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Occurrence of Microplastics in the Sediments of an Irish River and Their Effects on Nematode Survival and Biodiversity

Loriane Murphy *, Kieran Germaine, John Cleary and Thomais Kakouli-Duarte *

Molecular Ecology and Nematode Research Group, enviroCORE, Department of Applied Science, South East Technological University, Kilkenny Road Campus, Kilkenny Road, R93 V960 Carlow, Ireland
* Correspondence: c00165715@setu.ie (L.M.); thomae.kakouli@setu.ie (T.K.-D.)

Abstract: Microplastics (MPs), defined as plastic particles of less than 5 mm, pose a significant global environmental threat, particularly in aquatic ecosystems, due to their persistence and potential harmful effects on wildlife and human health. They can absorb persistent organic pollutants (POPs), like polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), raising concerns about their impact on biota. To elucidate this impact, the present study employed attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) to analyse the characteristics of MPs sourced from commercial cosmetics. We investigated the toxicity of MPs on *Caenorhabditis elegans* and two entomopathogenic nematode species, *Steinernema feltiae* (the enviroCORE strain SB12(1)) and *Steinernema carpocapsae* (a commercial strain from e-NEMA) in laboratory bioassays. Nematodes were exposed to various concentrations of MPs and other pollutants, including atrazine, 1,3-dichloropropene, naphthalene, and fluorene, in controlled settings over 72 to 96 h. Additionally, high-throughput 18S rDNA sequencing was used to analyse nematode biodiversity in sediments from the River Barrow (RB) in SE Ireland. Our findings revealed that MPs increased nematode mortality and adversely affected community structure, as indicated by nematode maturity and sigma maturity indices, suggesting a potential disruption of the ecological balance in river sediments. This highlighted the ecological risks posed by MP pollution and emphasised the urgent need for further research into the health of benthic ecosystems in Ireland, particularly in relation to how MPs may influence nematode community dynamics and biodiversity.

Keywords: microplastics; nematodes; River Barrow; ecotoxicology; biodiversity; persistent organic pollutants

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1. Introduction

Microplastics are becoming persistent pollutants that are of growing concern [1] due to their potential toxicity to aquatic biota and human health, as well as their association with pathogenic bacteria and effects on the food web [2,3]. Microplastics have become ubiquitous worldwide, in the oceans, on land, and even in polar glaciers where human activity is minimal, and they are regarded as a rising threat to ecological and environmental sustainability [4]. The occurrence of MPs in water is an emerging worldwide environmental issue, with an estimated annual release of approximately 0.7 to 1.8 million tonnes of MPs into the environment being reported in Europe [5]. Microplastic particles have been reported in a variety of food and beverage items, including seafood, processed foods, beverages, honey, sugar, beer, milk, table salt, bottled water, tap water, and rice [6–8]. The large surface area and small volume of MPs, typically with particle sizes less than 5 mm, facilitate the adsorption and transfer of various environmental pollutants. These pollutants include PAHs, pesticides, polybrominated diphenyl ethers, pharmaceuticals, and personal care products [9], as well as heavy metals such as chromium (Cr), nickel (Ni) [6], copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), and titanium (Ti) [10–15]. When MPs enter aquatic environments, they can transport these contaminants into food webs, potentially

impacting aquatic organisms [16]. Additionally, plasticisers found in MPs can effectively adsorb toxic compounds, including PAHs and dichlorodiphenyltrichloroethane (DDT), which can then be transferred to living organisms, posing risks to human health through the food chain [17].

Microplastics can have physical effects on organisms when ingested, but they can also adsorb and transport chemical pollutants, including PAHs and pesticides. Polycyclic aromatic hydrocarbons are a large class of aromatic compounds produced through inadequate combustion or pyrolysis activities, such as automobile exhaust, cigarette smoking, industrial processes, the consumption of grilled and smoked food, fossil fuel combustion, and forest fires. Consequently, PAHs have been detected in high quantities in the air, water, and soil [18,19]. Similarly, pesticides are chemical mixtures employed to prevent, remove, repel, or neutralise organisms that pose challenges in agriculture, including microorganisms (bacteria, fungi, and viruses), insects, animals, and weeds [20]. The ecological impacts of pesticides vary based on pollutant concentration, quantity, and exposure duration, presenting significant hazards due to their persistence in the environment and propensity for bioaccumulation [20]. This leads to water and soil contamination, even at low application rates, coupled with poor biodegradability [21]. For instance, 1,3-dichloropropene is frequently utilised for its efficacy in controlling pathogenic nematodes and weeds [22]. In the field of herbicides, atrazine is a widely used, cost-effective pesticide known for its ability to control broadleaf and grassy weeds; however, it is also a major pollutant of soil and aquatic systems, demonstrating disruptive effects on aquatic flora and animal reproduction, which can adversely impact community structure [23,24].

In this study, nematodes were used as environmental indicators to investigate the effect of MPs and associated organic pollutants. Nematodes are the most abundant and diverse group of metazoans found in the sediment and soil, and they play a critical role in the food webs of benthic and soil environments [25]. Nematodes are non-segmented roundworms that live in almost every ecosystem on the planet and have considerable potential as environmental indicators. They are used as bioindicators in assessments of anthropogenic pollution because of their ecological significance, widespread prevalence, and exceptionally high individual densities [26]. Nematodes have been used to study gene expression in relation to environmental challenges, in laboratory ecotoxicity assays, and for in situ monitoring of the ecological impact of a variety of environmental problems [26,27].

Entomopathogenic nematodes (EPNs) from the families Steinernematidae (genus *Steinernema*) and Heterorhabditidae (genus *Heterorhabditis*) of the phylum Nematoda are soil-dwelling, lethal insect parasites, which can offer an alternative to chemical insecticides and are easily mass produced in vivo and in vitro [28,29]. Their symbiotic bacteria are released into their insect host hemocoel by nematode free-living infective juveniles (IJs), which penetrate insect hosts through natural body openings and pores in the cuticle. The subsequent resulting bacterial septicaemia kills the host quickly, and its cadaver is changed to a nutritious broth for the nematodes, mediated by the nematode symbiotic bacteria. By creating bacteriocins, antimicrobials, and other antibiotics, the bacteria protect the cadaver resource from rivals. Infective juveniles feed on the bacterial broth and mature into adults, which can give rise to multiple generations until resources are depleted. Then, new IJs emerge from the cadaver and disperse in the soil, looking for new hosts to infect [30–32].

Caenorhabditis elegans is a model organism that has been widely used to study genotoxic reactions to environmental contaminants from molecular to organismal levels [33,34]. Because of its small size, rapid life cycle (3–4 days), short average lifetime (2–3 weeks), and ease of rearing, this transparent, free-living nematode offers numerous benefits for genetic investigations [35,36]. *Caenorhabditis elegans* has shown a low-dose response to several chemicals after being used in assessing the toxicity of a range of environmental pollutants, including zinc oxide (ZnO) nanoparticles, which are commonly found in toothpaste, beauty products, sunscreens, and textiles, as well as bisphenol A (BPA), which is widely used in the manufacture of plastic products [36–38]. Ref. [35] emphasised the advantages of biological testing with *C. elegans*. *Caenorhabditis elegans* displays rapid growth on agar plates or in

liquid culture at temperatures ranging from 15 to 25 °C, using *Escherichia coli* as a food source. Its fast growth is evidenced by a half-day doubling time and a generation period lasting only 3 to 5 days. Additionally, advantages of using *C. elegans* include the possibility of self- or cross-fertilisation [39]. *Caenorhabditis elegans* is one of the species that has been used to study the toxicity of MPs [39,40]. In a number of previous studies, MPs have been shown to have negative physical and biological impacts on *C. elegans* [34,36,40,41].

However, there is limited research on the impact of MPs on other nematode indicators, such as EPNs, apart from the present study testing *C. elegans* and EPNs as sentinels of MP pollution. To assess sediment quality, nematodes have been used as an effective alternative to the existing conventional methods for this task, such as chemical analyses [42]. Both morphological and molecular (DNA-based) methods have been employed in the identification of nematodes in order to facilitate an effective assessment of biodiversity [43,44]. In the 1970s, ref. [45] studied the relationship between nematode community structure and sediment pollution (anthropogenic contamination with heavy metals and organic pollutants) and discovered that nematode community structure of river sediments was related to pollution and site structure [45,46]. To date, there is limited understanding of the specific effects of MPs on nematodes, particularly regarding their toxicity, mortality, and community structure in aquatic ecosystems.

Hence, the aims of the current study were (1) to improve understanding of MP properties and their interactions with organic pollutants, (2) to determine the toxic effects of MP particles using three nematode species (*C. elegans*, *Steinernema feltiae* (SB12(1)), and *Steinernema carpocapsae*) as sentinel organisms, and (3) to observe possible effects of MPs on nematode communities from Irish river sediments via high-throughput sequencing of nematode 18S rDNA for taxa identification.

2. Materials and Methods

This study investigated the effects of MP contamination on nematodes, utilising a comprehensive methodology divided into two key components: microplastic characterisation and studying nematode behaviour in response to MP exposure. The latter was achieved by carrying out laboratory MP toxicity bioassays on nematodes and also by analysing nematode communities in river sediments contaminated with MPs. Microplastics were isolated from two commercial facial scrubs, prepared by diluting the products in boiling water, followed by vacuum filtration and drying. These particles were characterised using ATR-FTIR spectroscopy for identification purposes and then treated with various pesticides and monitored for adsorption over a 12-month period. For toxicity bioassays, *C. elegans* and two species of EPNs (*S. feltiae* and *S. carpocapsae*) were cultured using nematode growth medium and insect hosts, respectively, and *C. elegans* were age synchronised to ensure uniformity in experimental conditions; all EPNs emerge from culture at the same age, so this step was not necessary. Toxicity assays used 96-well plates with nematode suspensions exposed to MPs and chemical contaminants, followed by nematode mortality assessments under a microscope. Sediments were collected from sites previously shown to be affected by MP contamination, as reported by [47]. Nematode communities in contaminated river sediments were analysed through DNA extraction and sequencing, providing insights into community diversity and the impact of MPs on ecosystem health. A one-way ANOVA was conducted to assess the significance of the observed effects, thus elucidating the relationship between MP pollution and nematode health in aquatic ecosystems.

