Article

Influence of *Eisenia fetida* on the Nematode Populations during Vermicomposting Process

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Abstract: Vermicomposting stands as a sustainable and environmentally friendly waste management practice, leveraging the metabolic prowess of earthworms to facilitate the decomposition of organic matter into nutrient-rich compost. The aim of this experiment was to study the influence of *Eisenia fetida* on the density and the tropic structure of a nematode community during the vermicomposting process over a period of 3 months. Sewage sludge and green waste served as composting substrates. Overall, six compost variants were prepared consisting of three variants incorporating *E. fetida* and three control variants lacking *E. fetida*. Throughout the investigation, samples were gathered on a monthly basis, with each variant undergoing three repetitions. The aim was to isolate nematodes, determine the population density of the five trophic groups, and identify the dominant community. The anaysis was conducted employing both microscopic examination and molecular metabarcoding (NGS). It was shown that the bacterial-feeding community maintained dominance. The introduction of *E. fetida* into the compost led to a significant rise in the abundance of Diplogasteridae. In the variant without *E. fetida*, the amount of Diplodasteridae exceeded 0.1% only after the 30th (C3) or the 60th (C1, C2) day of composting, while in the compost with *E. fetida*, they were present in large amounts (ranging from 11.0% to 28.0%) already on the 30th day of composting. The introduction of *Eisenia fetida* also led to a notable reduction in *H. gingivalis* with significant implications for mitigating the risk of halicephalobiosis. The introduction of *E. fetida* resulted in reducing *H. gingivalis* to levels below 0.1% in all compost variants.

Keywords: organic waste; earthworms; pathogen; *Halicephalobus gingivalis*

Citation: Zapałowska, A.; Skwiercz, A.; Puchalski, C.; Malewski, T. Influence of *Eisenia fetida* on the Nematode Populations during Vermicomposting Process. *Appl. Sci.* **2024**, *14*, 1576. https://doi.org/10.3390/app14041576

Academic Editors: Gerald Sims and Norman Q. Arancon

Received: 22 December 2023
Revised: 30 January 2024
Accepted: 14 February 2024
Published: 16 February 2024

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1. Introduction

Composting is a natural and controlled decomposition process that transforms organic materials into a nutrient-rich soil conditioner. This process involves the mineralization and partial humification of organic matter, in controlled aerobic conditions, to a stable, pathogen-free product with the properties of humus [1]. Composting occurs in two distinct phases—mineralization and humification—each further divided into two respective stages: meso- and thermophilic stages during the mineralization phase, and cooling and maturation stages during the humification phase. The energy produced during mineralization is accumulated in the form of heat, which increases the temperature of the compost and is a characteristic feature of the process. During cooling, humic substances are synthesized, which leads to the production of mature compost.

Earthworms play a pivotal role as crucial biological factors in the soil ecosystem. These organisms are central to the decomposition and mineralization of organic matter within the soil. The agricultural significance of earthworms has been recognized for an extended period, as they contribute significantly to soil fertilization processes and exert influence on the physicochemical properties of the soil [2]. Vermicompost is produced with the participation of earthworms. They feed on organic residues [3], promote the colonization of the soil by beneficial bacteria and fungi, and break down pathogens and pests [4]. Earthworms have been observed to enhance the mobility and accessibility of heavy metals. They achieve this by stimulating microbial activity and accelerating the decomposition of organic matter. Additionally, they contribute to soil pH acidification, diminish the dissolved organic carbon responsible for metal transportation, and directly uptake these metals [5,6].

The most popular earthworm breeding species among earthworms is *Eisenia fetida* [7,8]. This species is distinguished by a high activity in processing the substrate and the ability to manifest this activity all year round, with the shortest period of development and the highest rate of reproduction. *Eisenia fetida* is used throughout the world for this purpose as it is ubiquitous, can tolerate a wide range of temperature and can live in wastes with good moisture content.

Nematodes are small organisms abundant in soil, they present a high species diversity. Nematodes span various trophic levels within soil ecosystems, functioning as bacterivores, fungivores, plant feeders, predators, and omnivores. This diverse spectrum allows them to consume a broad array of food sources [9]. The number, species diversity, and trophic structure of nematode communities are significantly influenced by biotic (humidity, temperature, pH, abundance of organic matter), abiotic (interspecific competition, relationships with other organisms) and anthropogenic (soil, pollution) factors. Hence, nematodes possess several characteristics that make them excellent bioindicators [10–15]. Nematodes are closely related to plants, microorganisms, and other groups of soil fauna. They take part in the circulation of nutrients [16]. Their high nutritional activity and rapid metabolism play an important role in processing microbial biomass and releasing mineral nitrogen compounds into the soil [17]. Nematodes have the capacity to stimulate and facilitate the growth and proliferation of microorganisms, particularly those engaged in decomposing organic matter [18]. Conversely, controlling plant feeder nematodes is essential in agriculture due to their direct impact as pests or vectors of different plant diseases [19].

This study aimed to investigate the influence of *Eisenia fetida* presence on nematode communities during the compost process, specifically focusing on the potential changes in nematode density and structure within sites introduced with *Eisenia fetida*. It was hypothesized that certain nematode types would exhibit greater dominance in *Eisenia fetida*-inhabited sites compared to the control sites.

2. Materials and Methods

2.1. Experimental Design

The compost was prepared using different municipal wastes in various mass proportions (kg by mass). The composting process was conducted according to the methodology described in the paper by Zapałowska et al. [20].

Two sets of treatment were implemented:

- Without Earthworms:

  C1: 165 kg of sewage sludge and 35 kg of sawdust
  C2: 90 kg of sewage sludge, 10 kg of sawdust, and 100 kg of garden and park waste
  C3: 180 kg of garden and park waste and 20 kg of sawdust

- With Earthworms:

  C4: 165 kg of sewage sludge, 35 kg of sawdust, and *Eisenia fetida*
  C5: 90 kg of sewage sludge, 10 kg of sawdust, 100 kg of garden and park waste, and *Eisenia fetida*

C6: 180 kg of garden and park waste, 20 kg of sawdust, and *Eisenia fetida*

Following the blending process, the combined waste was placed into a bioreactor; a plastic container shaped like a thermally insulated cube with a 1 m^3 capacity. The entire mixture underwent a composting process with ongoing temperature monitoring inside the bioreactor. To aid the bioconversion of the substrates, a manual rotation of shovels was used for mechanical mixing. Temperature measurements helped confirm three distinct phases in the transformation of substrates into compost within the bioreactor. In the end, the result was a solid product characterized by a fatty composition.

The stocking density of *Eisenia fetida* in the experiment was determined by adding 10 L of *E. fetida* for each cubic meter of the container. Each liter supported a population density of 500 individual earthworms (1 g for mature earthworms and 0.1 g for larvae). The introduction of 2 kg of mature earthworms and 300 g of larvae, accounting for the unit weight of individual developmental stages, facilitated a systematic and well-regulated dispersion of *Eisenia fetida* across the experimental environment.

The analysis of compost samples followed the outlined procedure: assessments were conducted for 6 variations, with each variant subjected to three repetitions. The entirety of the analysis process spanned three consecutive months.

2.2. Nematodes Extraction

A total of 200 cm^3 of compost was placed in a beaker, and the remaining water was added to achieve a total volume of 500 mL. The compost was thoroughly mixed and allowed to settle at the bottom of the beaker. The sediment suspension was subsequently decanted and transferred to a 200 mL tube for centrifugation at 2000$\times$ g (RCF) for 3 min. The supernatant was then discarded, and the precipitate was resuspended with 80 mL of 1 molar sucrose solution.

