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Effects of long-term fertilization with contemporary Danish human urine, composted household waste and sewage sludge on soil nematode abundance and community structure

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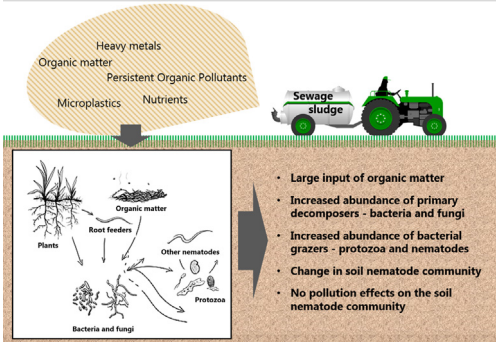
HIGHLIGHTS

- We studied soil health after long-term field fertilization with urban waste products.
- Urban waste can contain heavy metals, POP and microplastics, which causes concern.
- Abundance of most soil organisms increased in plots fertilized with urban waste.
- Urban waste fertilization altered the community structure of soil nematodes.
- We found no indications of negative pollution effects on nematode communities.

GRAPHICAL ABSTRACT



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ABSTRACT

It is desirable to recycle the urban waste products human urine, composted household waste and sewage sludge as fertilizers to agricultural fields. This could minimize the use of NPK fertilizer, improve soil structure and store carbon. However, waste products may contain heavy metals, persistent organic pollutants (POP) and plastics, and there are concerns that long-term build-up of these substances will cause unwanted effects on soil health.

Nematodes are ubiquitous and numerous in soil ecosystems. Abundance and community structure of soil nematodes can be used as indicators of soil health, as some species are vulnerable to pollution. There are well-developed methods for detecting environmental changes based on nematode community structure.

At the long-term CRUCIAL field experiment, where alternative fertilizer products have been applied since 2003, we measured effects of long-term fertilization with human urine, composted household waste and sewage sludge on soil properties (pH, soil organic matter and nitrogen availability), abundance of soil microorganisms (bacteria, fungi, small protozoa and ciliates) and nematode trophic groups compared to plots with unfertilized, NPK and cattle manure treatment. Sampling and assessments were done three times during a growth season. Further, we assessed the composition of nematode communities using metabarcoding.

Treatments with a high input of organic matter (cattle manure, composted household waste and sewage sludge) had high abundances of bacteria and thus bacterial grazers (small protozoa, ciliates, and bacterial feeding nematodes). We found a significant correlation between nematode community structure and pH and organic matter. We calculated

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the nematode Maturity Index 2–5 (pollution indicator) based on metabarcoding data, which did not differ significantly between the treatments.
We conclude that long-term fertilization with different types of contemporary Danish urban waste products affects both soil properties and abundance of soil organisms, the latter largely reflecting the organic matter input of the fertilizer treatments. We found no adverse effect on nematode communities that could indicate pollution-induced stress on nematofauna or decreased soil fertility.

1. Introduction

Recycling of organic waste products, such as human urine, composted organic household waste and sewage sludge, as fertilizers for agricultural fields has received attention, because it could have an important role in sustainable crop production (Fytili and Zabaniotou, 2008). From a short-term perspective, human urine, composted organic household waste and sewage sludge are suitable fertilizers, and maintain plant growth comparable to mineral (NPK) fertilizers (Singh and Agrawal, 2008). The use of NPK fertilizer must be reduced because phosphorus (P) is a critically limited resource (Cordell et al., 2009), and because synthetic production of mineral nitrogen (N) fertilizers is highly energy consuming, and thus both expensive and of environmental concern (Tanaka et al., 2016). In addition, composted organic household waste and sewage sludge fertilization add substantial amounts of organic matter to the soil, which improves soil structure (Peltre et al., 2017) and thus draining- and water holding capacity, and enhances soil carbon sequestration (Smith, 2004).

However, there are concerns because especially composted organic household waste and sewage sludge contain various xenobiotic substances which could affect soil health. Both products will inevitably contain some quantities of pollutants, for example heavy metals, persistent organic pollutants (POPs) and pieces of plastic/micro-plastic. The main worry is that this 'cocktail' of pollutants will build up in the soil and in a long-term perspective compromise soil biological processes and thus plant productivity. Historically, especially sewage sludge fertilization has been examined in eco-toxicological studies (Giller et al., 1998; Singh and Agrawal, 2008), and it was shown that this fertilizer had severe effects on several groups of soil organisms, e.g. *Rhizobium* bacteria (Chaudri et al., 1993; Hirsch et al., 1993), mycorrhizal fungi (Koomen et al., 1990) and nematodes (Georgieva et al., 2002), mainly due to a high content of heavy metals in the sludge (Giller et al., 1998). However, sewage sludge quality has improved over the last decades, and the heavy metal content has decreased. Amlinger et al. (2004) found that there has been a 3–5-fold decline in the content of cadmium (Cd), mercury (Hg), lead (Pb) and zinc (Zn) in European sewage sludge since the seventies. Liu et al. (2021) recently performed a critical statistical analysis of historical and contemporary sludge quality data from the UK and found, through a soil accumulation model, that Zn and Cu would be the first to approach their statutory soil limits in the long term. The study also found that nickel (Ni), Cd, Pb and Hg were no longer significant and, from a practical perspective, could be removed from the regulatory controls on agricultural use.

At the CRUCIAL experiment, located at University of Copenhagen's fields in Høje Taastrup, Denmark, field plots have been fertilized with various products from human waste streams for >20 years (some products also at highly accelerated levels corresponding, at this point in time, to >100 years of legal application) (Lopez-Rayo et al., 2016). In Danish agriculture, use of sewage sludge and composted household waste is regulated on the basis of the P content, and the annual application is limited to 30 kg P ha^{-1}. In the CRUCIAL long-term field experiment, we have intentionally breached this legal limitation, in order to represent 'worst case' scenarios for waste recycling through acceleration. Specifically, the waste products of interest are human urine, composted organic household waste and sewage sludge. In addition, the experiment has comparable plots with more conventional fertilization practices with NPK fertilizer and cattle manure, as well as plots that have not been fertilized. In 2012, amendment of sewage sludge, composted organic waste, NPK and cattle manure was terminated on one third of the area of each of the waste-treated plots. These subplots (retired plots) indicate the long-term after-effect in soils of the particular treatments. Subsequently, the retired plots were amended with nitrogen fertilizer (55 kg N ha^{-1}), to ensure moderate crop yields. The field experiment thus offers a unique possibility to study the long-term effects of alternative fertilization schemes, with the legacy of products that have been applied at high or even accelerated rates over two decades, and under more realistic agricultural conditions.

Evaluating the consequences of these alternative fertilization schemes on soil health is not straight-forward, given the complex nature of the soil environment, where essential functional processes are the result of interactions between highly diverse communities of soil organisms and soil physicochemical parameters. Thorough assessment of the impact on soil health therefore calls for complementary studies investigating associated aspects of soil biology and physicochemistry. Analyses of nematode community changes can be used to clarify biotic responses to soil management practices. Nematodes are an essential, ubiquitous, and by number of individuals the most abundant, group of animals in the soil food web. They are a diverse group of soil organisms which contain both bacterial feeders, fungal feeders, plant parasites, omnivores and predators (Yeates et al., 1993). Soil nematodes are active in the soil capillary water, where they, due to their permeable cuticle, are in intimate contact with dissolved compounds. The sensitivity to disturbances and pollution as well as the response to nutrient enrichment vary between nematode taxa, and nematode community changes have therefore been used as indicators of environmental changes due to disturbance, eutrophication and pollution (Neher, 2001; Yeates and Bongers, 1999). Much effort has been put into development of methods that describe the condition of soils in relation to pollutants and environmental disturbances based on nematode community structure (Bongers, 1990; Bongers et al., 1995), and to distinguish pollution effects from eutrophication effects (Ferris and Bongers, 2009). It is thus well-established that nematode community structure responds to soil heavy metal pollution, with a dominance of pollution-insensitive taxa at high heavy metal concentration and a higher representation of sensitive taxa at decreasing heavy metal concentrations (Georgieva et al., 2002; Ikoyi et al., 2021; Šalamún et al., 2012; Sánchez-Moreno and Navas, 2007). The colonizer-persister (cp) scale is used to calculate the Maturity Index 2–5 (MI2–5). The cp values describe each taxon on a scale ranging from extreme r-strategist ('colonizers') to extreme K-strategists ('persisters'), and the MI2–5 is the weighted mean of cp-groups in each sample (Bongers and Korthals, 1993). Present studies adapt MI calculations based on morphological identification to molecular meta-barcoding identification methods (Ikoyi et al., 2020; Kenmotsu et al., 2021; Porazinska et al., 2009).