2.1. Effects of MPs Contamination on Nematodes

2.1.1. Microplastic Samples' Preparation

Isolation of polyethylene MPs from a commercial exfoliating product was adapted from [48,49], with us selecting two of the most commonly used commercial facial scrubs: (i) Clean and Clear Exfoliating Daily Wash, and (ii) Clean and Clear Blackhead Clearing Daily Scrub. Each product was diluted in approximately 1 L of boiling water to extract MPs for further analysis. The mixture was then subjected to vacuum filtration using a

suction pump attached to a Buchner funnel with Whatman filter paper N°4. Filter papers retaining MPs were dried at room temperature overnight and then dried in an oven at 60 °C for 5 min. Once dried, the particles were transferred into labelled 1.5 cm centrifuge tubes. The recovered MPs were transferred onto slides analysed under a stereoscope (Optika microscopes) at a magnification of 40X. The total magnification was calculated by multiplying the objective lens magnification (4) by the ocular lens magnification (10), resulting in a total magnification of X40. Furthermore, MP particles were characterised using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. Spectra were collected from 4000 cm^{-1} to 500 cm^{-1} using a Perkin Elmer Spectrum 65 FT-IR Spectrometer sourced from Dulin, Ireland. A background scan was performed between each sample after cleaning the ATR diamond crystal with 70% 2-propanol. Absorption bands were recorded and compared to the reference spectra. Commercial clear polyethylene (PE) particles sized at 1000 µm http://www.cospheric.com/ (accessed on 18 September 2020) were used as a reference to identify the recovered MPs.

In this study, product 1, Clean and Clear Exfoliating Daily Wash, was selected for the toxicity bioassay due to the sizes of its MPs, which ranged from approximately 29 to 300 µm, compared to the MPs of product 2, measuring between 51.5 µm and 1 mm. Prior to toxicity assessments, the extracted MPs were transferred into clear sterilised 15 mL vials containing various treatments for 12 months, including Ringer's solution (untreated control), atrazine (100 ppb), 1,3-dichloropropene (100 ppb), naphthalene (100 ppb), and fluorene (100 ppb).

2.1.2. Nematode Cultures

Caenorhabditis elegans (sourced from cgc.umn.edu) and two species of entomopathogenic nematodes (EPNs), *Steinernema feltiae* (SB12(1) enviroCORE strain) and *Steinernema carpocapsae* (a commercial e-NEMA strain), were chosen as sentinel organisms to evaluate MP toxicity. Rearing techniques of EPNs and *C. elegans* were adapted from [35,50,51].

Culturing and Maintenance of *Caenorhabditis elegans*

Nematode growth medium (NGM) was used to grow *C. elegans* [35]. Nematode growth (NG) agar contained 3 g sodium chloride (NaCl), 17 g bacteriological agar (N°3), and 2.5 g bacteriological peptone and was made up to 975 mL with deionised water. The mixture was autoclaved (20 min, 121 °C) and was allowed to cool to 60 °C. It subsequently received 25 mL KH_2PO_4 pH 6.0, 1 mL of 1 M $MgSO_4$, 1 mL of 1 M $CaCl_2$, and 1 mL of 5 mg/mL cholesterol in ethanol. It was then aseptically poured into Petri plates. Prior to use, the plates were left to dry at room temperature for 2–3 days in a laminar airflow, for detection of any contaminants. A safety measure designed to detect and minimise the risk of contaminants, including MP contamination, has been detailed in the Supplementary Materials (Text S1).

Seeding of NGM Plates and Nematode Medium Preparation

To prepare NGM plates for growing *C. elegans*, approximately 20 µL of *Escherichia coli* OP50 liquid culture was aseptically added to 50 mL of Luria broth and incubated overnight at 37 °C in a shaking incubator. The culture was then seeded onto NGM plates in a laminar flow hood, allowed to dry, and a square-shaped piece of agar containing *C. elegans* was placed at the centre. The plates were sealed with parafilm and incubated at 21 °C for 24 h to ensure viable nematode growth before being used for toxicity tests. Additionally, a potassium phosphate buffer (pH 6.0) was prepared by mixing KH_2PO_4 and K_2HPO_4 solutions, while solutions of 1 M calcium chloride and 1 M magnesium sulphate were made and autoclaved, with cholesterol being prepared in ethanol without autoclaving [52].

Culturing and Maintenance of Entomopathogenic Nematodes

Two species of EPNs, *Steinernema feltiae* (the SB12(1) enviroCORE strain) and *Steinernema carpocapsae* (a commercial e-NEMA strain), were cultured, maintained, and kept at

9 °C in the laboratory. *Galleria mellonella* was sourced commercially from Live Foods Direct (Sheffield, UK) and was used as a host to culture EPNs. Two sheets of Whatman filter paper were used to line the lids of standard-sized Petri dishes with a diameter of 100 mm and a height of 15 mm. The filter paper was moistened with approximately 1–1.5 mL of a dense IJ suspension. Following this, five late instar larvae of *G. mellonella* were carefully placed on the filter paper, and the base of the Petri dish was added as a lid to close the dish. To keep the filter paper moist, the dish was loosely wrapped with parafilm. The plates were subsequently incubated for 3–7 days in darkness at a temperature of 21 °C until mortality of *G. mellonella* occurred. Nematode IJs were recovered using the white trap method [53] after insect mortality had occurred. An inverted Petri dish (60 × 15 mm) was placed in a clear container (150 × 30 mm). Filter paper was used to cover the dish and create a raised platform. Enough sterile deionised water was then placed in the clear container to completely submerge the filter paper's edges. The infected insects were placed on the platform and incubated for up to 14 days at room temperature. Nematodes were harvested after emergence by decanting the water into an empty clear dish. The infected host's transparent dish was refilled with sterile deionised water and incubated for another 1–3 days. Incubating for an additional 1–3 days ensures maximal emergence of nematodes from infected insects, thereby enhancing the experimental yield. Nematodes were then maintained at 9 °C until needed, with no more than two weeks between emergence and experimentation.

2.1.3. *Caenorhabditis elegans* Age Synchronisation Using Alkaline Hypochlorite Solution (Bleaching)

The bleaching technique is used for age synchronising *C. elegans* cultures at the first larval stage (L1). Age synchronising in the rearing of *C. elegans* ensures that all the nematodes in a culture reach the L1 stage at the same time, promoting consistency in experimental timing and comparisons. *Caenorhabditis elegans* can be synchronised by isolating newly hatched larvae or by treating gravid adults with bleach (by killing everything but embryos) and isolating eggs, which are resistant to bleach treatment. After treatment with alkaline hypochlorite solution composed of 3.75 mL sodium hydroxide, 3.0 mL bleach, and 8.25 mL double deionised water, embryos are incubated in liquid media without food, which allows hatching but prevents further development [50,52,54].

To age synchronise *C. elegans*, the nematodes were cultured on NGM plates until the adult stage. The nematodes were allowed to grow for two to five days to recover enough embryos. Then, seeded NGM plates were washed out to recover all the nematodes using Ringer's solution. The nematodes in Ringer's solution were transferred into 15 mL Falcon conical centrifuge tubes and centrifuged for 2 min at 1500 rpm, at room temperature. The supernatant was discarded, and this step was repeated three times to ensure that most of the worms were transferred into Ringer' s solution. Most of Ringer's solution was aspirated without disturbing the nematode pellet. Subsequently, 15 mL of the 20% alkaline hypochlorite solution was added to each tube. The tubes were gently inverted and agitated for 5 min and centrifuged for 1 min at 1500 rpm. Following that, 20% alkaline hypochlorite solution was aspirated without disturbing the nematode pellet. About 15 mL of Ringer's solution was added to the tube. The tube was furthermore centrifuged for 1 min, and this step was repeated three to four times since the treatment may still have been active. The embryos were transferred onto new unseeded NGM plates (without food—*E. coli* OP50) to allow the eggs to hatch. The culture plates were incubated for 3 days prior to MP toxicity tests.

2.1.4. Toxicity Bioassay of EPNs and *C. elegans* Using Microplastic Pollutants

Nematode suspensions were transferred into sterile Falcon tubes to obtain the required concentration for the assays. The Falcon tubes were left upright for 30 min to ensure complete settlement of nematodes at the bottom of the tubes. The supernatant was slowly decanted using a sterile glass pipette to obtain an approximately 10 IJ/100 µL suspension.

Subsequently, 96-well plates were used to conduct the toxicity assays using a fresh batch of EPNs and *C. elegans*. To investigate the effects of MPs on EPNs and *C. elegans*, each well was filled with 100 µL of sterilised deionised water with approximately 10 juveniles of EPNs and *C. elegans* at the L1 stage, and we added approximately 0.01 g MP particles. The particles had been treated with Ringer's solution, which was used as an untreated control, and atrazine (100 ppb), 1,3-dichloropropene (100 ppb), naphthalene (100 ppb), and fluorene (100 ppb), for 12 months prior to toxicity bioassays. Afterward, 100 µL of PAHs and the pesticides mentioned above were added to each well (see Table 1). Nematodes were then counted under a stereo microscope (Optica SZM-2), Ponteranica, Italy at magnifications ranging from 7× (minimum) to 45× (maximum). The total magnification was calculated by multiplying the objective lens magnifications (0.7 and 4.5) by the ocular lens magnification (10). The nematode mortality was assessed after 72 and 96 h exposure (Figure 1). The results were recorded as the mean percentage mortality of nematodes in each treatment.