The tubes were centrifuged again for 2 min at 2000$\times$ g (RCF). The supernatant, containing nematodes, was poured through a 25 µm sieve. Nematodes were extracted from the sieve and placed in glass containers. The nematodes were then thermally killed with 6% formalin (90 °C), fixed in an equal amount of water, and passed through a series of graded glycerin and ethanol solutions. Finally, they were stored on slides in anhydrous glycerin.

The isolated nematodes were transferred to a fixation vessel containing an S1 solution (20 mL of 96% ethanol, 1 mL of glycerol, and 79 mL of distilled water). The dishes were positioned in a desiccator with a thin layer of 96% ethanol and moved to an incubator set at 40 °C. After 24 h in the desiccator, the containers with nematodes in the S1 liquid were placed in the incubator with the addition of S2 liquid (93 mL of 96% ethanol and 7 mL of glycerin), introducing a few drops of S2 liquid every hour for 8 h. It is considered that the nematodes become saturated with glycerin after 24 h in the incubator.

Nematodes were prepared on glass slides using the paraffin ring method. Nematodes embedded in glycerin were transferred to microscope slides containing drops of anhydrous glycerin. The paraffin rings, melting at a temperature of 50 degrees, provided protection for the nematodes. Morphological features were utilized for nematode identification, and each nematode taxon was assigned to one of five trophic groups based on life characteristics and nutritional preferences: plant-parasites, bacterivores, fungivores, omnivores, and predators. Nematodes were identified to the species (plant-parasitic) and genus (bacterivores, fungivores, predators, omnivores) levels using a Carl Zeiss Jena A-Scope microscope and the diagnostic key of Brzeski [21] and Andrássy [22]. Nematode species were allocated to trophic groups according to Yeates et al. [23].

2.3. DNA Extraction and Libraries Preparation

DNA extraction and libraries preparation were conducted according to the methodology described in the paper by Zapałowska et al. [20]. Briefly, total DNA was extracted using a DNA Mini Kit (Syngen Biotech, Wrocław, Poland), following the manufacturer's protocol. The D2-D3 segment of the 28S rDNA region was amplified using the specific primers. The PCR product was purified and a library was constructed using the NEBNext Multiplex

Oligos for Illumina 96 Index Primers (New England Biolabs, Salisbury, UK). The resulting PCR products were pooled, and the final purified product was then quantified using qPCR according to the qPCR Quantification Protocol Guide (KAPA LibraryQuantification Kits for Illumina Sequencing platforms, Roche, Basel, Switzerland). The paired-end (300 bp with V3 chemistry) sequencing was performed using the MiSeq platform (Illumina, San Diego, CA, USA).

2.4. Processing and Analysis of Sequencing Data

The taxa of nematodes present in the analyzed samples were determined using the metabarcoding approach. Metabarcoding allows for simultaneous identification of many taxa within the same sample at all nematode developmental stages. The quality of the obtained reads was checked at FastQC and filtered using the Trimmomatic to exclude low-quality reads (Q < 20, sequences with any ambiguous (N) bases, more than six homopolymers) and chimera sequences. Community composition and taxonomic affiliations of the obtained sequences were performed by the software pipeline CCMetagen v1.2.3. The processing and analysis of sequencing data were conducted following the methodology described in the paper by Zapałowska et al. [20], using the criteria for taxonomic assignment in CCMetagen as follows: species-level similarity threshold of 99.00%, genus-level of 95.00%, family-level of 90.00%, order-level of 85.00%, class-level of 75.00%, and phylum-level of 55.00% [24].

3. Results and Discussion

In our treatment, 23 nematode genera were identified. Bacterial-feeders represented 80.7% of total nematofauna including 11 genera (Figure 1A). Fungal-feeders represented 11.0% of nematode abundance (five genera) (Figure 1B). Plant-parasitic nematodes (PPN) represented 0.2% of the total density, and three genera were identified (*Merlinius*, *Paratylenchus*, *Pratylenchus*). Notably, PPN presence was only detected on the initial day of composting. Omnivores (5.3%) were represented by *Eudorylaimus* and *Dorylaimus* (Figure 1C) and Predators (2.8%) by *Mononchoides* and *Pristionchus* genera (Figure 1D). The total nematode density was 1.46-fold higher in the treatments with *Eisenia fetida* than in the non-inoculated treatment.