Nematodes, mostly studied using the model organism *Caenorhabditis elegans*, are sensitive to both heavy metals (Johansen et al., 2019; Peredney and Williams, 2000), POPs (Čadková et al., 2020; Chen et al., 2019) and microplastics (Kim et al., 2020a; Kim et al., 2020b). However, the majority of these studies are performed in laboratories with single pollutant exposures, often under relatively short exposure duration and with higher than environmentally relevant exposure concentration, and thus have limited resemblance with the complexities of real field conditions.

In this study, the main aim was to examine the effects of long-term fertilization with human urine, composted household waste and sewage sludge on the nematode abundance and community structure. In particular, we wanted to investigate whether the composition of nematode communities indicated that soil health was compromised by these different fertilizer products, considering that xenobiotics were inevitably added with the products. We compared the waste treatments with plots treated with NPK

(mineral fertilizer abundantly used in conventional farming) and cattle manure (high organic matter content similar to sewage sludge and composted household waste) and unfertilized plots (no fertilizers used and thus serves as a negative control).

We evaluated the differences in soil properties (pH, soil organic matter and nitrogen availability) and differences in abundance of the different nematode feeding groups caused by long-term treatment with different fertilizers. We also quantified the main groups of microorganisms (bacteria, fungi, protozoa (amoebae/flagellates and ciliates)), to be able to relate responses of nematode trophic groups to responses in prey (bacteria and fungi) and competitors (protozoa). Further, we described the nematode community structure based on metabarcoding, and calculated a metabarcoding-based Maturity Index to detect possible pollution effects of the treatments.

2. Materials and methods

2.1. Soil sampling

We utilized the long-term experiment CRUCIAL at University of Copenhagen's field station in Høje Taastrup, Denmark (55° 40′ N, 12° 18′ E). In the CRUCIAL experiment, field plots (each 891 m^2) have been treated with different conventional and alternative fertilizers since 2003, including human urine, composted household waste and sewage sludge (Magid et al., 2006; Poulsen et al., 2013). For treatments with composted household waste and sewage sludge, we used plots treated with accelerated levels (amounting to three times the recommended N dosage), as these are expected to have the highest pollution load. Neighbouring plots are separated by 3 m wide strips of grass, to impede carry-over of fertilizers from one plot to another during agricultural practices. Table 1 summarises the treatments used in this study and the amount of fertilizers amended each year. The experimental setup is structured as a randomized block design, where each treatment is replicated three times. The various fertilizer products are very different in terms of organic content, type of organic matter and initial availability of nutrients, which may complicate the interpretation of studies on toxic effects of the products. Therefore, from 2013 amendments were suspended in subplots of essential treatments (called the "retired plots"). Since then, the retired plots have only been fertilized with a reduced amount of mineral fertilizer, to assimilate the treatments in terms of nutrient and organic matter, leaving a legacy of xenobiotics from the organic waste fertilizer products.

We sampled three times during the growth season of 2020, on May 11th, June 25th and September 11th. In 2020, spring wheat was grown in all plots. At each sampling time, we collected soil from plots with fertilization schemes: unfertilized, NPK, human urine, cattle manure, composted household waste (accelerated level) and sewage sludge (accelerated level), altogether samplings from 18 plots and a total of 54 samples. In addition, we sampled "retired plots" of NPK, cattle manure, composted household waste (accelerated level) and sewage sludge (accelerated level), altogether 12 plots and a total of 36 samples. Each sample consisted of 15 soil cores (ø 2 cm) of 20 cm depth taken in a diagonal of the plot and pooled together. Soil samples were kept in closed air-tight plastic bags from sampling in the field until the soil analysis could be performed in the laboratory.

2.2. Soil characterization

Soil pH was measured on fresh samples. A mixture of 10 g of soil and 50 ml ddH_2O was shaken thoroughly for 30 min on an orbital shaker and left to settle for 15 min. Then, pH of the solution was measured using a pH meter (Metrohm 827 pH lab) (Johansen et al., 2021).

To determine the water content (WC), we measured the mass loss after drying the soil at 80 °C for 48 h. Subsequently, soil total organic matter content (TOM) was determined as mass loss on ignition for 6 h at 550 °C.

To determine soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations, aliquots of 15 g of fresh soil was mixed with 50 ml 0.5 M K_2SO4 and shaken on an orbital shaker at 150 rpm for 1 h. Samples were then filtered through a Whatman 5 filter (pore size 2.5 μm) and subsequently analysed for ammonium and nitrate concentration on a FOSS FIAstar 5000 (Kindtler et al., 2019).

We measured the concentration of a range of potentially problematic heavy metals in soil sampled from the plots in autumn 2020 (dried 0–20 cm top-soil sieved at 2 mm and subsequently crushed with zirconium beads). Aliquots of approximately 100 mg were digested with 2.5 ml 70 % HNO_3 and 1 ml 15 % H_2O_2 in a microwave oven (Multiwave 3000, Anton Paar, GmbH, Graz, Austria), and diluted with milli-Q H_2O to a final volume of 50 ml. Then, samples were analysed for Ag, As, Cd, Cr, Cu, Ni, Pb and Zn on an Agilent 7900 ICP-MS (Zoorob et al., 1998).

2.3. Quantification of soil organisms

We quantified the population of soil protozoa with the most probable number (MPN) enumeration method (Jarvis et al., 2010; Rønn et al., 1995). In brief, 3 g of fresh soil was blended with 100 ml amoeba saline (AS) (Page, 1967) for 1 min. For amoeba and flagellates, eight replicated threefold dilution series of each soil suspension were prepared in 96 wells microtiter plates with 0.3 g L^{-1} tryptic soy broth (TSB) in AS medium. For ciliates, eight replicated tenfold dilution series of each soil suspension were prepared in 24 well micro plates, prepared with a sterile grain of barley as medium (Ekelund et al., 2002). All plates were incubated for 10 days at 12 °C, then each well was checked for the presence of protozoa under an inverted light microscope (Nikon Eclipse Ts2), and the MPN was calculated.

We extracted nematodes from 100 ml of fresh soil from each sample with the Oostenbrink elutriator method (Whitehead and Hemming, 1965). The nematode population was counted under an inverted light

Table 1
Overview of the treatments in CRUCIAL experimental field used in this study, and the amounts of fertilizers (nitrogen (N), phosphorus (P) and potassium (K)) given each year from 2003 to 2021 (Poulsen et al., 2013).