Table 1. Experimental design. The toxicity bioassays were run in 96-well plates. Each treatment was tested with a total of 10 replicates. For the control group, Ringer's solution served as the blank sample. Ringer's/Untreated refers to the untreated control group, while the rest are treated groups.

	Treatment								
Ringer's/Untreated	10	10	10	10	10	10	10	10	10
Ringer's/Untreated + MPs	10	10	10	10	10	10	10	10	10
Atrazine 100ppb	10	10	10	10	10	10	10	10	10
Atrazine 100ppb +MPs	10	10	10	10	10	10	10	10	10
Ringer's/Untreated	10	10	10	10	10	10	10	10	10
Ringer's/Untreated + MPs	10	10	10	10	10	10	10	10	10
Napht100ppb	10	10	10	10	10	10	10	10	10
Atrazine 100ppb +MPs	10	10	10	10	10	10	10	10	10

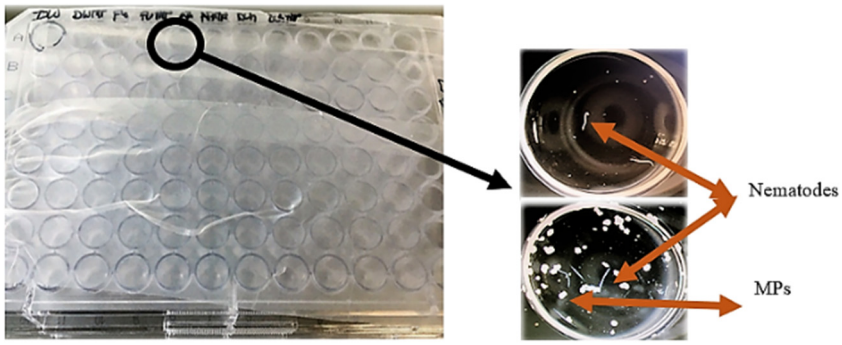

Figure 1. Nematode mortality was assessed using a stereoscope in 96-well plates, each containing various treatments and 10 nematodes.

2.1.5. Statistical Analysis

ANOVA was carried out to identify significant effects on nematode mortality of the induced MP treatments. The statistical analysis was conducted using Microsoft Office Excel (Version 2410, Build 18129.20158). The level of significance was set at $p \leq 0.05$.

2.2. Effects of MPs on Nematode Communities in Irish River Sediments

2.2.1. Sediment Sampling and Processing

River sediment samples were collected in triplicate from four previously studied locations: Dolmen Hotel—Carlow (DHC, 52°49′58.5″ N 6°55′30.0″ W), Milford—Carlow (MLF, 52°47′04.7″ N 6°57′60.0″ W), MacMurroughs—Wexford (MCR, 52°25′13.0″ N 6°56′04.9″ W), and Great Island (GI, 52°16′58.9″ N 6°59′52.3″ W). The samples were taken along the RB from a depth of 10 cm using a Dutch auger. The collected samples were stored at −20 °C prior to analysis. Subsequently, 25 g sediment sub-samples were transferred to sterile

Falcon tubes containing 25 mL deionised water and centrifuged for 2 min at 3500 rpm. The supernatant was discarded, and the remaining materials were placed in Petri dishes and dried overnight at room temperature. Furthermore, the dried sediment soil was thoroughly homogenised using a mortar and pestle. Afterward, total DNA was extracted from 0.25 g of the dried sediment sub-samples using the Qiagen DNeasy® PowerSoil® Pro kit, Hilden, Germany following the manufacturer's protocols. Prior to exporting to a sequencing company (Novogene, Beijing, China), the concentration and quality of the DNA were evaluated using an InvitrogenTM QubitTM 4 Fluorometer (Thermo Fisher Scientific, Hong Kong), OR, United States. DNA purity was then assessed using 1.5% agarose gels. The DNA samples were stored at −80 °C until being sent for sequencing. The primer pair MN18F and 22R [55] was used on the Illumina paired-end platform (Novogene, Beijing, China), and the nematode 18S V4V5 rRNA region was sequenced by the sequencing company.

2.2.2. 18S rDNA Gene Amplification and DNA Sequencing

The sediment DNA samples were sent to Novogene Ltd. (Beijing, China) for subsequent polymerase chain reaction (PCR), library preparation, and DNA sequencing. Specifically, a PCR primer set MN18F (forward) (5′-CGCGAATRGCTCATTACAACAGC-3′) and 22R (reverse) (5′-GCCTGCTGCCTTCCTTGGA-3′), adapted from [55], was provided by the authors to the company and used to amplify the V4V5 region of the 18S gene in nematodes. Following PCR amplification, libraries were produced by Novogene using the NEBNext® UltraTM DNA Library Prep Kit from Illumina, following the manufacturer's guidelines, and indexed. Library quantification was performed by the company using Qubit and qPCR, and sequencing was carried out on the Illumina platform, generating 250 bp paired-end reads.

2.2.3. Analysis of the DNA Sequencing Data

DNA extracted from sediment samples was submitted to Novogene for sequencing. Novogene provided the DNA sequence data, as well as diversity indices. Data provided by Novogene, including diversity indices, OTU table, and heatmap, was further analysed using Microsoft Excel. The alpha diversity indices of nematodes in river sediment, including observed operational taxonomic unit (OTU) number, Chao1, Simpson, and Shannon values, were analysed using QIIME software (Version 1.7.0) on Illumina MiSeq sequencing data by the sequencing company Novogene Ltd. (Beijing). The paired-end reads were assigned to samples based on their unique barcodes and truncated by cutting off the barcode and primer sequences. For alpha diversity, the number of OTUs was used to evaluate the diversity of the river sediment nematode communities. Paired-end reads were merged using the fast and accurate tool FLASH (V1.2.7), specifically designed to combine reads with overlapping sequences into raw tags. Quality filtering on the raw tags was performed under specific filtering conditions to obtain the high-quality clean tags according to the Qiime (V1.7.0) quality-controlled process. Sequence analysis was performed by Uparse software (Uparse v7.0.1090) using all the effective tags and clustered into operational taxonomic units (OTUs) at ≥97% similarity, in order to analyse the species diversity in each sample. For each representative sequence, Qiime (Version 1.7.0) in the Mothur method was used to perform the assignment of taxonomy against the SSU rRNA of the SILVA138 database. The species annotation was conducted at each taxonomic rank (kingdom, phylum, class, order, family, genus, species) with a threshold of 0.8~1. A principal component analysis (PCA) was performed to investigate the clustering of samples based on OTUs. The more similar the composition of communities among the samples, the closer the distance of their corresponding data points on the PCA graph. Analyses of similarity (Anosim) and permutational MANOVA (ADONIS) in R software (Version 2.15.3) [56–59] were also used to evaluate the significance in nematode communities between sediment samples from the four selected locations. Heatmaps were generated using the FactoMineR package in R software (Version 2.15.3). Boxplots were formed to analyse the difference in alpha and beta diversity indices across nematode community groups from the selected locations. T-

tests and Wilcoxon and Tukey tests were used to investigate the significance of MP impacts on nematode communities.

The maturity indices (MIs) based on the nematode coloniser–persister (c-p) scale [60] the ecological triangle model, and food web analysis of nematode communities were calculated to assess the health of river sediments. This was performed by submitting the sediment nematode data obtained from 18S rDNA sequencing to NINJA (Nematode INdicator Joint Analysis [60], provided at https://shiny.wur.nl/ninja/ (accessed on 5 February 2023).

3. Results

3.1. Effects of Contaminated MP on Nematodes

3.1.1. Interactions Between Microplastics and Organic Pollutants

Figure 2a and Table 2 present a comparison between the absorption bands obtained from the recorded data and those found in polymer reference spectra. ATR-FTIR analysis of MPs exposed to atrazine for a 12-month period showed clear evidence of atrazine being retained on the MP surface. Figure 2b shows that, in addition to the four peaks associated with the original MPs (Figure 2a), twelve additional peaks appear that correlate with peaks on the atrazine reference spectrum (Figure 2c). These peaks are at 3300 cm^{-1}, 3150 cm^{-1}, 1618 cm^{-1}, 1544 cm^{-1}, 1401 cm^{-1}, 1343 cm^{-1}, 1304 cm^{-1}, 1264 cm^{-1}, 1165 cm^{-1}, 1128 cm^{-1}, 1056 cm^{-1}, 991 cm^{-1}, 809 cm^{-1}, and 803 cm^{-1}. For MPs exposed to 1,3-dichloropropene (Figure 2d,e) and naphthalene (Figure 2f,g), the same type of correlation was not observed; however, a number of additional peaks appear on MP spectra after exposure (Figure 2d,f), which are not present for the original MPs (Figure 2a). The additional peaks that appear in Figure 2d,f do not appear in the spectra for MPs exposed to atrazine or fluorene, so they cannot be ascribed to degradation of the polymer. These peaks are, therefore, attributed to sorption of 1,3-dichloropropene and naphthalene on the surface of the MPs; however, these pollutants do not seem to be retained on the MPs to the same extent as atrazine.