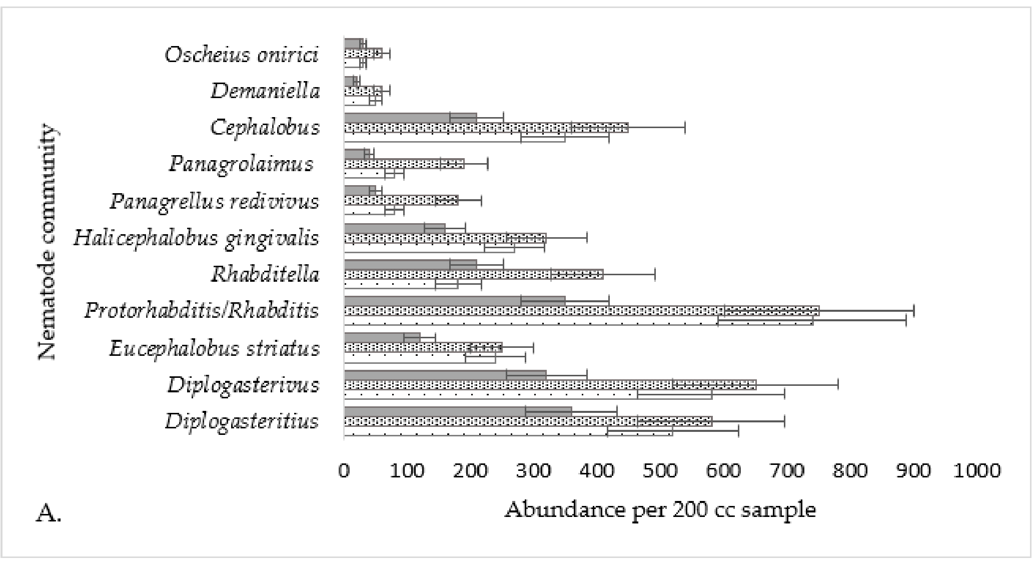


Figure 1. *Cont.*

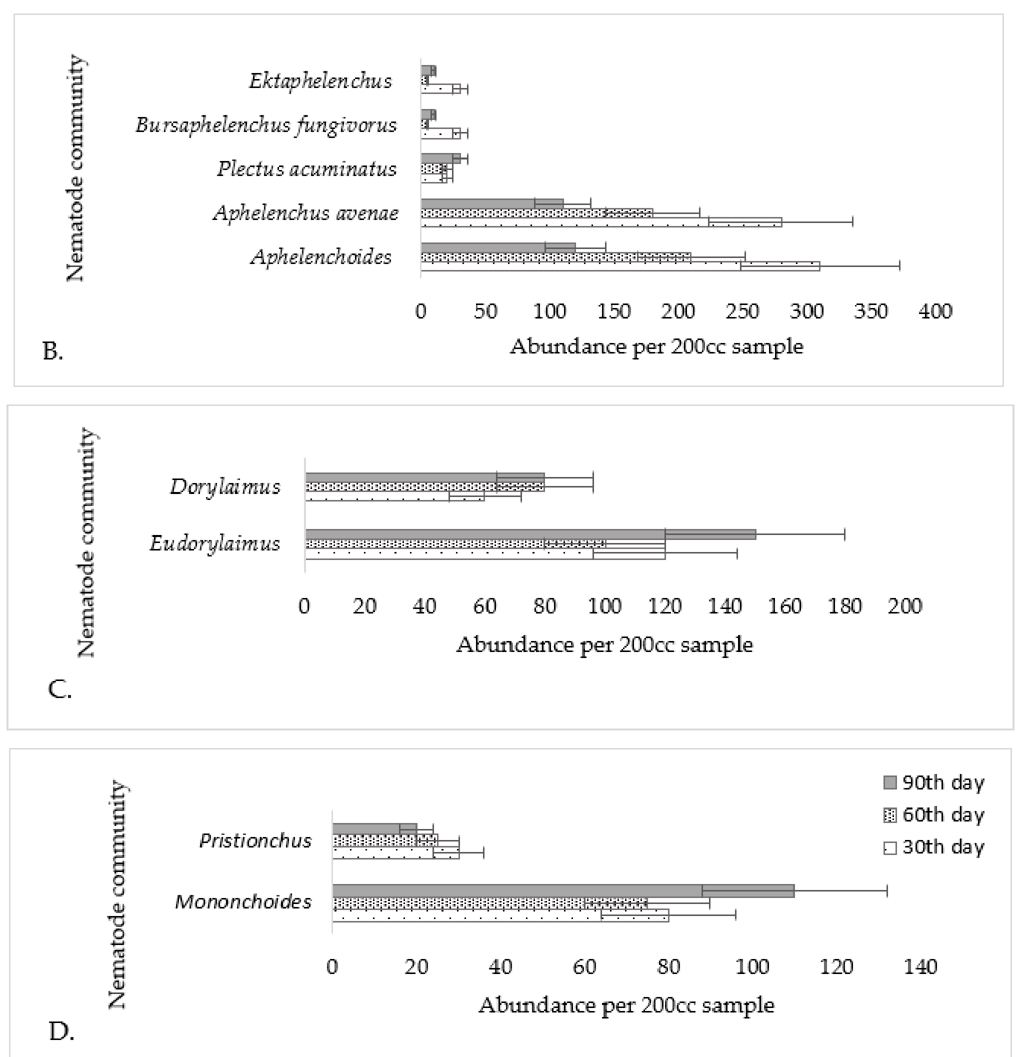


Figure 1. Abundance of the nematode community at the genera level per 200 cc compost sample: bacterivores (**A**), fungivores (**B**), omnivores (**C**) and predators (**D**) on the 30th, 60th and the 90th day of composting (n = 3).

Molecular metabarcoding has been proposed as a promising method to rapidly measure the community composition based on the genetic identification of species [25,26] The morphometric method for identifying nematode species revealed a higher count compared to the molecular metabarcoding technique. In the examined substrate samples, approximately 10,000 nematode individuals were discovered, predominantly bacterivores and fungivores, encompassing larval stages. This conclusion aligns with the findings of Bogers and Bongers [27], who remarked that fully identifying all nematodes in a soil sample to determine the total species count is nearly impractical. The discrepancy or absence of a specific nematode species uncovered through molecular analysis compared to classical taxonomy's morphometric identification can be attributed to this limitation. Although metabarcoding is a highly promising technique, it has its drawbacks, including sensitivity of the results to marker choice and the fact that reference databases are incomplete [28,29].

Next-generation sequencing reads (NGS) have been assigned at different taxa levels: family, genera, species and even strains or isolates. Examples of the reads assignment are presented in Figure 2.

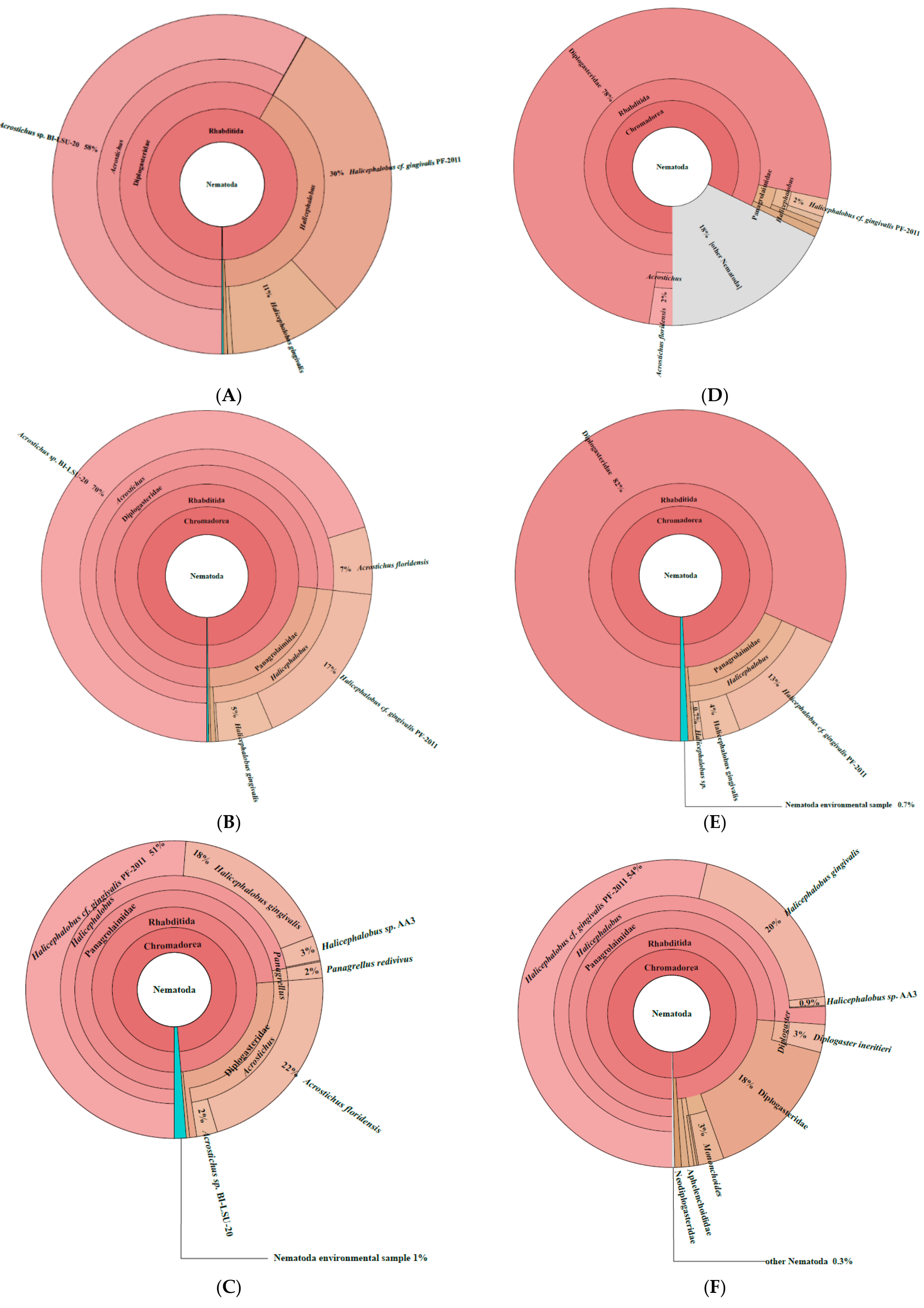


Figure 2. Krona graph representing the nematode community abundance estimated by NGS sequencing of pulled (n = 3) specimens in three compost variants without earthworms: C1 (**A**), C2 (**B**), C3 (**C**) and three compost variants with *Eisenia fetida*: C4 (**D**), C5 (**E**), and C6 (**F**) on the 30th day of composting.