Treatment	Abbreviation	Yearly organic C input (t ha^{-1})	Yearly N input (t ha^{-1})	Mineral fertilizer equivalent (MFE)	Yearly P input (t ha^{-1})	Yearly K input (t ha^{-1})
Unfertilized	U	0	0	–	0	0
Human urine	HU	0	0.7	65	0.00006	0.0002
NPK	NPK	0	0.4	100	0.015	0.052
Cattle manure	CM	34.7	1.7	65	0.11	0.30
Composted household waste	HHC	68.8	5.2	20	0.30	0.49
Sewage sludge	SS	19.7	3.2	45	0.42	0.023
NPK (retired)	NPK RED	2003–2012: 0 2013–2020: 0	2003–2012: 0.4 2013–2020: 0.055	2003–2012: 100 2013–2020: 100	2003–2012: 0.015 2013–2020: 0	2003–2012: 0.052 2013–2020: 0
Cattle manure (retired)	CM RED	2003–2012: 34.7 2013–2020: 0	2003–2012: 1.7 2013–2020: 0.055	2003–2012: 65 2013–2020: 100	2003–2012: 0.11 2013–2020: 0	2003–2012: 00.30 2013–2020: 0
Composted household waste (retired)	HHC RED	2003–2012: 68.8 2013–2020: 0	2003–2012: 5.2 2013–2020: 0.055	2003–2012: 20 2013–2020: 100	2003–2012: 0.30 2013–2020: 0	2003–2012: 0.49 2013–2020: 0
Sewage sludge (retired)	SS RED	2003–2012: 19.7 2013–2020: 0	2003–2012: 3.2 2013–2020: 0.055	2003–2012: 45 2013–2020: 100	2003–2012: 0.42 2013–2020: 0	2003–2012: 0.023 2013–2020: 0

microscope (Nikon Eclipse Ts2), and each nematode was assigned to a feeding group of bacterial feeders, fungal feeders, plant parasites, Tylenchidae (root-hair feeders/fugal feeders), omnivores or predators based on morphological characteristics of mainly the buccal cavity and oesophagus (Dam et al., 2017; Yeates et al., 1993).

To quantify bacterial and fungal abundances in the soil, we performed quantitative PCR (qPCR) targeting the bacterial 16S rRNA gene and the fungal ITS region, respectively. From all soil samples, we extracted DNA from 10 g fresh weight with the DNeasy PowerMax Soil Kit (Qiagen, Hilden, Germany), following the manufacturers protocol. Subsequently, DNA was purified and concentrated with the Genomic DNA Clean & Concentrator-25 kit (Zymo Research, Irvine, CA, USA). DNA samples were stored at −20 °C.

For the 16S rRNA assay, a standard curve was made from a 10-fold dilution series of *Escherichia coli*. The number of 16S rRNA gene copies was assessed using the primers EUB338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and EUB518R (5′-ATTACCGCGGCTGCTGG-3′), targeting a 215 bp fragment of the V3 region of the 16S rRNA gene (Haugwitz et al., 2014). PCR cycling conditions were: 50 °C for 2 min and 95 °C for 12 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s, with a final melting cycle of 15 s at 95 °C, 60 °C for 60 s, and 95 °C for 15 s.

The number of fungal ITS copies was quantified using the primers gITS7 (5′ GTGARTCATCGARTCTTTG-3′) and ITS4 (5′TCCTCCGCTTA TTGATA TGC-3′) (Christiansen et al., 2017). A standard curve was made from a 10-fold dilution series of a plasmid containing the ITS2 region amplified from *Aureobasidium pullulans* (de Bary) G. Arnaud. PCR cycling conditions were: 50 °C for 2 min and 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 56 °C for 20 s, and extension at 72 °C for 45 s, with a final melting cycle of 15 s at 95 °C, 60 °C for 60 s, and 95 °C for 15 s.

2.4. Metabarcoding of nematode community

For the metabarcoding of nematode communities, we used the purified and concentrated DNA extracts described above (see Section 2.3). DNA was amplified using the primers Nemf (5′-GGGGAAGTATGGTTGCAAA-3′) and 18Sr2b (5′-TACAAAGGGCAGGGACGTAAT-3′) (Porazinska et al., 2009; Sapkota and Nicolaisen, 2015), which targets the V6-V8 region of the 18S rRNA gene. Library preparation and amplicon sequencing was performed on each sample on a NovaSeq 6000 platform PE250 (100 K tags per sample) by Novogene Europe (Cambridge, UK). Sequences were processed with DADA2 version 1.14 (Callahan et al., 2016). Briefly, raw reads were quality filtered and trimmed (*filterAndTrim* - maxN = 0, maxEE = 2, truncQ = 2), followed by error learning (*learnErrors*), de-replication (*derepFastq*) and merging of forward and reverse reads (*mergePairs*) prior to the construction of the sequence table (*makeSequenceTable*). Chimera sequences were removed (*removeBimeraDenovo*) and taxonomic classification of chimera-free ASVs was performed with the naïve Bayesian classifier, as implemented in DADA2, with a bootstrap threshold of 80, by searching against the PR2database V1.14.0 (Guillou et al., 2012). Due to the incomplete taxonomies of many nematode entries in the PR2 database, nematode ASVs that were not assigned to genus were searched against the NCBI Genbank. Top hits with coverage of 100 % in the BLAST search and sequence similarities of 100 % at species and ≥99 % at genus rank were considered. ASVs identified as singletons were removed and the generated sample-wise ASV abundance table was used for further analysis.

2.5. Statistical analyses

We tested the overall significance of fertilization schemes on soil characteristics, abundance of soil organisms and the nematode feeding groups using ANOVA. Soil characteristic or organism abundance were used as dependent variable and treatment, time of sampling and block was used as independent variables, all as category variables. We also tested for interaction effects between treatment and time of sampling. Subsequently, we used Tukey's post-hoc test to compare treatments in an all-pairwise comparison during the growing season. Block was included in the analyses to test for effects of the landscape variation at the field where the CRUCIAL experiment is situated. These analyses were made in SAS Enterprise Guide 7.1.

Statistical analyses of the sequenced data were conducted in software R, version 4.0.5, using vegan (v2.5.7) (Oksanen et al., 2019), phyloseq (v1.34.0.) (McMurdie and Holmes, 2013), and ggplot2 (v3.3.2) (Wickham, 2016) packages. Samples with <500 reads were removed before nematode composition and diversity estimation. Relative abundance of taxonomic groups (genus level) was determined using the transform_sample_counts() function in phyloseq. Alpha diversity (ASV richness and Shannon) was computed using the plot richness function.

We examined the edaphic factors affecting nematode communities in soils with different treatments using canonical correspondence analysis (CCA) in "vegan" (version2.6–2) (Oksanen et al., 2022). Significantly correlating variables were fitted onto the ordination space using the 'envfit' function in R.

To calculate nematode Maturity Index 2–5 (MI2–5) we removed unassigned taxa from the nematode data. Each genus was assigned to the "colonizer-persister" cp-scale (Bongers, 1990), and the metabarcoding-derived MI2–5 was calculated using the online Nematode INdicator Joint Analysis (NINJA) calculation system (https://shiny.wur.nl/ninja/). This tool uses a database of nematode genera and assigns these a cp-score between 1 and 5, then it calculates MI2–5 as a weighted mean of cp-scores of nematode genera with cp 2–5 (leaving out cp-1 genera). Originally, the MI was calculated as the weighted mean cp-score of the free-living nematodes based on counts of nematodes; here we calculated the metabarcoding-derived MI2–5 based on reading counts of taxa (Desmedt et al., 2022). An ANOVA was performed to test the overall significance of fertilization schemes on MI2–5.

3. Results

3.1. Soil properties

Plots fertilized with composted household waste, where lime is added in the composting process, had the highest pH close to 7.5. In contrast, plots fertilized with sewage sludge had the lowest pH of 6.5. The remaining plots had, with minor variation, a pH just below 7 (Table 2). Soil pH was significantly affected by the fertilizer treatment (ANOVA, $p < 0.001$). There was a significant interaction between treatment and time (ANOVA, $p < 0.001$), probably driven by the time pattern for the unfertilized plot, which deviates from the remaining treatments. For the remaining treatments, time is of little importance for pH, and thus treatment is driving the differences in pH. The content of organic matter in the plots, assessed by loss on ignition, varied significantly between treatments (ANOVA, $p < 0.001$). The means of organic matter fall into three blocks (Tukey HSD), the plots with no or low carbon fertilizers (unfertilized, human urine and NPK) treatments group together with the lowest content of organic matter content, the treatments with cattle manure and sewage sludge fertilizers are grouped together, and composted household waste plots has the highest content of organic matter (Table 2). The water content of the soils was mainly affected by the last rainfall, therefore we see large variations in this parameter during the season (ANOVA, $p < 0.001$). Although water content is not a direct measure for water holding capacity, in this comparison it can be considered a proxy for the relative ability of the differently treated soils to hold water, since samples were protected from loss of water via evaporation from sampling until analysis. We do see a significant effect of the fertilizer treatments (ANOVA, $p < 0.001$), where plots treated with fertilizers with high organic matter (cattle manure, composted household waste and sewage sludge) generally held more water (Table 2).