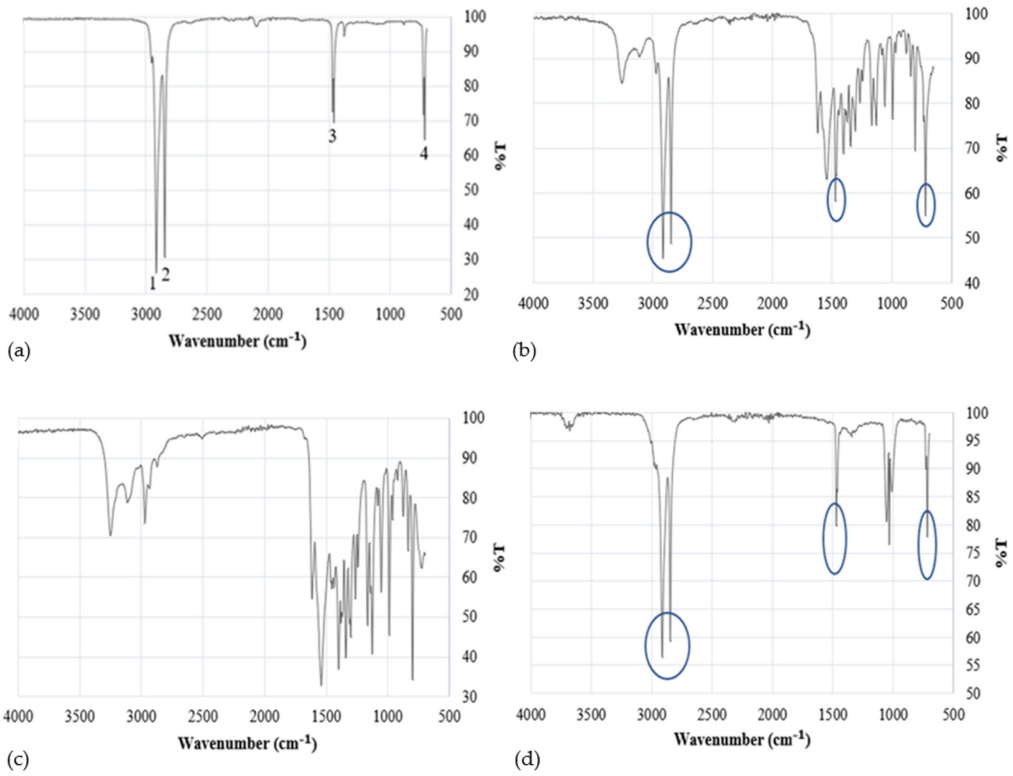

Figure 2. *Cont.*

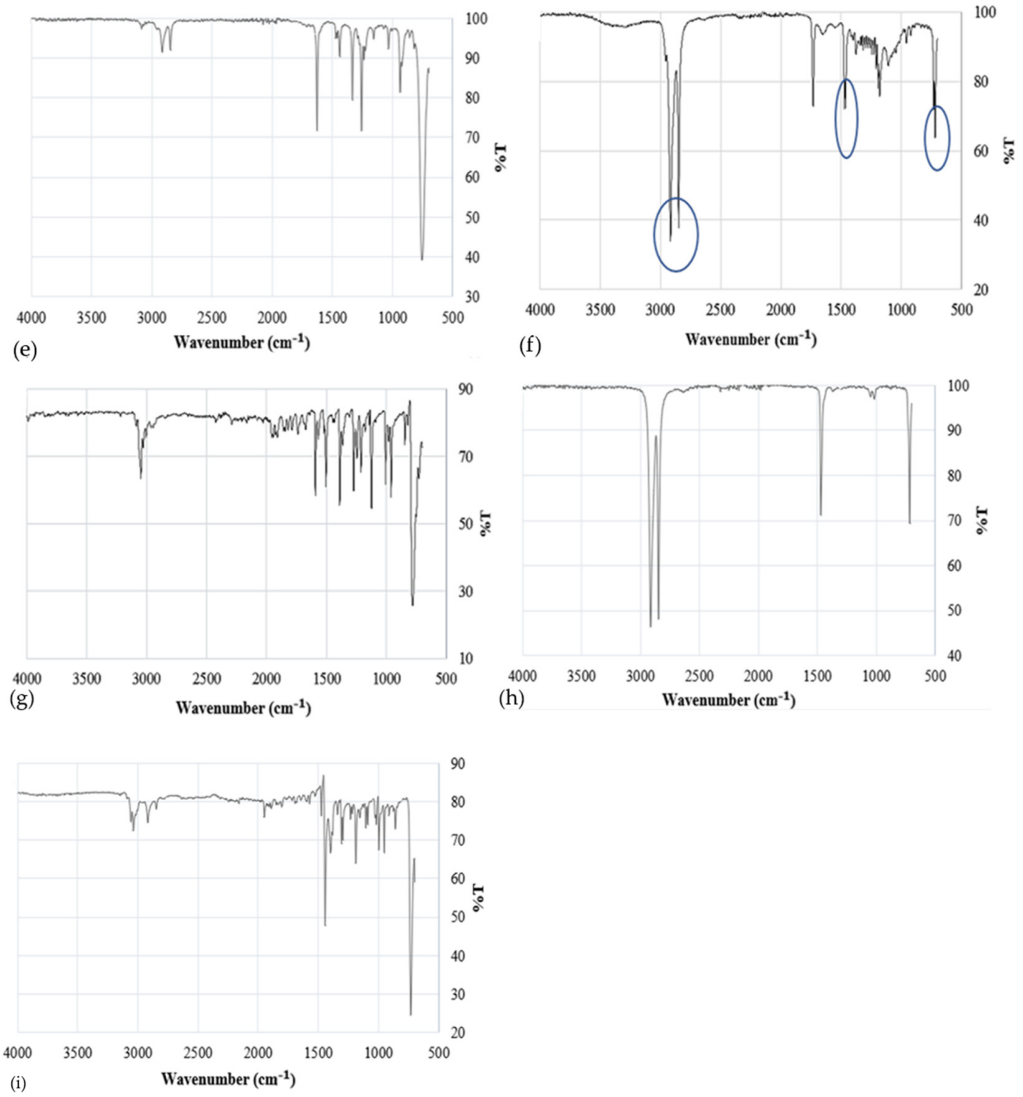

Figure 2. (**a**) ATR-FTIR spectrum of commercial clear polyethylene; ATR-FTIR spectra of MPs after immersion in (**b**) 100ppb atrazine, (**c**) atrazine reference spectrum; (**d**) 100 ppb 1,3-dichloropropene, (**e**) 1,3-dichloropropene reference spectrum; (**f**) 100 ppb naphthalene, (**g**) naphthalene reference spectrum; and (**h**) 100 ppb fluorene, (**i**) fluorene reference spectrum, showing MP absorption of toxic chemicals. The blue circles on the spectra represent original peaks before MP exposure to treatments.

Table 2. ATR-FTIR peak data and assignments for polyethylene.

Polymer	Absorption Band (cm^{-1})	Assignment
Polyethylene (PE)	2915	C-H stretch
	2848	C-H stretch
	1472	CH_2 bend
	717	CH2 rock

There were no significant changes or additional peaks on the spectrum for the MPs after exposure to fluorene (Figure 2h) compared to the spectrum of the original MPs (Figure 2a), suggesting that fluorene was not significantly retained on the MPs.

3.1.2. Toxicity Bioassays of MPs on Nematodes

After 72 h exposure, the results showed high mortality in *S. carpocapsae* IJs and *C. elegans* J3 in all the treatments containing MPs compared to mortality in *S. feltiae* SB12(1) IJs. However, *S. carpocapsae* IJs were the most sensitive to all the treatments during both

72 and 96 h time exposures. In addition, *S. carpocapsae* IJs were more significantly affected by MPs with all the treatments compared to *S. feltiae* SB12(1) IJs and *C. elegans* J3 ($p < 0.05$) (Figure 3). *Steinernema feltiae* SB12(1) IJs were the least affected in all the treatments. There was only a significant difference between the mortality of *S. feltiae* SB12(1) IJs exposed to the combination treatments of atrazine with no MPs and the mortality in the treatment with MPs ($p < 0.05$), which confirms that MP particles have some impacts on nematodes. Mortality of the three nematode species was significantly higher in the combination treatments of 1,3-dichloropropene with no MPs and with MPs (F = 2.861, $p = 0.002724$) in comparison to the treatments with atrazine, naphthalene, and fluorene. Investigating nematode exposure to MP particles in different treatments revealed that chemicals adhere to MPs and are subsequently transferred into nematodes. Acute exposure to contaminated MP particles resulted in a time- and dose-dependent increase in nematode mortality. All three nematode species were readily affected by MP treatments; however, the mean mortality percentage was significantly different among all the treatments (Figure 3).

A single-factor one-way ANOVA of the data showed significant mortality variation among the three nematode species. The analysis of variance (ANOVA) was conducted on the three nematode species and various treatments, with the results presented in Table 3. Notably, Table 3 presents a significant effect of an increasing exposure time to naphthalene on the three nematode species. Specifically, following 96 h exposure to naphthalene-treated MPs, there was a statistically significant difference in nematode mortality (one-way ANOVA: F = 1.954, $p = 0.008807$).

Table 3. F and *p*-values of nematode mortality in various treatments.

