et al., 2020), which may also have been the case in our study. Disentangling the effect of crop type from that of management type is, however, not possible in our case and would require further research. Comparison of yield levels between farming types and production systems is difficult as crop species varied between the treatments and years. However, average ley yield was ca. 12 % lower and barley yield ca. 24 % lower in organic than conventional dairy farming types over the years (2001–2020). Similarly, rye and oat yields were 45 % and 33 % (respectively) lower in organic than conventional cereal farming types (data not shown). It is conceivable that lower yield levels in organic systems contributed to our results but discerning its effect is also difficult.

A further difference between the farming types was the application rate of organic fertilizers, being markedly higher in the dairy than in the cereal farming type, especially in the conventional production plots. The conventional cereal farming types were fertilized only with synthetic fertilizers, and the organic cereal farming types received cattle slurry only in the first and last year of the four-year crop rotation. The amount of slurry received by the organic cereal farming type was almost eight times lower during the four-year crop rotation period compared with the dairy farming types. For earthworms, the lowest total densities and masses were measured in the conventional cereal farming type, which did not receive cattle slurry. Slurry application can have an immediate negative effect on earthworms, but the effect is transitory and in the longer term the effect can be distinctively positive (Curry, 2004; Viketoft et al., 2021). However, in our study it is difficult to disentangle the effect of organic fertilizer application from the effects of crop or cover plants species, plant protection measures or soil tillage frequency.

To conclude, in our study conducted under boreal conditions, differences in the studied soil faunal groups between organic and conventional production systems were clearly smaller than those between cereal and dairy farming types. Of the various differences between farming types, the crop species was the most important one as it defines the management practice and thus the soil conditions where soil biota lives. The results underline that farm type needs to be considered when assessing the impacts of organicproduction on the soil environment and the biota therein.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.104944.

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