The morphological approach of nematodes allows fast and relatively inexpensive identification to the genus level but identification to the species level requires the involvement of experts for every family of detected nematodes and appropriate nematode museum collections. Moreover, the identification of specimens is limited to this sex and developmental stage for which morphological keys have been developed. To overcome this limitation, we identified the nematodes present in compost sampled by metabarcoding. This approach is sex- and stage-insensitive but limited by the presence of molecular marker sequences in the appropriate databases, marker sequence intragenic variability and the "barcoding gap"—differences in similarity between sequences belonging to the same and other species. Moreover, in some genera (*Bursaphelenchus*, *Ektaphelenchus*, *Panagrellus*, *Panagrolaimus*) there are inconsistencies between nematode species, included in the World Database of Nematodes and TaxId of NCBI (Table 1). For *Bursaphelenchus* World Database of Nematodes listen 43, while in GenBank there are sequence records for 92 species. To a lesser extent, this also applies to *Ektaphelenchus*, *Panagrellus*, *Panagrolaimus*. For other genera there is consistency between World Database of Nematodes and TaxId of NCBI, but number of species with sequenced markers (18S and 28S rDNA) varies from 0% (*Demaniella*, *Protorhabditis*) to 93% (*Pristionchus*).

Table 1. Current molecular resources for molecular identification of nematode species nematode community. Number of species according to data in Nemys: World Database of Nematodes ver. (11/2023); [30] GenBank accessed 14 November 2023.

Number of Species in Genus. N = Number of Species	Species with GenBank Records. n = Number of Records (Records Include at Least 18S and 28S rRNA Sequences)
Demaniella N = 5	identified to genus level, n = 4
Cephalobus N = 19	*C. cubaensis*, n = 5
	C. oryzae, n = 1; 18S rRNA only
	C. persegnis, n = 17
	identified to genus level, n = 34
Panagrolaimus N = 11	*P. artyukhovskii*, n = 1; 1818S rRNA only
Disagreement between species list in WDN and supplementary publications and GenBank TaxI Species listed in WDN and supplementary publications: *P. davidi* Timm, 1971 *P. detritophagus* Fuchs, 1930 *P. hygrophilus* Bassen, 1940 *P. kolymaensis* Shatilovich, Gade, Pippel, Hoffmeyer, Tchesunov, Stevens, Winkler, Hughes, Traikov, Hiller, Rivkina, Schiffer, Myers & Kurzchalia, 2023 *P. magnivulvatus* Boström, 1995 *P. neotropicus* Poinar, 2011 *P. papillosus* Loof, 1971 *P. rigidus* Schneider, 1866 *P. subelongatus*, Cobb, 1914 *P. superbus* Fuchs, 1930 *P. thiennemanni* Hirschmann, 1952 *Panagrellus* N = 4	*P. davidi*, n = 1
	P. detritophagus, n = 25
	P. facetus, n = 2; 18S rRNA only
	P. kolymaensis **
	P. labiatus, n = 10
	P. paetzoldi, n = 10
	P. cf. *papillosus*, n = 2; 18S rRNA only
	P. rigidus, n = 6
	P. cf. *rigidus*, n = 8
	P. subelongatus, n = 36; 18S rRNA only
	P. superbus, n = 7620
	P. trilabiatus, n = 6; 18S rRNA only
	identified to genus level n = 315
	P. ceylonensis, n = 3

Table 1. *Cont.*

Number of Species in Genus. N = Number of Species	Species with GenBank Records. n = Number of Records (Records Include at Least 18S and 28S rRNA Sequences)
Disagreement between species list in WDN and supplementary publications and GenBank TaxI Species listed in WDN and supplementary publications: *P. filiformis* Sukul, 1971, Andrássy, 1984 *P. nepenthicola* Menzel, 1922, Goodey, 1945 *P. redivivus* Linnaeus, 1767, Goodey, 1945 *P. ulmi Abolafia*, Alizadeh & Khakvar, 2016	*P. dubius*, n = 9, 28S rRNA only
	P. levitatus, n = 2
	P. pycnus, n = 2
	P. redivivoides, n = 11
	P. redivivus, n = 687
	identified to genus level n = 9
Halicephalobus N = 10	*H. gingivalis*, n = 33
	H. cf. *gingivalis*, n = 4
	H. mephisto, whole genome shotgun sequence identified to genus level, n = 2550
Rhabditella N = 7	*R. axei*, n = 9 identified to genus level, n = 4, 18S rRNA only
Protorhabditis	identified to genus level, n = 14
Rhabditis N = 40	*R. belari*, n = 1, 28S rRNA only
	R. blumi, n = 4
	R. brassicae, n = 4
	R. dolichura, n = 1, 28S rRN *R.* cf. *longicaudata*, n = 1, 18S rRNA
	R. nidrosiensis, n = 1, 28S rRNA
	R. remanei, n = 1, 28S rRNA only
	R. terricola, n = 1, 28S rRNA only
	R. cf. *terricola*, n = 49, 18S rRNA only
	R. tokai, n = 1, 5S rRNA only
	identified to genus level, n = 208
Eucephalobus N = 10	*E. hooperi*, n = 1, 18S rRNA only
	E. laevis, n = 4, 18S rRNA only
	E. mucronatus, n = 2
	E. oxyuroides, n = 17
	E. cf. *oxyuroides*, n = 33, 18S rRNA
	E. striatus, n = 86
	E. cf. *teres*, n = 1, 18S rRNA only
	identified to genus level, n = 20
Ektaphelenchus N = 7	*E. apophysatus*, n = 9, 28S rRNA only
Disagreement between species list in WDN and supplementary publications and GenBank TaxI *E. berbericus* Alvani, Mahdikhani Moghadam, Giblin Davis & Pedram, 2016 *E. cupressi* Golhasan, Abdollahpour, Fang, Abolafia & Heydari, 2019 *E. kanzakii* Pedram, 2019 *E. koreanus* Gu, Maria, Fang & Liu, 2019 *E. masseyi* Heydari & Pedram, 2021 *E. oleae* Miraeiz, Heydari, Adeldoost & Ye, 2017 *E. phoenicis* Keramat, Mahboubi, Atighi, Pourjam, Abolafia, Alghanimi & Pedram, 2023	*E. berbericus*, n = 5
	E. cupressi, n = 3
	E. joyceae, n = 18S rRNA only
	E. kanzakii, n = 3
	E. masseyi, n = 4
	E. obtusus, n = 3
	E. oleae, n = 4
	E. phoenicis, n = 2