Treatment	Exposure Time (Hours)	F	*p*-Value
Nematode medium, atrazine, and MP	96	0.964	0.527931
Nematode medium, naphthalene, and MP	72	0.938	0.563178
Nematode medium, naphthalene, and MP	96	1.954	0.008807
Nematode medium, fluorene, and MP	72	1.645	0.039446
Nematode medium, fluorene, and MP	96	0.639	0.913678
Nematode medium, 1,3-dichloropropene, and MP	72	0.718	0.843136
Nematode medium, 1,3-dichloropropene, and MP	96	0.948	0.548654

When comparing the mortality rates of *S. carpocapsae* IJs, *C. elegans* J3, and *S. feltiae* SB12(1) IJs to those of the untreated control (nematode medium with MP), it was observed that the mortality rates were 26%, 21%, and 23%, respectively. The mortality of *S. carpocapsae* IJs and *C. elegans* J3 increased significantly to 60%, and 41%, respectively, in MPs treated with 1,3–dichloropropene, at 96 h exposure. However, *S. feltiae* SB12(1) IJ mortality was lower than that of *S. carpocapsae* IJs, and for *C. elegans* J3, reached 38%. Furthermore, the mortality rates of *S. carpocapsae* IJs, *C. elegans* J3, and *S. feltiae* SB12(1) IJs, after 96 h of exposure to MPs treated with atrazine, were 46%, 41%, and 35%, respectively, compared to 32%, 29%, and 25%, respectively, when exposed to MPs that were not treated (control). The mortality rates of *S. carpocapsae* IJs, *C. elegans* J3, and *S. feltiae* SB12(1) IJs were also affected in MPs treated with naphthalene at 96 h, reaching 40%, 35%, and 30%, respectively, compared to the control treatment containing MPs and showing mortality rates of 34%, 27%, and 23%, respectively. Moreover, the mortality rates of *S. carpocapsae* IJs, *C. elegans* J3, and *S. feltiae* SB12(1) IJs were impacted by exposure to MPs treated with fluorene, resulting in rates of 41%, 37%, and 33%, respectively. These rates were notably higher than the control rates of 30%, 26%, and 22%, respectively.

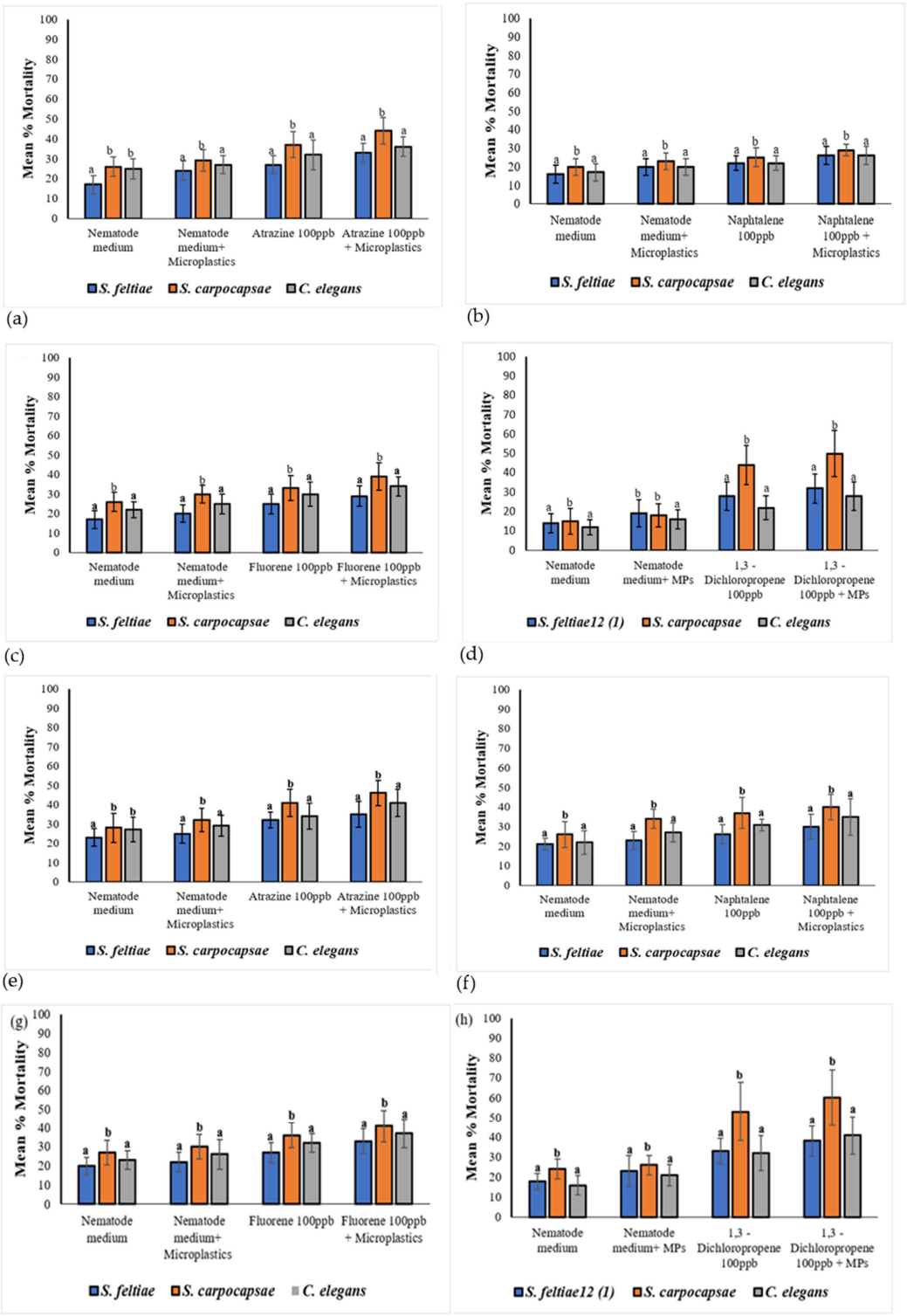

Figure 3. Comparison of mortality of *S. feltiae* SB12(1) IJs, *S. carpocapsae* IJs, and *C. elegans* J3 exposed to a mixture of MPs in various treatments after 72 h (**a**–**d**) or 96 h (**e**–**h**). Different letters above the bars indicate statistically significant differences ($p < 0.05$) as determined by one-way ANOVA. The data represent the average of 10 replicates per sample (n = 10). A *p*-value greater than 0.05 is indicated by the letter 'a', while a *p*-value less than 0.05 is indicated by the letter 'b'.

Significant differences in mortality of all species were observed among certain treatments containing MPs. For example, after 96 h exposure, *S. carpocapsae* IJ mortalities caused by atrazine and 1,3-dichloropropene were significantly ($p < 0.05$) higher compared to those

caused by naphthalene and fluorene in the same nematodes. Overall, there was a significant impact of MPs on the three nematode species among the treatments. Microplastics treated with 1,3-dichloropropene were found to be the most toxic treatment in this bioassay, as most nematodes were affected, which was not the case for the rest of treatments (Figure 3a–h).

3.2. Characteristics of DNA Sequences from Sediment Nematodes

The results of electrophoresis of sediment-extracted DNA samples are illustrated in Figure 4. After Novogene Ltd. (Beijing) optimised the sequencing results of sediment nematode DNA fragments on the Illumina paired-end platform, effective DNA sequences encoding vital biological functions within an organism were generated from 20 sediment samples. A total of 2,499,108 clean reads, defined as high-quality sequencing reads that are free from errors, artifacts, or contamination, were obtained through analysis provided by the sequencing company Novogene Ltd. (Beijing). The mean number of reads obtained for DHC samples was 119,975 ± 4251.099, for GI samples was 125,182 ± 4426.391, for MCR samples was 129,313 ± 5852.505, and for MLF samples was 125,352 ± 3313.495.

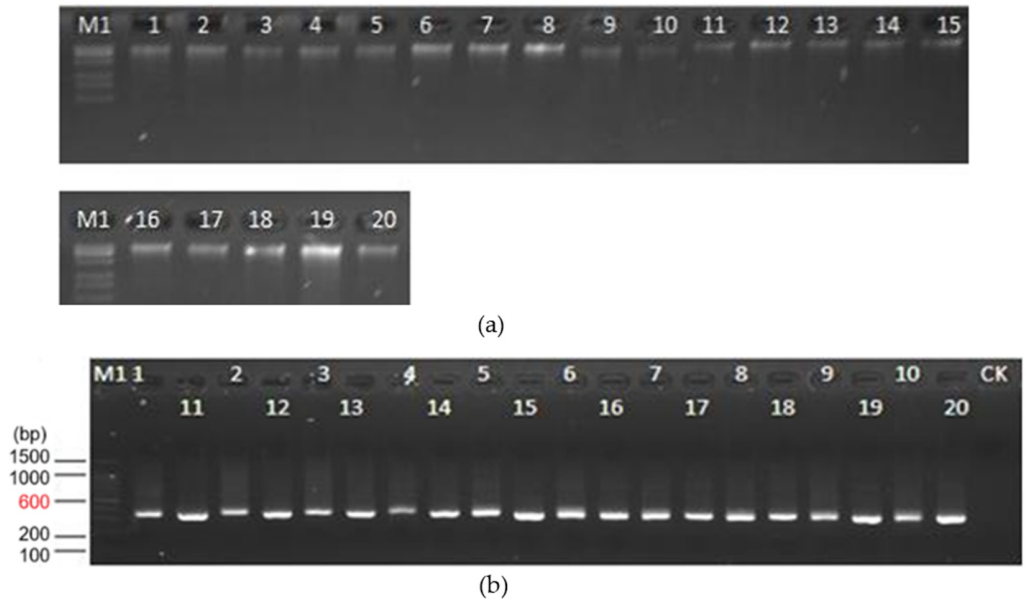

Figure 4. (**a**) Agarose (1.5%) gel electrophoresis of river sediment DNA at 100 volts for 40 min. Note: M1 indicates Trans 15K plus marker, lanes 1 to 20 represent samples; (**b**) PCR product gel electrophoresis of samples processed by Novogene Ltd. (Beijing) sequencing company. Note: M1 indicates Trans 100 bp ladder. PCR amplicons amplified in different samples; lanes 1–5: DHC samples, lanes 6–10: MLF samples, lanes 11–15: MCR sample, and lanes 16–20: GI samples.

The dilution curves of the sediment samples tended to be flat at all four locations, while the overall OTU coverage of the samples appeared to have reached saturation, suggesting that the sequencing depth adequately reflected the structure of the nematode community (Figure 5).