The concentration of inorganic nitrogen (N) in the soil, in the forms of both NH_4^+ and NO_3^-, varied with treatments and season. Whereas NH_4^+ concentration varied significantly among both treatment (ANOVA, $p = 0.003$) and season (ANOVA, $p < 0.001$), there was a significant interaction effect for NO_3^-, (ANOVA, treatment*time, $p < 0.001$). For NO_3^- this means that the seasonal pattern differed among treatments. The unfertilized plots

Table 2
Mean values (n = 3) and standard errors (SE) of soil parameters measured three times during the growing season of 2020 on May 11th, June 25th and September 11th. Data was analysed with ANOVA GLM, where we tested for effects of treatment, time, the interaction treatment x time and block ($p < 0.05$:*; $p < 0.01$:**; $p < 0.001$:***). Additionally, a post-hoc Tukey HSD test was performed to group the treatments in an all-pairwise analyses. Data for NH_4^+ were log transformed prior to analyses.

	Unfertilized	Human urine	NPK	Cattle manure	Compost	Sewage sludge	
pH							*ANOVA* ***
May 11th	6.97 ± 0.01	6.83 ± 0.03	6.77 ± 0.03	6.90 ± 0.04	7.36 ± 0.06	6.54 ± 0.11	*Treatment* ***
June 25th	6.73 ± 0.01	6.87 ± 0.03	6.74 ± 0.01	6.96 ± 0.04	7.56 ± 0.02	6.52 ± 0.09	*Time* ***NS***
September 11th	7.03 ± 0.02	6.86 ± 0.02	6.66 ± 0.03	6.82 ± 0.05	7.48 ± 0.04	6.53 ± 0.05	*Treatment x Time* ***
Tukey group	c	c	b	c	d	a	*Block* ***NS***
Water content (% of DW)							*ANOVA* ***
May 11th	4.0 ± 0.7	5.2 ± 0.2	4.9 ± 0.7	8.8 ± 0.8	13.2 ± 0.4	8.1 ± 1.1	*Treatment* ***
June 25th	6.2 ± 0.2	6.4 ± 0.4	6.9 ± 0.2	9.9 ± 0.8	12.6 ± 0.5	9.5 ± 0.9	*Time* ***
September 11th	14.4 ± 0.3	15.6 ± 0.5	16.2 ± 0.2	19.5 ± 0.5	23.3 ± 0.5	19.1 ± 0.4	*Treatment x Time* ***NS***
Tukey group	a	a	a	b	c	b	*Block* *
Loss on ignition (% of DW)							*ANOVA* ***
May 11th	3.5 ± 0.1	4.1 ± 0.3	4.0 ± 0.1	5.7 ± 0.1	9.7 ± 0.1	5.4 ± 0.2	*Treatment* ***
June 25th	3.5 ± 0.1	4.2 ± 0.3	4.1 ± 0.1	5.8 ± 0.2	9.7 ± 0.2	5.3 ± 0.3	*Time* *
September 11th	3.3 ± 0.1	3.8 ± 0.4	3.8 ± 0.1	5.5 ± 0.1	9.5 ± 0.1	5.2 ± 0.2	*Treatment x Time* ***NS***
Tukey group	a	b	b	c	d	c	*Block* *
NH_4^+ (mg kg^{-1} soil DW)							*ANOVA* ***
May 11th	1.9 ± 0.8	2.5 ± 1.1	9.8 ± 3.6	4.0 ± 1.4	4.9 ± 3.1	5.2 ± 1.1	*Treatment* **
June 25th	1.2 ± 0.8	0.7 ± 0.2	1.0 ± 0.2	1.7 ± 0.4	2.2 ± 0.2	1.7 ± 0.1	*Time* ***
September 11th	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	1.0 ± 0.2	0.9 ± 0.1	*Treatment x Time* ***NS***
Tukey group	a	ab	ab	ab	b	b	*Block* ***NS***
NO_3^- (mg kg^{-1} soil DW)							*ANOVA* ***
May 11th	4.1 ± 0.5	13.3 ± 0.7	24.8 ± 0.3	9.9 ± 0.1	16.2 ± 1.6	21.8 ± 1.5	*Treatment* ***
June 25th	2.9 ± 0.7	2.3 ± 0.1	2.8 ± 0.5	4.1 ± 1.2	6.7 ± 0.5	10.5 ± 1.7	*Time* ***
September 11th	2.5 ± 0.1	3.9 ± 0.2	3.9 ± 0.1	10.8 ± 0.9	14.1 ± 1.3	16.2 ± 4.1	*Treatment x Time* ***
Tukey group	a	b	bc	b	c	d	*Block* ***NS***

did not receive any form of N, and therefore had generally low concentrations of both NH_4^+ and NO_3^-. The human urine and NPK plots received readily mineralized N (urine) or mineral N with the fertilizer at the beginning of the season, and had high concentrations at the first sampling, which later declined. Fertilizers with high organic matter (cattle manure, composted household waste and sewage sludge), had high amounts of organically bound N, which most likely was mineralized by microbial activity during the growing season (Table 2).

We found significant effects of the treatments on the metal concentration of Ag, Cu and Zn, but not As, Cd, Cr, Ni and Pb (see supplementary materials Table S1). The concentration of Ag was significantly increased in the sewage sludge and retired sewage sludge treatments, with at least 10 times higher concentration than the unfertilized, NPK, human urine and cattle manure treatments. The concentrations of Cu and Zn were significantly increased in the composted household waste treatments.

Table 3
Mean values (n = 3) and standard errors (SE) of abundances of soil organisms (gene copies or individuals per gram of dry soil) measured three times during the growing season of 2020 on May 11th, June 25th and September 11th. Data was analysed with ANOVA GLM, where we tested for effects of treatment, time, the interaction treatment x time and block ($p < 0.05$:*; $p < 0.01$:**; $p < 0.001$:***). Additionally, a post-hoc Tukey HSD test was performed to group the treatments in an all-pairwise analyses. Data for total nematodes were log transformed prior to analyses.