Table 1. *Cont.*

Number of Species in Genus. N = Number of Species	Species with GenBank Records. n = Number of Records (Records Include at Least 18S and 28S rRNA Sequences)
	E. taiwanensis, n = 3
	identified to genus level, n = 13
Bursaphelenchus N = 43	*B. abietinus*, n = 5
Disagreement between species list in WDN and supplementary publications and GenBank TaxI *B. andrassyi* Dayi, Calin, Akbulut, Gu, Schroder, Vieira & Braasch, 2014 *B. carpini* Kanzaki, Masuya, Ichihara, Maehara, Aikawa, Ekino & Ide, 2019 *B. cryphali*, Fuchs, 1930, Meyl, 1960 *B. decraemerae* Wang, Gu, Maria, Fang & Li, 2018 *B. fagi* Tomalak & Filipiak, 2014 *B. firmae* Kanzaki, Maehara, Aikawa & Matsumoto, 2012 *B. geraerti* Wang, Maria, Gu, Fang, Wang & Li, 2018 *B. gillanii* Schoenfeld, Braasch, Riedel & Gu, 2014 *B. hirsutae* Kanzaki, Ekino, Ide, Masuya & Degawa, 2018 *B. irokophilus* Torrini, Strangi, Mazza, Marianelli, Roversi & Kanzaki, 2019 *B. juglandis* Ryss, Parker, Alvarez-Ortega, Nadler & Subbotin, 2021 *B. kesiyae* Kanzaki, Aikawa, Maehara & Pham, 2016 *B. kiyoharai* Kanzaki, Maehara, Aikawa, Masuya & Giblin Davis, 2011 *B. koreanus* Gu, Wang & Chen, 2013 *B. laciniatae* Kanzaki, Masuya, Ichihara, Maehara Aikawa, Ekino & Ide, 2019 *B. manipurensis* Chanu & Meitei, 2014 *B. michaelseni* Tomalak & Filipiak, 2019 *B. microcarpae* Kanzaki, Ekino, Kajimura & Degawa, 2021 *B. moensi* Wang, Maria, Gu, Fang, Wang & Li, 2018 *B. mucronatus* Mamiya & Enda, 1979 *B. niphades* Tanaka, Tanaka, Akiba, Aikawa, Maehara, Takeuchi & Kanzaki, 2014 *B. osumiana* Kanzaki, Akiba, Kanetani, Tetsuka & Ikegame, 2014 *B. paraburgeri* Wang & Gu, 2012 *B. paraluxuriosae* Gu, Wang, Braasch, Burgermeister & Schroeder, 2012 *B. parantoniae* Munawar, Fang, He, Gu & Li, 2015 *B. penai* Kanzaki, Giblin Davis, Carillo, Duncan & Gonzalez, 2014 *B. piceae* Tomalak & Pomorski, 2015 *B. populi* Tomalak & Filipiak, 2010 *B. posterovulvus* Gu, Wang, He, Wang, Chen & Wang, 2014 *B. pterocarpi* Gu, Fang, Liu, Pedram & Li, 2019 *B. rockyi* Wang, Fang, Maria, Gu & Ge, 2019 *B. sakishimanus* Kanzaki, Okabe & Kobori, 2015 *B. saudi* Gu, Maria, Fang, He, Braasch & Li, 2016 *B. similus* Poinar, 2011 *B. sycophilus* Kanzaki, Tanaka, Giblin Davis & Davies, 2014 *B. tadamiensis* Kanzaki, Taki, Masuya & Okabe, 2012 *B. taprhorychi* Tomalak, Malewski, Gu & Fa Qiang, 2017 *B. tiliae* Tomalak & Malewski, 2014 *B. trypophloei* Tomalak & Filipiak, 2011 *B. ulmophilus* Ryss, Polyanina, Popovichev & Subbotin, 2015 *B. yuyaoensis* Gu, He, Wang & Chen, 2014 *B. cryphali okhotskensis* Kanzaki, Masuya, Ichihara, Maehara, Aikawa, Ekino & Ide, 2019 *B. mucronatus kolymensis*, Braasch, Gu & Burgermeister, 2011	*B. abruptus*, n = 6
	B. acaloleptae, n = 2
	B. africanus, n = 6
	B. anamurius, n = 3
	B. anatolius, n = 3
	B. andrassyi, n = 8
	B. antoniae, n = 26
	B. arthuri, n = 4
	B. arthuroides, n = 6
	B. borealis, n = 9
	B. braascha, n = 4
	B. carpini, n = 4
	B. chengi, n = 4
	B. cocophilus, n = 2
	B. conicaudatus, n = 28
	B. corneolus, n = 39
	B. crenati, n = 15
	B. cryphali, n = 10
	B. cryphali okhotskensis, n = 2
	B. debrae, n = 4; 28S rRNA only
	B. doui, n = 31
	B. eggersi, n = 21
	B. eremus, n = 8
	B. eucarpus, n = 3
	B. fagi, n = 2
	B. firmae, n = 3
	B. fraudulentus, n = 23
	B. fungivorus, n = 16
	B. gerberae, n = 5
	B. gillanii, n = 1; 18S rRNA
	B. hellenicus, n = 4
	B. hildegardae, n = 6
	B. hofmann, n = 16
	B. hylobianum, n = 6
	B. kesiyae, n = 2

Table 1. *Cont.*

Number of Species in Genus. N = Number of Species	Species with GenBank Records. n = Number of Records (Records Include at Least 18S and 28S rRNA Sequences)
	B. kevin, n = 4
	B. kiyoharai, n = 6
	B. koreanus, n = 3
	B. laciniatae, n = 2
	B. leoni, n = 8
	B. luxuriosae, n = 14
	B. macromucronatus, n = 5
	B. masseyi, n = 2
	B. mazandaranense, n = 4
	B. michalskii, n = 4
	B. microcarpae, n = 2
	B. minutus, n = 18
	B. mucronatus *
	B. obeche, n = 2
	B. okinawaensis *
	B. paraburgeri, n = 6
	B. paracorneolus, n = 4
	B. paraluxuriosae, n = 7
	B. parantoniae, n = 3
	B. paraparvispicularis, n = 7
	B. parapinasteri, n = 3
	B. parathailandae, n = 4
	B. parvispicularis, n = 7
	B. penai, n = 6
	B. pinasteri, n = 7
	B. piniperdae, n = 1
	B. pinophilus, n = 3
	B. platzeri, n = 4
	B. poligraphi, n = 10
	B. populi, n = 6
	B. posterovulvus, n = 5
	B. pterocarpi, n = 4
	B. rainulfi, n = 23
	B. ratzeburgii, n = 5
	B. rufipennis, n = 4
	B. sakishimanus, n = 2
	B. saudi, n = 4
	B. seani, n = 9
	B. sexdentati, n = 100

Table 1. *Cont.*

Number of Species in Genus. N = Number of Species	Species with GenBank Records. n = Number of Records (Records Include at Least 18S and 28S rRNA Sequences)
	B. sinensis, n = 6
	B. singaporensis, n = 4
	B. sycophilus, n = 2
	B. tadamiensis, n = 6
	B. taphrorychi, n = 8 28S rRNA only
	B. thailandae, n = 10
	B. tiliae, n = 29
	B. tokyoensis, n = 13
	B. trypophloei, n = 2
	B. tusciae, n = 9
	B. ulmophilus, n = 3
	B. vallesianus, n = 23
	B. willibaldi, n = 9
	B. wuae, n = 5
	B. xylophilus *
	B. yongensis, n = 6
	B. zvyagintsevi, n = 2
	identified to genus level, n = 251
Plectus N = 77	*P. acuminatus*, n = 19
	P. cf. *acuminatus*, n = 3
	P. andrassyi, n = 2, 18S rRNA, only
	P. antarcticus, n = 7
	P. cf. *antarcticus*, n = 3
	P. aquatilis, n = 20
	P. cf. *aquatilis*, n = 3
	P. belgicae, n = 10
	P. cirratus, n = 11
	P. cf. *cirratus*, n = 2, 18S rRNA, only
	P. exinocaudatus, n = 1, 28S rRNA, only
	P. frigophilus, n = 36, COI, only
	P. cf. *frigophilus*, n = 73, COI, only
	P. infundibulifer, n = 1, 28S rRNA, only
	P. longicaudatus, n = 9, 28S rRNA, only
	P. cf. *meridianus*, n = 5
	P. minimus, n = 8
	P. murrayi, n = 2819
	P. opisthocirculus, n = 3
	P. parietinus, n = 19

Table 1. *Cont.*

Number of Species in Genus. N = Number of Species	Species with GenBank Records. n = Number of Records (Records Include at Least 18S and 28S rRNA Sequences)
	P. cf. *parietinus*, n = 2, 18S rRNA, only
	P. parvus, n = 9
	P. pusillus, n = 1, 18S rRNA, only
	P. cf. *pusillus*, n = 3, 18S rRNA, only
	P. pusteri, n = 1, 28S rRNA, only
	P. rhizophilus, n = 2, 18S rRNA, only
	P. sambesii, whole genome shotgun sequencing
	P. tenuis, n = 2, 18S rRNA, only
	P. velox, n = 28
	identified to genus level, n = 214
Aphelenchus N = 10	*A. avenae* *
	A. cf. *avenae*, n = 1, 18S rRNA, only
	identified to genus level, n = 60
Aphelenchoides N = 83	*A. besseyi* *
	A. cf. *besseyi*, n = 2, 18S rRNA, only
	A. bicaudatus, n = 100
	A. cf. *bicaudatus*, n = 1, 18S rRNA, only
	A. blastophthorus, n = 18
	A. capsuloplanus, n = 2
	A. centralis, n = 1, 18S rRNA, only
	A. cibolensis, n = 1, 28S rRNA, only
	A. clarus, n = 1, 18S rRNA, only
	A. composticola, n = 2, 18S rRNA, only
	A. eldaricus, n = 2
	A. fragariae, n = 53
	A. fuchsi, n = 2
	A. fujianensis, n = 660
	A. giblindavisi, n = 2
	A. gorganensis, n = 2
	A. graminis, n = 1, 28S rRNA, only
	A. haguei, n = 1, 28S rRNA, only
	A. hamospiculatus, n = 3
	A. heidelbergi, n = 16
	A. iranicus, n = 2
	A. limberi, n = 1, 18S rRNA, only
	A. macronucleatus, n = 1, 18S rRNA, only
	A. medicagus **
	A. obtusicaudatus, n = 1, 28S rRNA, only