3.2.1. Sediment Nematode Community Composition

At the class level, the relative richness of Enoplea was 32% and of Chromadorea 68%. However, Enoplea richness was only higher than that of Chromadorea at the DHC location. In general, Chromadorea was the dominant class, accounting for 22.60%, 35.70%, 55.10%, 46.10%, 23.00%, 53.80%, 24.50%, 37.30%, 89.40%, 80.40%, 57.10%, 97.00%, 90.30%, 91.50%, 82.80%, 89.90%, 98.10%, 92.50%, 98.60%, and 94.60% of the nematode community at DHC1, DHC2, DHC3, DHC4, DHC5, MLF1, MLF2, MLF3, MLF4, MLF5, MCR1, MCR2, MCR3, MCR4, MCR5, GI1, GI2, GI3, GI4, and GI5, respectively (Figure 6). At the order level, Chromadorida (21.4%), Desmodorida (6.3%), Monhysterida (36.5%), Dorylaimida

(14.6%), and Triplonchida (13.7%) were the dominant orders, accounting for 92.4% of the total nematode sequences obtained (Figure 6a). Of the nine most frequently occurring nematode orders, significant differences were found in the abundances of Monhysterida, Dorylaimida, and Chromadorida compared to Triplonchida and Desmodorida (Figure 6b).

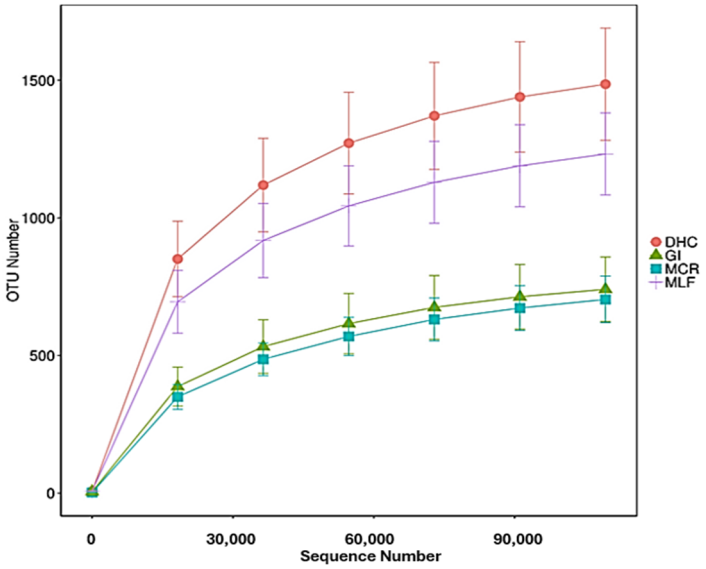

Figure 5. Rarefaction curve of the number of operational taxonomic units (OTUs) of a sediment nematode community. Operational taxonomic units were delineated at 97% sequence similarity: DHC: Dolmen Hotel Carlow, GI: Great Island, MCR: MacMurroughs, MLF: Milford. The *X*-axis represents the sequence number, while the *Y*-axis depicts the observed number of OTUs.

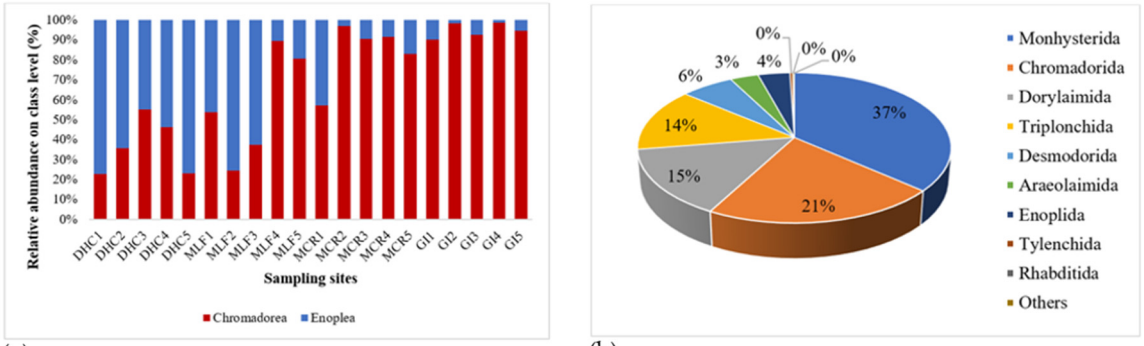

Figure 6. River sediment nematode abundances. (**a**) Relative abundance of sediment nematode classes in samples from the locations of this study; (**b**) relative distribution of sequences in the nematode dataset within different nematode orders for all sediment samples.

The relative abundances of the 10 most prevalent nematode genera in the RB sediment samples obtained by 18S rDNA gene sequencing are summarised in Figure 7a. The dominant genera groups in the river sediment nematode community of DHC were Triplonchida and Mermithida, both with a relative abundance of 28%, followed by Monhysterida (21%). Chromadorida was less abundant in MCR (9%), but dominated in MLF (28%), followed by Monhysterida (25%). Desmodorida was mainly identified in MCR (6%) and GI (17%). Araeolaimida was not present in the other two locations, with lower abundance observed in the MLF (2%) and MCR (11%). However, the main nematode group in the sediment nematode communities of MCR and GI was Monhysterida, with relative abundances of 57% and 44%. On the other hand, Chromadorida was dominant in the GI (34%) and MLF (28%) samples, followed by Dorylaimida, which was in abundance in the MLF (20%) and

DHC (34%) samples. Desmodorida was in a higher abundance in the GI (17%) samples and lower in the DHC (1%) samples. Monhysterida and Araeolaimida were mostly dominant as nematode orders in MCR compared to the rest of the locations (Figure 7a). The heatmap shows that the orders Monhysterida and Araeolaimida were mostly dominant in the sediment samples from MCR compared to sediment samples from the other three locations, followed by Dorylaimida, Triplonchida, and Tylenchida, which were in higher abundances in the DHC and MLF samples (Figure 7b).

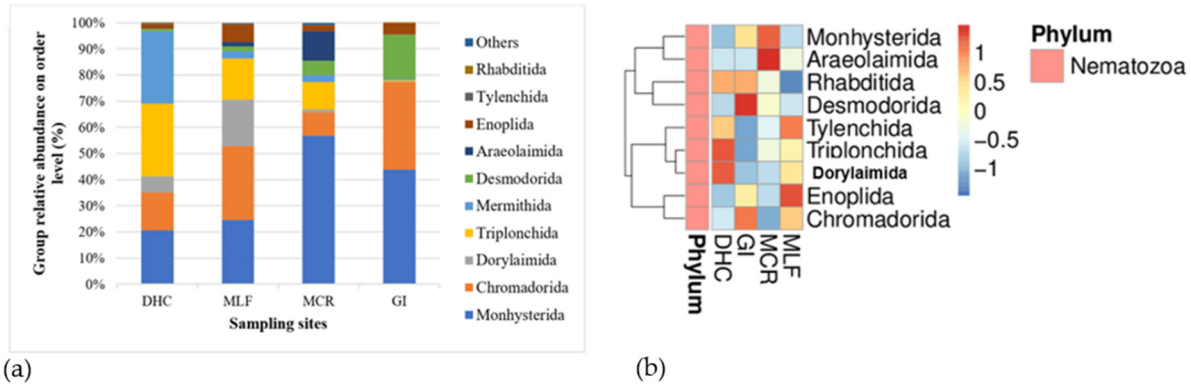

Figure 7. River sediment nematode community composition. (**a**) The most frequently occurring nematode genera in the study sites, and (**b**) heatmap representing the abundances of the nine most prevalent nematode orders among the four locations. Location group names: DHC: Dolmen Hotel—Carlow; GI: Great Island—Wexford; MCR: MacMurroughs—Wexford; and MLF: Milford—Carlow. The colour coding indicates the heatmap scale from 1 to −1.

At the genus level, there was a high diversity of nematodes within the DHC, MLF, and MCR samples, and a much lower diversity was observed in the GI samples. Monhysterida OTUs were identified in all the samples from the four locations, representing averages of 21 ± 11%, 25 ± 12%, 57 ± 22%, and 44 ± 26% of the total genera from the DHC, MLF, MCR, and GI samples, respectively (Figure 8a). In order to study the differences in nematode communities, the distribution of OTUs was measured. The analysis revealed that 63 OTUs were shared among all the samples. In addition, 16 unique OTUs were found in GI, 21 in MCR, 27 in MLF, and 65 in DHC (Figure 8b).

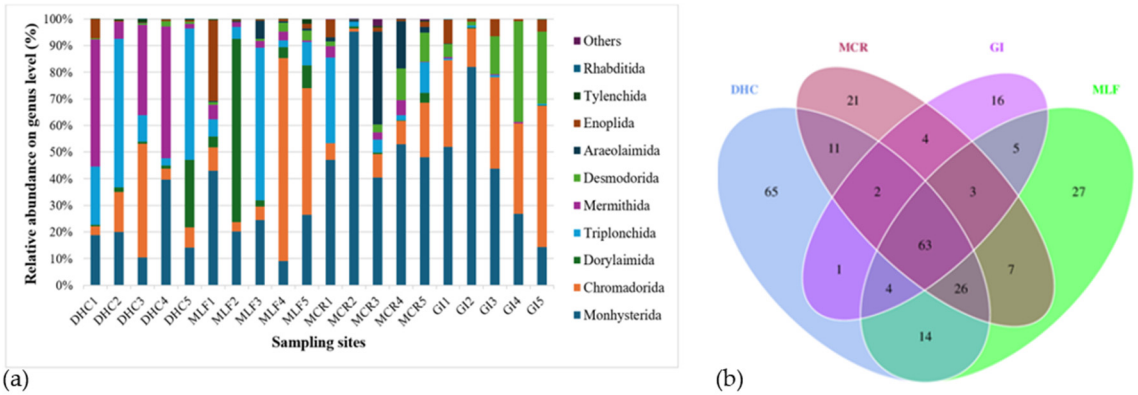

Figure 8. Relative abundance of the various orders in the nematode communities at the various study sites. (**a**) The relative distribution of sequences in the nematode dataset within different nematode orders for each of the 20 analysed sediment samples, and (**b**) Venn diagram displaying the overlap of shared OTUs among the four locations (at 97% similarity).