	Unfertilized	Human urine	NPK	Cattle manure	Compost	Sewage sludge	
Bacteria (log 16S copies g^{-1} soil DW)							*ANOVA* ***
May 11th	8.67 ± 0.04	8.75 ± 0.03	8.70 ± 0.03	8.84 ± 0.05	8.89 ± 0.01	8.81 ± 0.04	*Treatment* ***
June 25th	8.66 ± 0.04	8.80 ± 0.03	8.72 ± 0.03	8.81 ± 0.01	8.90 ± 0.02	8.78 ± 0.06	*Time* **
September 11th	8.76 ± 0.06	8.89 ± 0.04	8.83 ± 0.03	8.91 ± 0.01	8.90 ± 0.03	8.79 ± 0.04	*Treatment x Time* ***NS***
Tukey group	a	bcd	ab	cd	d	bc	*Block* ***NS***
Fungi (log ITS copies g^{-1} soil DW)							*ANOVA* ***
May 11th	6.56 ± 0.02	6.64 ± 0.09	6.61 ± 0.01	6.83 ± 0.08	6.99 ± 0.12	6.88 ± 0.08	*Treatment* ***
June 25th	6.41 ± 0.03	6.58 ± 0.01	6.66 ± 0.04	6.82 ± 0.07	6.74 ± 0.12	6.77 ± 0.15	*Time* ***NS***
September 11th	6.46 ± 0.15	6.67 ± 0.04	6.75 ± 0.03	7.05 ± 0.03	6.99 ± 0.13	6.83 ± 0.16	*Treatment x Time* ***NS***
Tukey group	a	ab	bc	c	c	c	*Block* ***NS***
Flagellates/amoeba (log g^{-1} soil DW)							*ANOVA* ***
May 11th	4.11 ± 0.22	4.30 ± 0.03	4.52 ± 0.09	4.65 ± 0.04	4.94 ± 0.12	4.91 ± 0.03	*Treatment* ***
June 25th	4.08 ± 0.11	4.27 ± 0.12	4.62 ± 0.12	4.80 ± 0.04	4.84 ± 0.16	4.86 ± 0.26	*Time* ***NS***
September 11th	4.23 ± 0.13	4.54 ± 0.04	4.67 ± 0.02	4.80 ± 0.18	4.64 ± 0.19	4.72 ± 0.11	*Treatment x Time* ***NS***
Tukey group	a	ab	bc	cd	d	d	*Block* ***NS***
Ciliates (log g^{-1} soil DW)							*ANOVA* ***
May 11th	1.41 ± 0.10	2.22 ± 0.05	1.77 ± 0.07	2.35 ± 0.19	2.75 ± 0.13	2.64 ± 0.08	*Treatment* ***
June 25th	1.61 ± 0.14	2.00 ± 0.15	1.91 ± 0.10	2.53 ± 0.21	2.81 ± 0.15	2.38 ± 0.22	*Time* ***
September 11th	1.42 ± 0.11	1.83 ± 0.21	1.58 ± 0.23	2.28 ± 0.10	2.11 ± 0.18	2.14 ± 0.15	*Treatment x Time* ***NS***
Tukey group	a	b	ab	c	c	c	*Block* *
Nematodes (g^{-1} soil DW)							*ANOVA* ***
May 11th	3.4 ± 0.5	5.7 ± 0.5	5.9 ± 0.6	21.5 ± 5.2	12.3 ± 1.3	13.1 ± 1.2	*Treatment* ***
June 25th	2.1 ± 0.1	5.2 ± 0.4	6.3 ± 0.6	9.9 ± 2.7	8.3 ± 1.4	13.7 ± 1.8	*Time* ***
September 11th	4.4 ± 0.8	7.7 ± 0.9	9.0 ± 0.4	16.8 ± 5.9	21.3 ± 3.6	17.5 ± 4.1	*Treatment x Time* ***NS***
Tukey group	a	b	b	c	c	c	*Block* ***NS***

3.2. Abundance of soil microorganisms

For all soil microorganisms there seemed to be a relation between treatments contributing to high soil organic matter and the abundance of organisms. Bacterial and fungal abundances were measured on the DNA extracts with qPCR targeting the 16S rDNA and ITS region, respectively. For bacteria, we found a significant effect of both treatments (ANOVA, $p < 0.001$) and sampling time (ANOVA, $p < 0.001$), with the highest abundances in two of the high organic matter fertilizer treatments (cattle manure and composted household waste) (Table 3). Likewise, the highest abundances of fungi were found in the high organic matter fertilizer treatments (i.e., cattle manure, composted household waste and sewage sludge) (ANOVA, $p < 0.001$) (Table 3). Populations of small soil protozoa (flagellates and amoeba) and ciliates were determined with the MPN method and showed a significant effect of treatment (ANOVA, $p < 0.001$ for both protozoa and ciliates), with the highest populations of small soil protozoa and ciliates also in the treatments with high content of organic matter. In addition, there was a significant seasonal effect on ciliates (ANOVA, $p < 0.001$), with the smallest ciliate populations in September.

3.3. Total nematode abundance

We found mean abundances between 2.1 and 21.5 nematodes g^{-1} soil DW over the season and among treatments. There were significant effects of treatment (ANOVA, $p < 0.001$) and time of season (ANOVA, $p < 0.001$) on total nematode abundance in the soil. Generally, the plots that had received fertilizers with no or low carbon (unfertilized, human urine and NPK) had lower nematode abundance than plots that had received fertilizers high in organic matter (cattle manure, composted household waste and sewage sludge) (Table 3).

3.4. Abundance of nematode feeding groups

Nematodes were divided into six feeding groups (bacterial feeders, fungal feeders, plant parasites, Tylenchidae (root-hair feeders/fungal feeders), omnivores, predators) based on morphological characteristics. We found a significant seasonal effect on all feeding groups, and a treatment effect on all groups except fungal feeders (Fig. 1). The treatments had a marked effect on bacterial feeders, where plots with high organic matter fertilizers (cattle manure, composted household waste, sewage sludge) had 2–4 fold higher abundances of bacterial feeders compared to unfertilized, NPK- or urine fertilized plots (Fig. 1). For fungal feeders, we only observed a seasonal effect, with the lowest abundances in the middle of the season. At the first sampling date, mean abundances of fungal feeding nematodes were ca. 50 % lower in accelerated household compost and accelerated sewage sludge than in the other fertilized treatments, but this difference was not significant. The abundance of plant parasites increased during the growing season (ANOVA, $p < 0.001$), but we also found an effect of the fertilizer treatments (ANOVA, $p < 0.001$), with high abundances in the composted household waste and sewage sludge plots and the lowest abundances in unfertilized plots (Fig. 1). Both season (ANOVA, $p < 0.01$) and fertilizer treatment (ANOVA, $p < 0.001$) affected the root-hair- and fungal feeding Tylenchidae, with lower abundances in the sewage sludge plots. For omnivores, we observed significant seasonal (ANOVA, $p < 0.001$) and treatment (ANOVA, $p < 0.001$) effects, with lower abundances in the composted household waste and sewage sludge plots (Fig. 1). For predators, we found a general significant effect of both treatments (ANOVA, $p < 0.01$), time of the season (ANOVA, $p < 0.05$) and block (ANOVA, $p < 0.05$), but pairwise comparisons over the season only revealed a significant difference between unfertilized and NPK treatment (Fig. 1).

3.5. Community structure of nematodes

Sequencing of soil samples amplified with nematode specific primers yielded 119,324 reads (range: 623–6527; median: 2044) that produced 1995 ASVs. For reads distribution and rarefaction curves, see Supplementary Fig. S3. Alpha diversity (observed and Shannon) was significantly different for the different treatments at different time points (Supplementary Fig. S4).

CCA ordination showed that nematode community composition varied between treatments and identified physio-chemical factors that could explain variations between the nematode communities at the different time points (Fig. 2). A PERMANOVA confirmed that treatments impacted the community composition significantly at all sampling dates (May 11th: $p = 0.005$; June 25th: $p = 0.010$; September 11th: $p = 0.007$). We found that nematode communities of the same treatment tended to cluster together (Fig. 2), and to some extent that treatments with similarities clustered together, e.g. unfertilized, human urine and NPK treatments tended to cluster separated from cattle manure, sewage sludge and composted household waste. These tendencies are most evident at the third sampling point in September 11th (Fig. 2B). The differences in nematode communities between treatments were significantly ($p < 0.05$) correlated with the environmental factors pH, total organic matter, water content and NO_3 concentration. Soil pH strongly correlated with nematode community at all the different time points.

3.6. Nematode metabarcoding-derived Maturity Index 2–5

We determined the number and relative abundance of nematode genera in each sample, based on the metabarcoding data. We found a total of 44 nematode genera, with plant parasites included (see supplementary materials Table S2). The metabarcoding-derived Maturity Index 2–5 (MI2–5) was calculated for each sample at the three different sampling times. The result of MI2–5 calculation and the relative abundance of each cp group 2–5 is displayed in Fig. 3. MI2–5 ranged from 2.52 to 3.66, and we found no significant differences in MI2–5 at the three time points.

4. Discussion

Recirculation of municipal waste products, in particular sewage sludge, as fertilizers for agricultural fields has historically been subject for many studies, because it was shown to have severe negative effects on soil health (Giller et al., 1998; McGrath et al., 1995; Singh and Agrawal, 2008). The main reason for this was the concentrations of heavy metals in the sludge, which were very high in the past. Therefore, there are currently strong restrictions on the use of municipal waste products, to avoid any unwanted effects on soil health. However, due to environmental regulation and perhaps de-industrialization in parts of Europe, the quality of sewage sludge has improved considerably. Notably, heavy metal concentrations in sewage sludge from some countries have been reduced to levels that are not likely to pose a risk to the soil environment (Liu et al., 2021). Thus, Magid et al. (2020) assessed that the risk associated with agricultural use of contemporary Danish sewage sludge is comparable to that of pig slurry, once the EU limits for Zn and Cu addition to pig feed have been fully implemented. It may therefore be time to re-evaluate the use of municipal waste products, as they are valuable nutrient resources, and they could play an important role in the transition towards sustainable crop production.