Table 1. *Cont.*

Number of Species in Genus. N = Number of Species	Species with GenBank Records. n = Number of Records (Records Include at Least 18S and 28S rRNA Sequences)
	A. obtusus, n = 2
	A. cf. *obtusus*, n = 1, 18S rRNA, only
	A. pannocaudus, n = 7
	A. paradalianensis, n = 19
	A. parietinus, n = 2, 28S rRNA, only
	A. cf. *parietinus*, n = 1, 18S rRNA, only
	A. primadentus, n = 2, 18S rRNA, only
	A. pseudobesseyi, n = 14
	A. pseudogoodeyi, n = 4
	A. ritzemabosi, n = 26
	A. rotundicaudatus, n = 3
	A. rutgersi, n = 4
	A. salixae, n = 4
	A. saprophilus, n = 2, 18S rRNA, only
	A. varicaudatus, n = 3
	A. xui, n = 3
	A. xylocopae, n = 6
	identified to genus level, n = 504
Dorylaimus N = 10	*D. elegans*, n = 1, 28S rRNA, only
	D. stagnalis, n = 79
	D. aff. stagnalis, n = 52, 18S rRNA, only
	identified to genus level, n = 2
Eudorylaimus N = 114	*E. altherri*, n = 4, 28S rRNA, only
	E. carteri, n = 7
	E. cf. *carteri*, n = 3, 18S rRNA, only
	E. centrocercus, n = 1, 28S rRNA, only
	E. centrocercus, n = 5, 18S rRNA, only
	E. cf. *coloradensis*, n = 3, 18S rRNA, only
	E. coniceps, n = 12, 18S rRNA, only
	E. cf. *meridionalis*, n = 3, 18S rRNA, only
	E. cf. *silvaticus*, n = 5, 18S rRNA, only
	E. cf. *sodakus*, n = 6, 18S rRNA, only
	E. cf. *subdigitalis*, n = 2, 18S rRNA, only
	identified to genus level, n = 23
Pristionchus N = 58	*P. aerivorus*, n = 40
	P. americanus, n = 42
	P. arcanus *
	P. atlanticus, n = 3
	P. auriculatae *

Table 1. *Cont.*

Number of Species in Genus. N = Number of Species	Species with GenBank Records. n = Number of Records (Records Include at Least 18S and 28S rRNA Sequences)
	P. boliviae, n = 28
	P. borbonicus, n = 25
	P. brevicauda, n = 4
	P. bucculentus, n = 28
	P. bulgaricus, n = 28
	P. chinensis *
	P. clavus, n = 28
	P. degawai, n = 1 18SRNA, n = 39 COI
	P. dorci, n = 2, 18SRNA only
	P. elegans, n = 6
	P. entomophagus *
	P. entomophilus, n = 2, 18SRNA only
	P. exspectatus *
	P. fissidentatus *
	P. fukushimae, n = 28
	P. hongkongensis, n = 1, 18SRNA only
	P. hoplostomus, n = 28
	P. japonicas *
	P. kurosawai, n = 1, 18S RNA only
	P. laevicollis, n = 4, 18S RNA only
	P. lheritieri, n = 61
	P. lucani, n = 3
	P. magnolia *
	P. marianneae, n = 32
	P. maupasi, n = 54
	P. maxplancki *
	P. mayeri *
	P. musae *
	P. neolucani, n = 1, 18S RNA only
	P. nudus, n = 1, 18S RNA only
	P. occultus, n = 1, 18S RNA only
	P. pacificus *
	P. cf. *pacificus*, n = 4
	P. paranudus *
	P. passalidorum, n = 2, 18S RNA only
	P. pauli, n = 32
	P. paulseni, n = 1, 18S RNA only
	P. pseudaerivorus, n = 35
	P. purgamentorium *

Table 1. *Cont.*

Number of Species in Genus. N = Number of Species	Species with GenBank Records. n = Number of Records (Records Include at Least 18S and 28S rRNA Sequences)
	P. quartusdecimus, n = 5
	P. racemosae, n = 2
	P. riukiariae, n = 1, 18S RNA only
	P. sikae, n = 1, 18S RNA only
	P. sycomori, n = 5
	P. taiwanensis, n = 1, 18S RNA only
	P. trametes, n = 2
	P. triformis, n = 3
	P. uniformis, n = 121
	P. yamagatae, n = 1, 18S RNA only
	identified to genus level, n = 317
Mononchoides N = 51	*M. americanus*, n = 2
	M. cf. *americanu*, n = 2
	M. colobocercus, n = 1, 18S rRNA, only
	M. composticola, n = 6
	M. iranicus, n = 2
	M. kanzakii, n = 2
	M. macrospiculum, n = 18
	M. striatus, n = 18, 18S rRNA, only
	identified to genus level, n = 35

* genome sequenced, ** mitochondrial genome sequenced.

Among bacterial-feeders, the most numerous genera were *Halicephalobus* and *Acrostichus*. Depending on the type of compost and composting time, the number of *Acrostichus* sp. ranged from 1.9% to 72.9%. In the compost made of sewage sludge and sawdust during composting, the abundance of *Acrostichus sp*. decreased from 59.2% to 46.9%. The change in sewage sludge for garden and park waste reversed this relationship and during composting, abundance of this genus increased from 1.9% to 25.6%. In compost made from sewage sludge, garden and park waste and sawdust, changes in the abundance of *Acrostichus* sp. mirrored those in the compost prepared from sewage sludge and sawdust. The number of sequence reads decreased from 72.9% to 49.9%. After their introduction into the compost, *E. fetida* led to a noticeable increase in abundance of *Acrostichum* sp. In the compost, there was a detected presence of *A. floridensis*. An abundance of this species varied from <0.1% to 30.5%. To our knowledge, this was the first time the species was reported in compost.

Previous research suggested that earthworms could influence the structure of nematode communities and decrease nematode density by engaging in grazing and ingesting nematodes. The trophic groups only consisted of fungivores and bacterivores since no plant-parasitic, dorylaimid, or mononchid nematodes were recovered [31,32]. In our study, nematode communities were dominated by bacterivories and their abundance was reduced by earthworm activity in variant C5 (Figure 3E). We found that nematode density was higher in the treatments inoculated with earthworms in variant C6 (Figure 3F). Furthermore, fungal feeders were also more abundant there. This interesting issue would need further development to be better understood. Some reports [33,34] showed that vermicompost had the ability to suppress several groups of plant-parasitic nematodes. Rostami et al. [35] found

that most of the bacteria isolated from earthworms or vermicomposts showed biocontrol potentials against the root-knot nematode. In our study there was a complete reduction in plant-parasitic nematodes. During the heat phase of compost maturation, the majority of PPN were debilitated by the substrate's high temperatures. The remaining nematodes were affected by bacteria and fungi known to be harmful to parasitic nematodes [36]. Furthermore, they were deprived of their usual food source as they typically feed on the sap of actively transpiring plants. In contrast to PPN nematodes, bacterivorous nematodes exhibited a significantly higher capacity for generating subsequent populations (number of nematode generations). Therefore, the temperature experienced during the initial maturation phase did not hinder their ability to replenish the population. Parasitic nematodes, whose physiological traits rely on access to plant-based food, underwent slower individual development compared to other trophic groups. Therefore, in our temperate European climate, species that form cysts typically complete one full generation, with the second one rarely reaching maturity [23,27,37]. PPN were detected at the outset of composting. If the complete reduction in PPN occurred throughout the process, it implies that composting effectively eliminated these harmful nematodes, resulting in a compost that is both safe and beneficial for plant growth. The early detection of PPN reduction, particularly on the initial day of composting, indicates the efficiency of the composting process from its inception. This identification holds significant importance in agricultural practices, as it ensures that the produced compost is devoid of harmful nematodes, thereby decreasing the likelihood of plant diseases and enhancing overall crop health and productivity.