3.2.2. Nematode Alpha and Beta Diversity

The alpha diversity of the nematode communities was determined using the Chao1, Simpson, ACE, Good's coverage, and Shannon diversity metrics (Table 4). Both observed OTU numbers and Chao1 showed a high level of species richness in the DHC location, followed by the MLF and MCR samples. However, a low level of species richness was observed in the GI samples. Likewise, both Shannon and Simpson indices showed a high level of species richness and evenness in the MCR and DHC locations compared to the MLF and GI samples.

Table 4. Alpha diversity indices of nematode communities from river sediment samples (n = 5). The data show the averages and ± standard deviations.

Sample Name	Observed Number of Species	Shannon	Simpson	Chao1	ACE	Good's Coverage
DHC	80.8 ± 19.84	3.46 ± 0.55	0.7908 ± 0.054	108.82 ± 27.295	114.798 ± 34.818	0.980 ± 0.006
MLF	69.6 ± 14.57	3.33 ± 1.202	0.7042 ± 0.218	105.741 ± 31.369	107.27 ± 21.354	0.9818 ± 0.004
MCR	59.8 ± 9.81	3.66 ± 0.828	0.8226 ± 0.137	91.347 ± 35.021	81.037 ± 16.566	0.9874 ± 0.004
GI	43.6 ± 3.29	2.85 ± 0.274	0.7746 ± 0.056	79.628 ± 43.50	72.519 ± 15.127	0.9874 ± 0.003

The impacts of MPs were assessed on nematode composition at the OTU level, which represents the highest taxonomic resolution. Significant variations in nematode community diversity among the four locations were identified when comparing the alpha diversity, specifically observed species, Chao1, Simpson, and Shannon diversity indices, as illustrated in Figure 9a–d.

Beta diversity, shown in Figure 10, also confirmed significant differences in nematode communities among the four locations. The two principal component axes explained 55.17% of the total variation in the nematode community; specifically, the PC1 axis explained 38.55% while the PC2 axis explained 16.62% of this variation (Figure 10a). Nematode communities from three locations (GI, MCR, and MLF) were clustered together, separate from that in DHC. In addition, some DHC and MLF samples were clustered together. The nematode community structures in samples from each location were significantly different. Sediment samples from DHC, for example, were well dispersed, but two samples from this location were clustered with four samples from the MLF location. Principal component analysis (PCA) demonstrated sample separation at the OTU level from the four locations. Furthermore, analysis of similarity (Anosim) and permutational MANOVA indicated that significant differences existed among the nematode communities in the twenty river sediment samples across the four locations (Figure 10b–g), indicating that MP pollutants in the sediments have a significant impact on nematode communities.

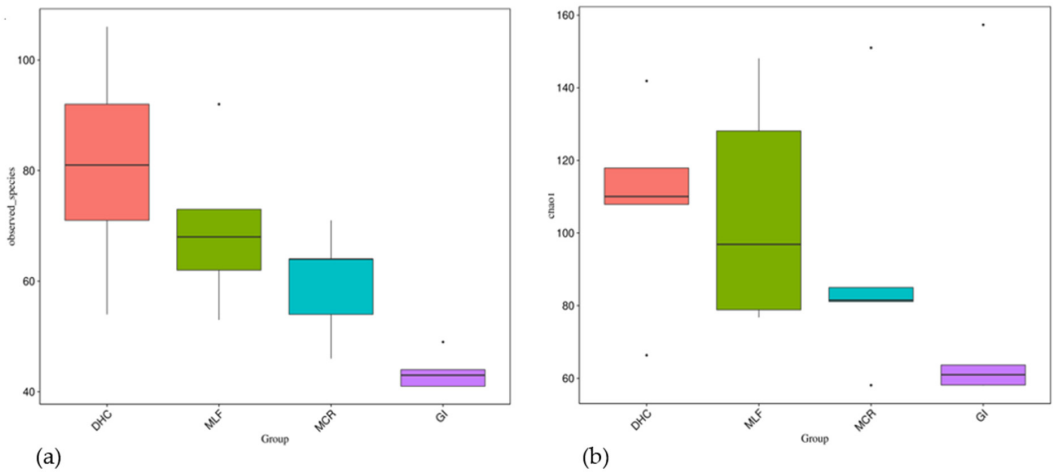

Figure 9. *Cont.*

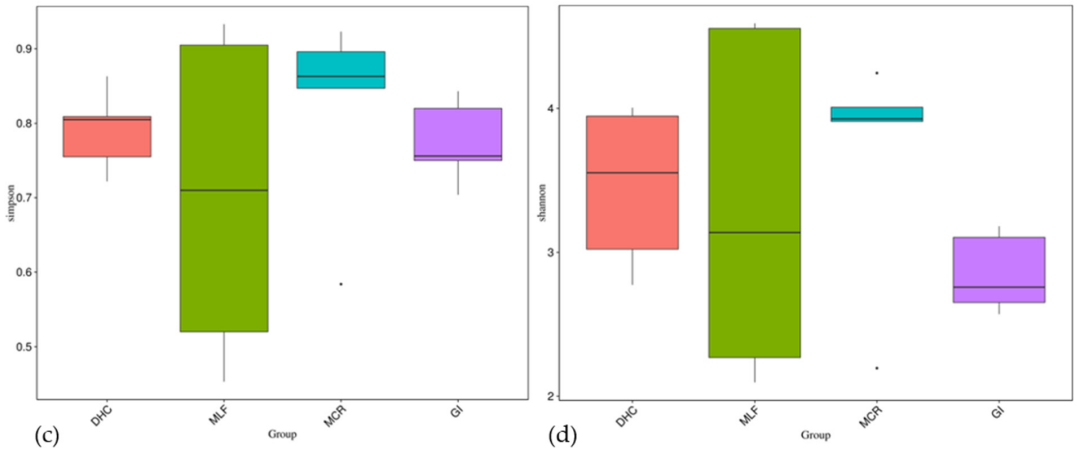

Figure 9. Alpha diversities of the nematode communities associated with the river sediment samples from DHC, MLF, MCR, and GI; observed species, Chao1, Simpson, and Shannon are displayed in (**a**,**b**,**c**,**d**), respectively.

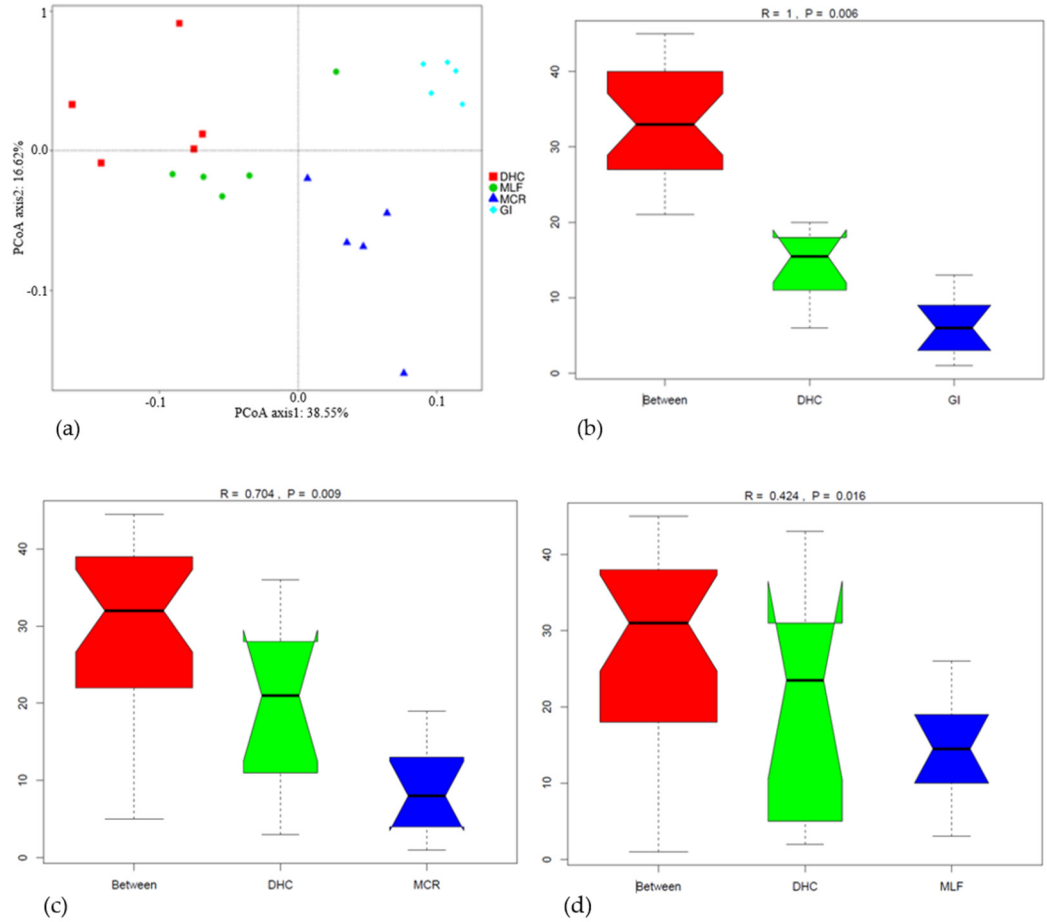

Figure 10. *Cont*.