The CRUCIAL field experiment reflects a long-term pollution load from municipal waste fertilizers, with concentrations in the waste products reflecting the past two decades. Field plots have been treated with human urine, composted household waste and sewage sludge for 20 years, and composted household waste and sewage sludge have been applied in accelerated dosages, leaving a pollution load corresponding to >100 years of normal application. The CRUCIAL experiment has been subject to several studies, which describe different components of the pollution legacy of the various fertilization schemes. Lopez-Rayo et al. (2016) measured heavy metals in the CRUCIAL soils in 2013 and found that the concentration of Cu was higher in the accelerated compost treatment and the concentration of Zn was elevated in both compost and sewage sludge treatments. However, the concentrations of metals were well below national safety limits. The measurements of Cu and Zn in the present study are in line with the findings of Lopez-Rayo et al. (2016). Additionally, we measured

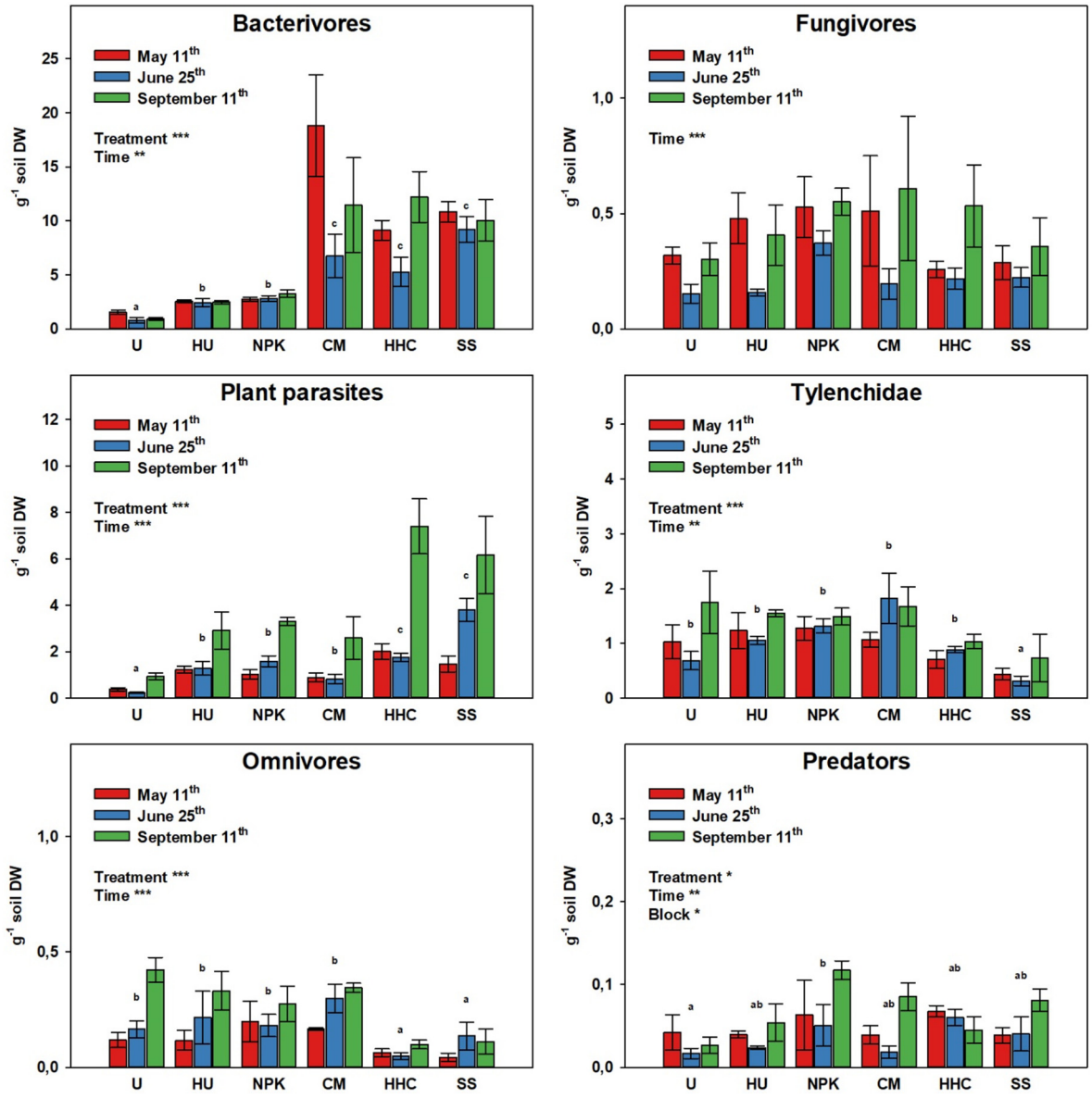

Fig. 1. Abundance of the six feeding groups of nematodes at the three timepoints May 11th, June 25th and September 11th in unfertilized (U) field plots and field plots fertilized with human urine (HU), NPK (NPK), cattle manure (CM), composted house waste (HHC) or sewage sludge (SS) for 20 years. The bars are average abundances (n = 3) ± 1 SE in individuals per gram of dry soil. ANOVA was used to test for effects of Treatment, Time, Treatment x Time and Block ($p < 0.05$:*; $p < 0.01$:**; $p < 0.001$:***), and significant effects are listed in the figure. In addition, an all-pairwise comparison was performed using Tukey HSD, and significant differences ($p < 0.05$) among treatment means are indicated by letters above columns. Abundance of all feeding groups except predators have been log transformed prior to analyses.

the concentration of Ag and Cd, and found a substantial increase of Ag in the sewage sludge treatments, but no treatment effects on Cd concentrations. All concentrations of heavy metals were well below the Danish threshold values for soil quality (Larsen et al., 2016). Vuaille et al. (2022) measured persistent organic pollutants (POP), components in e.g. personal care products and pharmaceuticals, and found increased concentrations in human urine and sewage sludge treated plots. Riber et al. (2014) studied the presence of antibiotic- and mercury-resistant Pseudomonad bacteria, and found a transient increase immediately after sludge amendment, which vanished within three weeks after amendment. Additionally, we know that microplastics are present in compost and sewage sludge (Braun et al., 2021; Simon et al., 2018), and preliminary unpublished measurements of microplastic in the soil from plots treated with compost and sewage sludge suggest a substantial increase of the microplastic content in these plots compared to NPK fertilized and unfertilized plots (pers comm Annemette Palmqvist). While additional investigations can further expose how this long-term exposure of various xenobiotics, and their interactions, affect soil biology, altogether, the pollution load in the soil treatments seems to be moderate.

At the establishment of the CRUCIAL experiment (Magid et al., 2006), the plots were laid out on a single field with uniform management history, however, 20 years of continuous application of different fertilizers have altered physio-chemical soil parameters and thus also the conditions for the soil organisms. The different fertilization schemes have resulted in differences in soil pH between treatments, and quite large differences in organic matter content. For instance, the composted household waste treatment has 2–2.5 times higher content of organic matter than the unfertilized and mineral NPK fertilizer treatments. The higher organic matter content of soil enhances the draining and water holding capacity, which was reflected in the enhanced water content in plots amended with cattle manure, sewage sludge and composted household waste (Table 2). Further, the higher organic matter content enhances carbon storage in the soil.