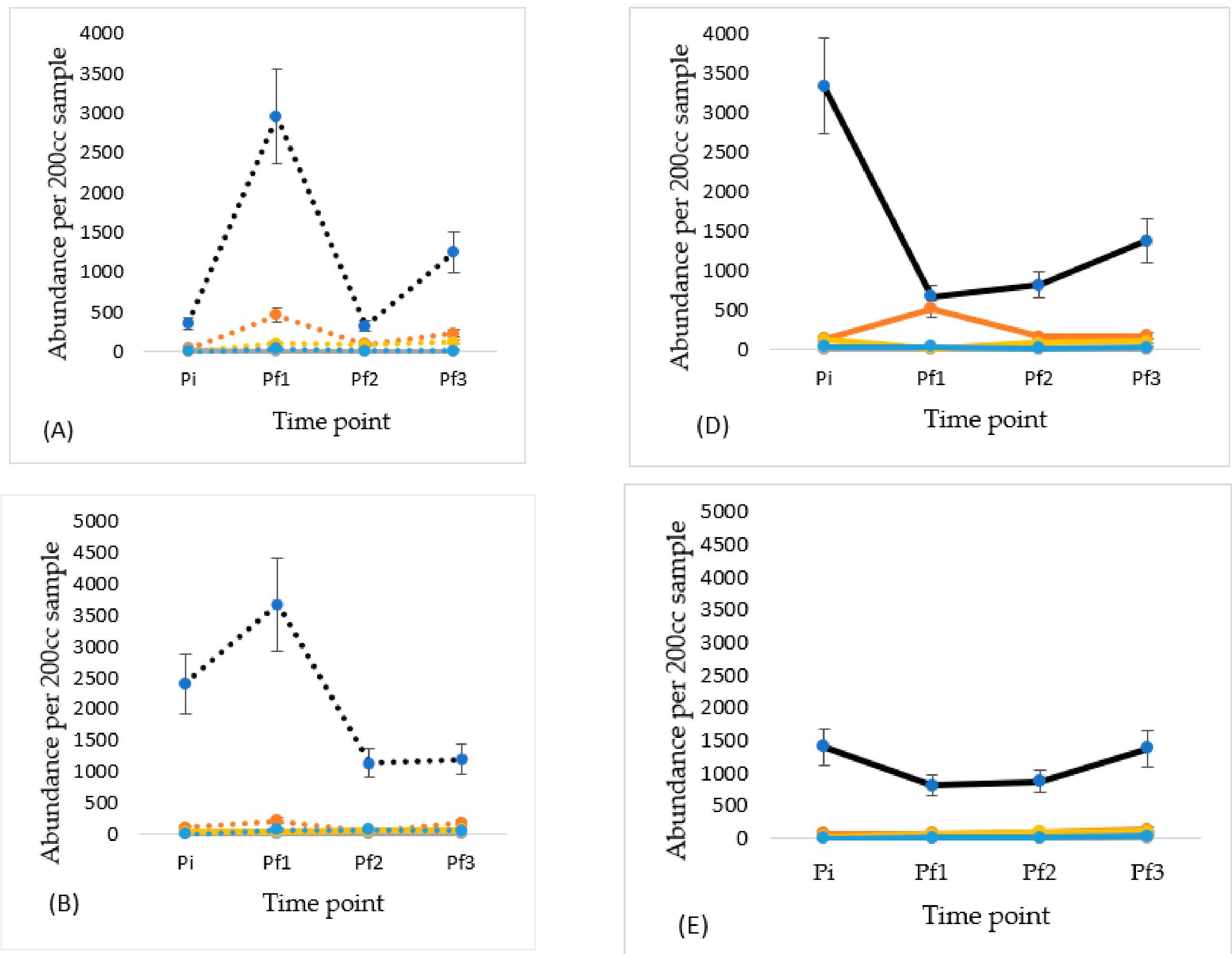
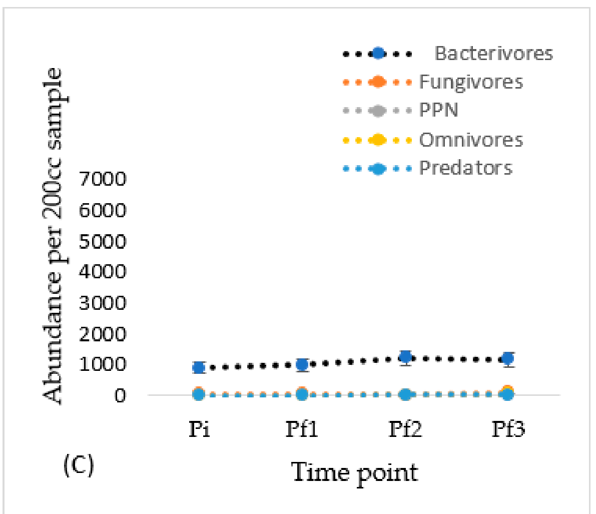


Figure 3. *Cont.*



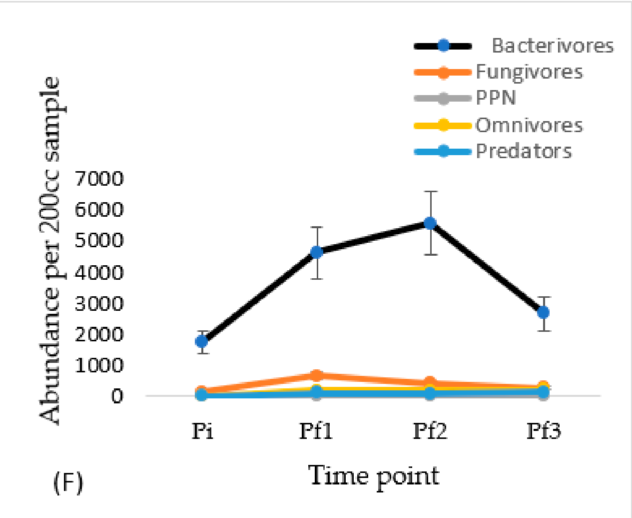


Figure 3. The nematode density per 200 cc compost sample at the level of five trophic group: bacterivores, fungivores, plant-parasitic nematodes (PPN), omnivores and predators in three compost variants without earthworms: C1 (**A**), C2 (**B**), C3 (**C**), and three compost variants with *Eisenia fetida*: C4 (**D**),C5 (**E**), C6 (**F**) during the fourth data collection period (Pi- on the initial day of composting, Pf1- on the 30th day of composting, Pf2- on the 60th day of composting, and Pf3- on the 90th day of composting) (n = 3).

Acrostichus is a genus with species inhabiting various habitats. They have been found to inhabit soil [38,39] dead wood [40] leafs, dung, and polluted water. Most species are bacteriophagous but may also feed on protozoa, fungi, and nematodes [41]. Our data are in-line with previously published. Unexpectedly, the analyzed compost samples detected the presence of sequences reads of *Acrostichus floridensis*, which occurrence previously had been reported only in Florida [42]. BLASTn analysis of the randomly selected 100 OTU assigned to this species showed that most of them had 100% identity with reference sequences from the GenBank database. This suggests that the range of this species is most likely much wider.

Panagrolaimidae can live in extreme conditions, including desiccation [43,44] freezing [45] and an unusual range of pH conditions [46]. Introduction into the compost *E. fetida* led to a noticeable reduction in *H. gingivalis* and *H.* cf. *gingivalis*, which also belongs to the Panagrolaimidae family (Figure 3). This species has been described as saprophytic bacteriovorous [47], which was detected in potting soil in Germany, in vegetable compost pile in the USA [48], in soil samples from the paddocks and meadows surrounding the stables and manure heap in Belgium [49] and in compost in Belgium [50]. It is referred to as a facultative parasite which infects horses [51–54], zebras (*Equus grevyi*) [55], humans [56,57] and some other species [52], causing granulomatous and neurological lesions. It has been detected worldwide but the majority of the reports in humans and equids so far come in Europe, North America and North East Asia [52]. Several routes of infection have been described, including the oral route via the ingestion of contaminated plant material [53], the respiratory route via inhalation of nematodes [52], and cutaneous infection [58]. Although *H. gingivalis* is a facultative parasite, infection from it is usually fatal. So far, treatment has been reported to be successful in only a few cases [47,58]. Moreover, it is tolerant to commonly use anthelmintic drugs, which does not allow us to expect better results of the therapy [49–59]. Halicephalobiosis, considered rare, can be a fatal disease that can result in mortality and morbidity in horses and humans, respectively. Our data align with those previously obtained [47,50], offering quantitative insights into the abundance and its alterations throughout the maturation of compost. Based on our experience, the incorporation of *E. fetida* leads to a decrease in *H. gingivalis* in all compost variants below 0.1% (Figure 4).

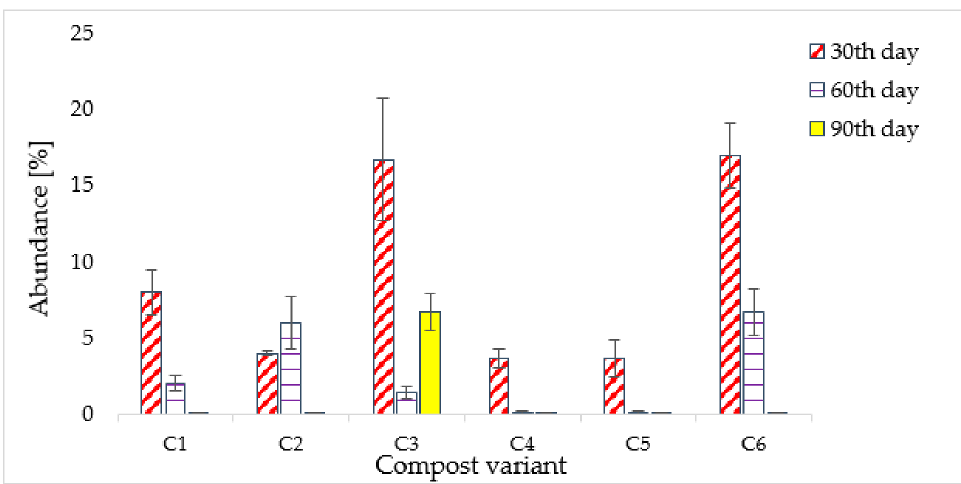


Figure 4. The *Halicephalobus gingivalis* abundance (%) in three variants without earthworms: C1, C2, C3, and three variants with *Eisenia fetida*: C4, C5, C6 on the 30th, 60th, and 90th day of composting (n = 3).

The presence of *E. fetida* in the compost significantly increased abundance of Diplogasteridae. In the compost without *E. fetida*, the amount of Diplodasteridae exceeds 0.1% only after the 30th (C3) or the 60th (C1, C2) day of composting, while in the compost with *E.fetida* they are present in large amounts (11.0–28.0%) already on the 30th day of composting (Figure 5).

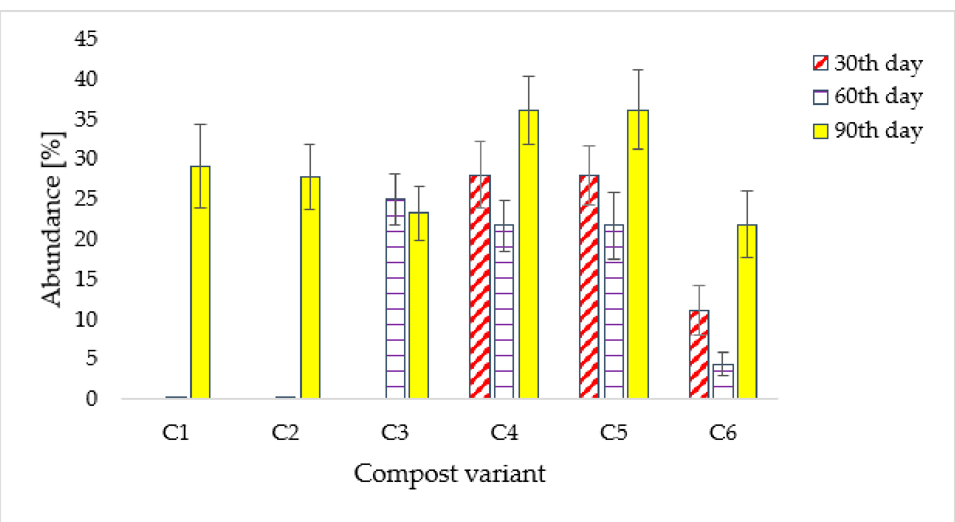


Figure 5. Diplodasteridae abundance (%) in three variants without earthworms: C1, C2, C3, and three variants with *Eisenia fetida*: C4, C5, C6 on the 30th, 60th, and 90th day of composting (n = 3).

4. Conclusions

The conducted research has provided insight into the dynamics of nematode communities during composting, highlighting the impact of *Eisenia fetida*. It was shown that the introduction of *Eisenia fetida* led to a significant increase in the abundance of *Acrostichum* sp., with the presence of *A. floridensis*, ranging from <0.1% to 30.5%.

The presence of *Eisenia fetida* in the compost significantly increased the abundance of Diplogasteridae, with significant numbers observed as early as day 30 of composting. In variants without *E. fetida*, Diplodasteridae exceeded 0.1% only after 30 (C3) or 60 (C1, C2) days of composting. However, in composts inoculated with *E. fetida*, significant amounts (from 11.0% to 28.0%) were observed already on the 30th day.

Additionally, the introduction of *Eisenia fetida* also led to a noticeable reduction in the number of *H. gingivalis* belonging to the Panagrolaimidae family, which is important for reducing the risk of halicephalobiosis.

A total elimination of plant-parasitic nematodes was observed, primarily ascribed to the elevated temperatures during the compost maturation phase, impacting their physiological characteristics and food sources.

In conclusion this study emphasizes the role of earthworms in shaping nematode populations in compost environments. The finding highlights the potential benefits of including *Eisenia fetida* are for effective nematode management and improving overall compost quality.

Author Contributions: Conceptualization, investigation, methodology, writing—original draft preparation A.Z.; investigation A.S.; formal analysis, C.P.; methodology, investigation, visualization, and writing—original draft preparation, T.M. All authors have read and agreed to the published version of the manuscript.

Funding: The project is financed by the program of the Minister of Education and Science named "Regional Initiative of Excellence" in the years 2019–2023, project number 026/RID/2018/19, the amount of financing PLN 9 542 500.00.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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