Another fundamental difference between the treatments is the type of nitrogen (N) that is amended and the variation in plant available inorganic

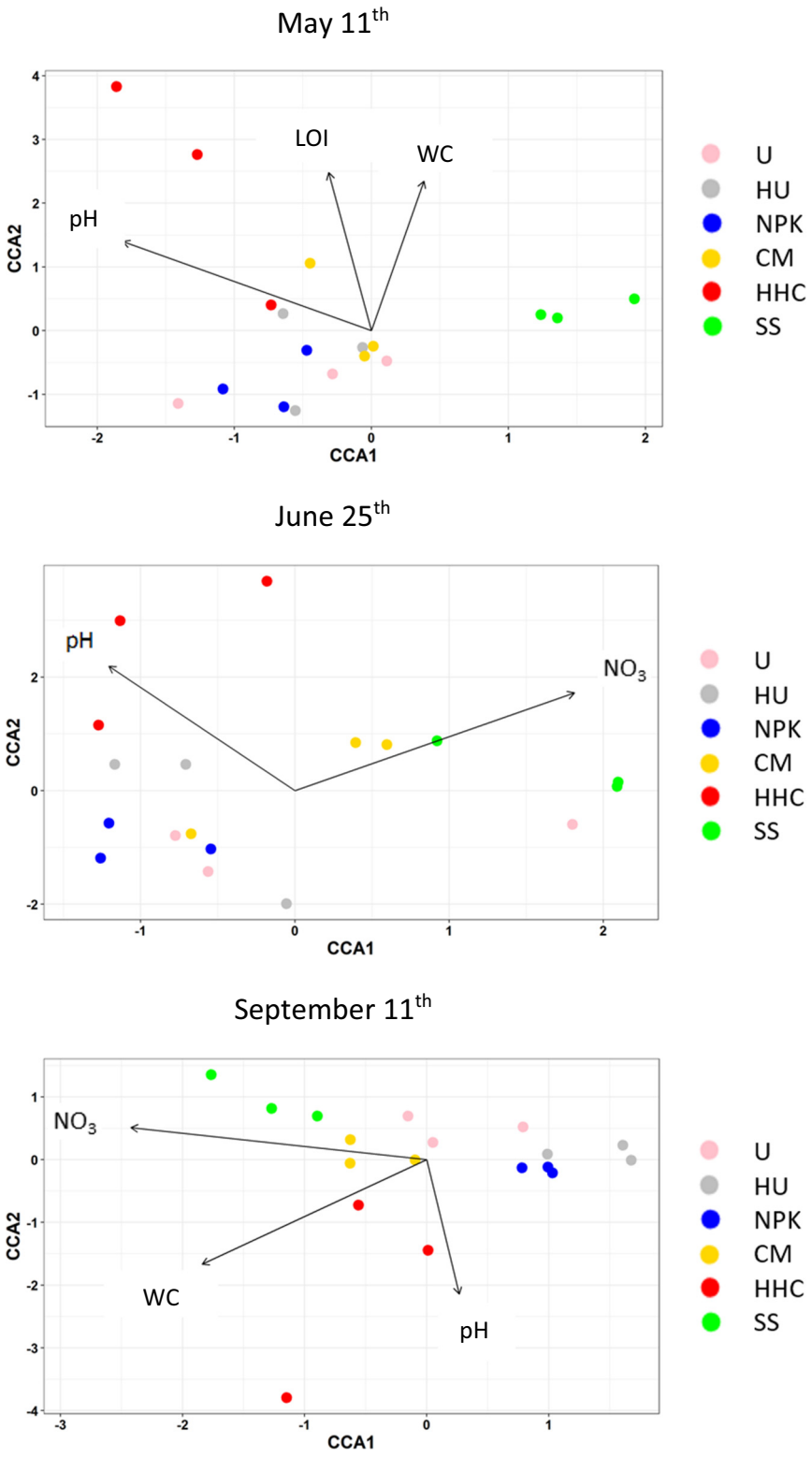

Fig. 2. CCA plots of the nematode community structure (based on ASV presence and abundance) in unfertilized (U) plots and in plots treated with human urine (HU), NPK (NPK), cattle manure (CM), composted household waste (HHC) or sewage sludge (SS) for 20 years. The plots have been sampled three times during the growing season of 2020: May 11th (top plot), June 25th (middle plot) and September 11th (bottom plot). Each datapoint represents one sample. Vectors of environmental variables have been plottet on the graph, provided they correlate significantly ($p < 0.05$) with the variation in the data.

N during the growing season. Whereas treatments with human urine and NPK receives an immediately plant-available form of N (NO_3^-, NH_4^+, or urea), the treatments with cattle manure, composted household waste and sewage sludge receives much of the total N in an organically bound form. Thus, the initially high inorganic N contents in human urine and NPK treated plots decreased markedly from mid-May to the end of June,

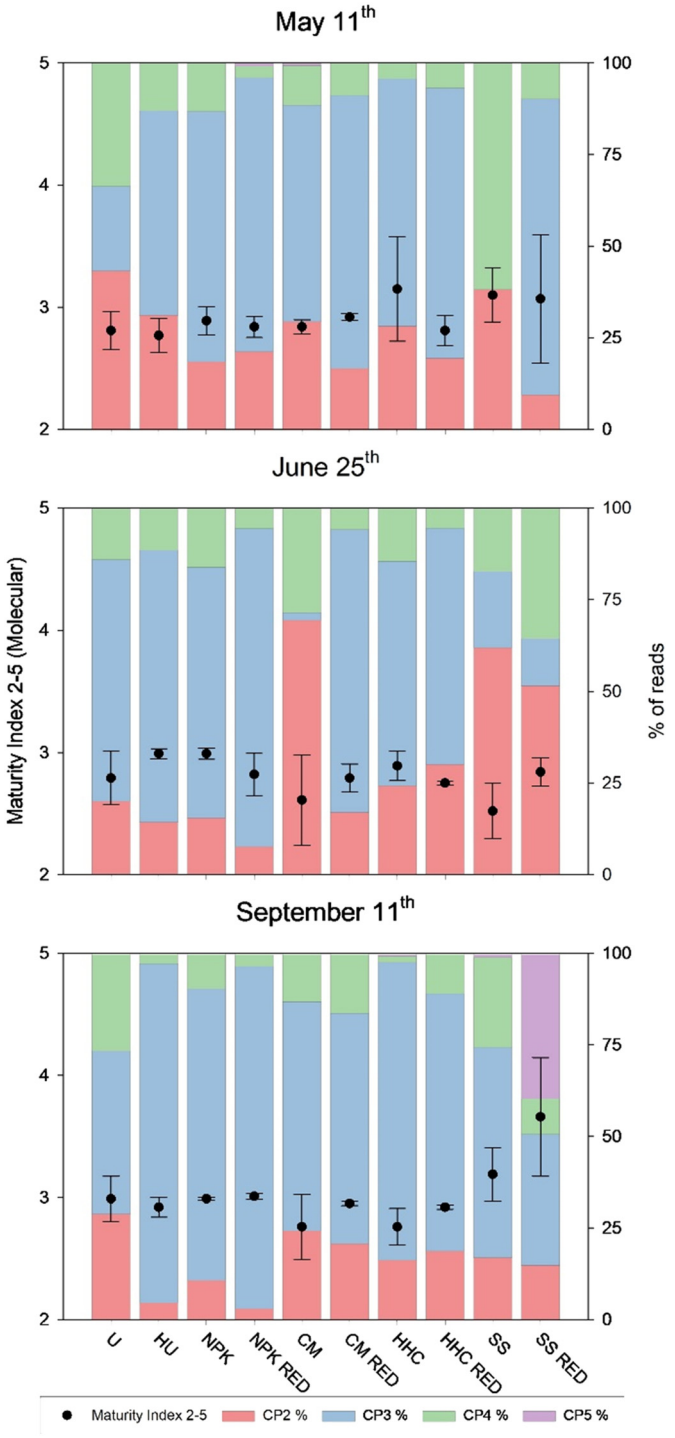

Fig. 3. Relative abundance of non-plant feeding nematode cp groups 2–5 and metabarcoding-derived Maturity Index 2–5 on the three timepoints May 11th, June 25th and September 11th in unfertilized (U) field plots and in plots fertilized with human urine (HU), NPK (NPK), retired NPK (NPK RED), cattle manure (CM), retired cattle manure (CM RED), composted household waste (HHC), retired composted hosehold waste (HHC RED), sewage sludge (SS) or retired sewage sludge (SS RED). The points show metabarcoding-derived Maturity Index 2–5 ± 1 SE (n = 3) on the on left y-axis and bars show the relative abundance (of number of reads) of cp groups on the right y-axis. Effects of the treatments on the Maturity Index 2–5 have been tested with a one-way ANOVA, and there were no effect at any of the timepoints (May 11th: p = 0.961; June 25th: p = 0.685; September 11th: p = 0.181).

whereas the microbial mineralization of organic N in the treatments with cattle manure, composted household waste and sewage sludge resulted in moderate inorganic N fluctuations during the growing season.

Soil organism responses to the different long-term fertilization schemes may reflect quantitative and qualitative differences in organic matter and nutrient input as well as physio-chemical differences between soils treated with different fertilizers, including the potential effects of xenobiotic components in the fertilizers (Bünemann et al., 2006; Vestergård et al., 2018).

We expected that 20 years of treatment with high organic matter fertilizers (cattle manure, composted household waste and sewage sludge) would enhance the abundance of microorganisms and higher trophic levels in the soil food web. Indeed, we found a significant increase in the abundances of bacteria and bacterial grazers (small protozoa, ciliates and bacterial feeding nematodes) in plots treated with these high organic matter fertilizers. The increase in bacterial abundance was modest, but the increase in bacterial grazers was more pronounced and indicates that the high organic matter fertilizers stimulated the activity of the bacterial-based decomposition pathway of the food web (Vestergård et al., 2018). In contrast, the build-up of organic matter in cattle manure, composted household waste and sewage sludge treatments did not result in corresponding increases in fungal feeding nematode (including Tylenchidae) abundances, but only in abundance of fungi. In all treatments, the abundance of fungal feeding nematodes was very low (< 1 individual g^{-1} soil), suggesting moderate fungal growth across the field. It is well-established that intensively managed agricultural soils favour bacterial-based over fungal-based decomposition, probably reflecting that hyphal growth and activity are sensitive to agricultural practices, e.g. tillage (Six et al., 2006). Abundances of plant parasitic nematodes were lowest in the unfertilized plots, intermediate in plots fertilized with human urine, NPK and cattle manure and highest in household compost and sewage sludge fertilized plots. This difference can be explained by the differences in plant growth, the food source for plant parasitic nematodes, which Lopez-Rayo et al. (2016) report is significantly higher (measured in grain yield) in household waste and sewage sludge treatments compared to unfertilized treatment. We also found a general increase of plant parasitic nematodes during the growing season, which also reflects plant growth.

Despite the high input of organic matter, the abundance of omnivorous nematodes and Tylenchidae was lowest in the accelerated composted household waste and sewage sludge treatments. The food sources for these two groups of nematodes are not well-defined; Tylenchidae encompass both root-hair feeding and fungal feeding taxa (Okada et al., 2005; Vestergård, 2004), and omnivorous nematodes may feed on a variety of food sources including fungi, bacteria, protozoa and small invertebrates (Yeates et al., 1993). For the compost treatment, this aligns with the previously reported low content of fungal-derived residues in compost treated plots at the CRUCIAL filed site (Peltre et al., 2017).

As omnivorous nematodes are K-strategists and considered sensitive to environmental disturbances and pollutants (Bongers, 1990; Vestergård et al., 2018) the low abundance in composted household waste and sewage sludge could indicate that components in these fertilizers compromised the soil environmental conditions for sensitive taxa. However, the abundance of predatory nematodes, which are also generally sensitive to pollutants and disturbance (Bongers, 1990), was not lower in these treatments, suggesting that the soil environment did not generally affect pollution- and disturbance-sensitive taxa adversely.

The Maturity Index 2–5 (Bongers, 1990; Wilson and Khakouli-Duarte, 2009) is designed to detect whether e.g. soil management practices affects the contribution of pollution- or disturbance sensitive nematode taxa, and thereby distinguish between eutrophication and pollution effects. We calculated the MI2–5 based on the meta-barcoding data (Kenmotsu et al., 2021) and found no significant differences between treatments. Hence, this analysis indicates that waste product fertilization did not impose an overall stress- or pollution response on the nematode community compared to NPK or cattle manure fertilization. Our study corroborates the results of Georgieva et al. (2002), where sewage sludge amendment did not affect MI2–5, unless the sludge was artificially spiked with Zn or Cu resulting in

soil concentrations that were considerably higher than soil concentrations in any of the treatments at the CRUCIAL site. We note that we only retrieved very few sequences read numbers for omnivorous nematode taxa. This means that the reduced omnivorous abundances in accelerated household compost and accelerated sewage sludge treatments revealed by the count data were not represented in the metabarcoding-derived MI2–5 index. The metabarcoding analysis was based on DNA extracted from 10 g soil, and as the overall abundance of omnivorous nematodes was low (< 0.5 individuals g^{-1} soil), this suggests that this strategy was below the detection limit of omnivorous nematode taxa in the sampled agricultural bulk soil. In rhizosphere samples, where nematode abundances are generally higher than in bulk soil, we have successfully detected omnivorous taxa using a similar metabarcoding strategy (Desmedt et al., 2022; Sikder et al., 2021a; Sikder et al., 2021b).

Whereas the MI2–5 did not vary between treatments, the PERMANOVA and CCA plots showed that the different fertilizer treatments resulted in nematode communities with different taxonomic composition. The variation between nematode communities was related to the environmental factors pH, water content, soil organic matter, and NO_3^-. It is well-known that land use practices change soil pH and organic matter content, and that this affects microbial production and diversity (Cruz-Paredes et al., 2021; Frostegård and Bååth, 1996; Lauber et al., 2008), with cascading effects in microbial feeding protozoa and nematodes (Mortensen et al., 2020; Vestergård et al., 2018). Thus, the nematode population could be affected both by the physiochemical parameters (including pollution effects) in the soil and the derived effects of increased food sources (e.g. bacteria, fungi and plant roots). This study shows that the fertilization schemes do alter the nematode populations and community composition, possibly reflecting altered food sources, changes in soil parameters or pollution effects. However, since the Maturity Index 2–5 did not vary between the fertilization treatments, and other factors such as pH and water content could explain the change in community, we do not suspect pollution effects on the community.

5. Conclusion

Altogether, we found that the fertilization schemes applied over two decades had measurable effects on soil characteristics and soil organisms. The most interesting question for this study is if the fertilization with municipal waste products affected soil fertility and health negatively. We found that the amount of organic matter has increased in the plots fertilized with products with high organic matter content (cattle manure, composted household waste, sewage sludge), which enhances the drainage and water holding capacity of the soil. Further, the enhanced organic matter content stimulated the bacterial-based decomposition pathway in the soil, as we found increased abundances of both bacteria and bacterial grazers in these treatments. The low abundance of omnivorous nematodes in composted household and sewage sludge treatments could indicate that components in soils treated with these waste products could compromise the growth of pollution- and disturbance-sensitive soil organisms. However, the abundance of predatory nematodes, which are generally sensitive to pollutants, was not negatively affected in compost and sewage sludge amended plots. Further, the Maturity Index 2–5, which is designed to detect pollution effects, did not vary between the fertilizer treatments. Thus, we conclude that fertilization with composted household waste and sewage sludge in accelerated dosages have had mainly beneficial effects on soil health in terms of soil structure and abundance of decomposer organisms.

CRediT authorship contribution statement

Jesper Liengaard Johansen: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Marie Dam:** Investigation, Data curation, Writing – review & editing. **Enoch Narh Kudjordjie:** Software, Formal analysis, Data curation, Writing – review & editing. **Susana Silva Santos:** Software, Formal analysis, Data curation, Writing – review & editing. **Annemette Palmqvist:** Conceptualization, Writing – review & editing, Funding acquisition. **Jakob Magid:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Mette Vestergård:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.160485.

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