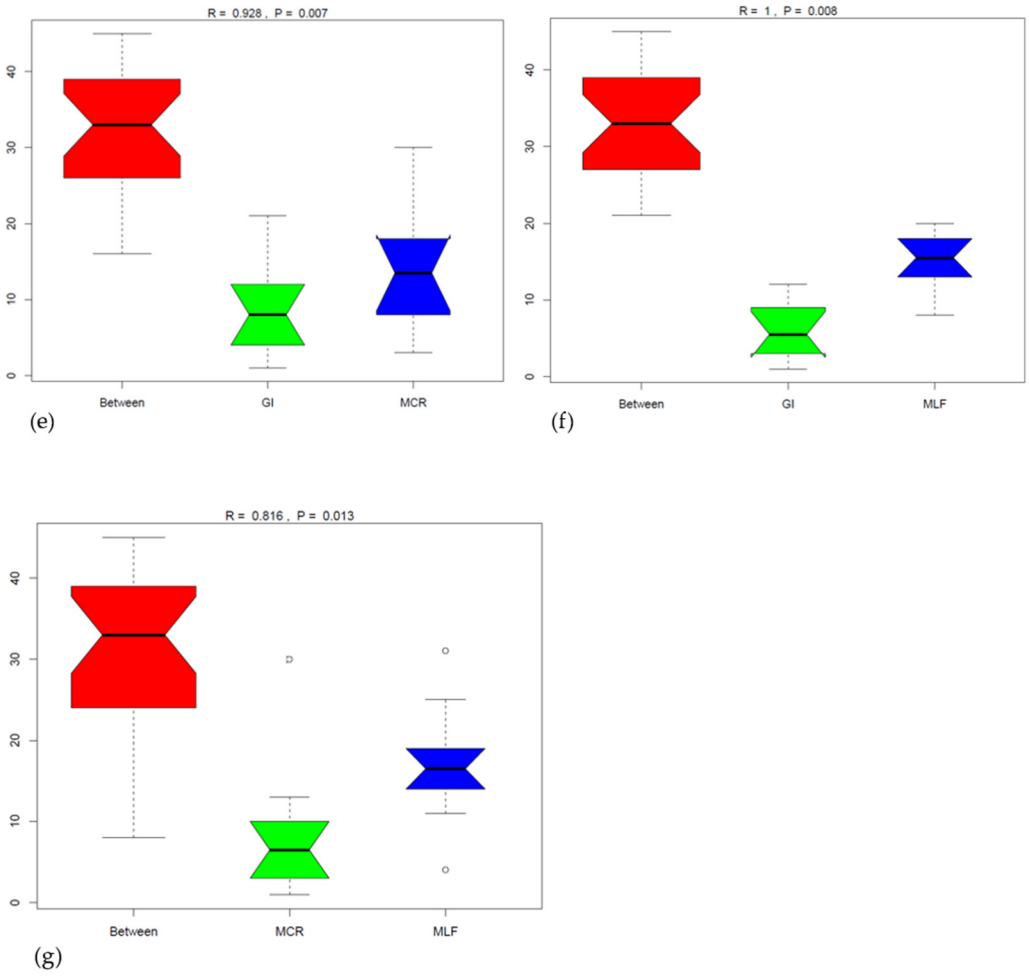

Figure 10. Beta diversities of the nematode communities associated with the river sediment. (**a**) Principal coordinate analysis (PCoA) of sediment nematode communities based on Bray-Curtis distances; PCoA plots are based on Bray-Curtis distances at the OTU level (97% sequence similarity). Red are Dolmen Hotel-Carlow samples, Green are Milford samples, Blue are MacMurroughs samples, and cyan are Great Island samples. (**b**–**g**) Anosim results of samples.

3.2.3. Trophic Guilds and c-p Scale Association Across Locations

Nematode communities were significantly affected by the presence of MP particles in the river sediment. The occurrence of nematode trophic groups differed significantly among the four locations ($p < 0.05$). Coloniser–persister (c-p) classification is based on life cycle properties. Nematodes of c-p-1 are regarded as enrichment opportunists; they have short life cycles and are often found in disturbed environments. In contrast, nematodes of c-p-5 have long life cycles and tend to inhabit stable, mature ecosystems. Coloniser–persisters of herbivores are called p-p [61]. In the present study, bacterivores were the most diverse and abundant trophic group across locations, whereas omnivores were least abundant. Plant parasites (p-p) were abundant across all the locations (see Table 5).

Sediment samples from the four locations presented a significant difference in nematode trophic group abundance. Sediment samples from DHC—site A (38.37%) had the highest abundance of herbivores, followed by those from MLF—site B (23.97%). The lowest numbers of herbivores were observed in MCR—site C (13.29%) and GI—site D (4.9%) samples. The abundance of bacterivores was significantly ($p = 0.01$) higher in MCR—site C (70.47%) and GI—site D (44%), followed by DHC—site A (33.4%). The lowest number was observed in MLF—site B (27.3%). The abundance of predators was the highest in sediment samples from GI—site D (50.9%) and MLF—site B (30.8%), followed by DHC—site A (21.8%). The lowest number was observed in sediment samples from MCR—site C

(15.1%). Moreover, omnivores were found to decrease in abundance in line with the most polluted locations, DHC—site A (6.5), MCR—site C (1.1), and GI—site D (0.1), compared to MLF—site B (17.9), which had the highest abundance (Figure 11).

Table 5. Feeding types and coloniser–persister class assignments of nematode taxa in river sediment.

Family	c-p Class	p-p Class	Feeding Type
Trichodoridae	0	4	Herbivores—ectoparasites
Tylenchidae	0	2	Herbivores—epidermal/root hair feeders
Monhysteridae	2	0	Bacterivores
Plectidae	2	0	Bacterivores
Rhabditidae	1	0	Bacterivores
Achromadoridae	3	0	Predators
Cyatholaimidae	3	0	Predators
Dorylaimidae	4	0	Omnivores

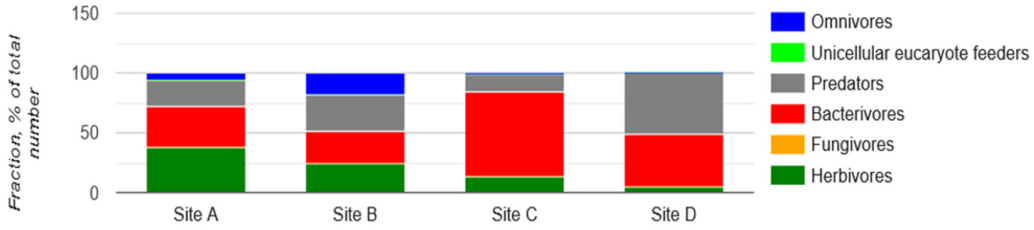

Figure 11. Nematode abundance of various feeding (trophic) groups (guilds) across locations. Feeding type composition of nematode assemblage in the River Barrow sediment samples. Site A (DHC), site B (MLF), site C (MCR), and site D (GI). The percentage was determined by summing the abundances of all samples collected at each site and subsequently presenting the total cumulative abundance of all samples within each respective site.

Nematodes were also classified into five c-p groups (1–5) according to their coloniser–persister life strategy. Throughout the locations, c-p2 nematodes contributed the major proportion. Meanwhile, c-p4 nematodes were the least occurring and only observed at sites A and B, not at sites C and D. The majority of the nematode groups were classified under the c-p2 and c-p3 categories, with c-p2 predominately represented. Sediment samples from MCR—site C (81.3%) and DHC—site A (55.4%) had the highest abundances of c-p2 nematodes, followed by GI—site D (46.3%). The lowest abundance was observed in samples from MLF—site B (35.6%). However, sediment samples from GI—site D (53.6%) and MLF—site B (40.4%) had the highest abundances of c-p3, followed by DHC—site A (35.3%). The lowest abundance was observed in samples from MCR—site C (17.4%). Lastly, sediment samples from MLF—site B (23.6%) had the highest abundance of c-p4, and sediment samples from DHC—site A (10.5%) had the lowest. No c-p4 nematodes were observed in sediment samples from MCR—site C (0%) and GI—site D (0%) (Figure 12).

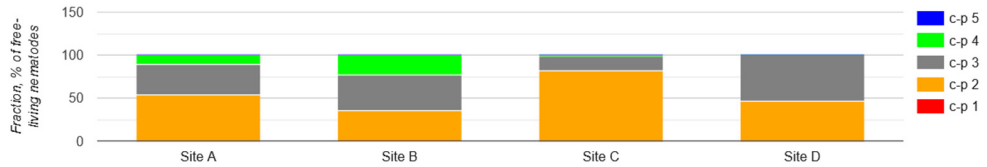

Figure 12. Relative abundance (%) of nematode assemblage C–P groups at four different locations. The percentage was determined by adding up the abundances of all samples at each site and presenting the total cumulative abundance of all samples at each site.

The maturity indices at site B for MLF (2.77 ± 0.51) exhibited a slightly greater value in comparison to site A for DHC (2.29 ± 0.44), site C for MCR (2.2 ± 0.16), and site

D for GI (2.54 ± 0.27), as depicted in Figure 13. There was no significant difference in maturity indices among the four locations (p= 0.154). However, the maturity indices of the four locations were less than 3, indicating low sediment food web maturity indices and persistence of MP pollutants among the four locations [43]. In contrast, there were significant differences among the sigma maturity indices (ΣMI) between the four locations ($p < 0.05$) (Figure 14): site B for MLF (3.13 ± 0.27), site A for DHC (3.10 ± 0.51), site C for MCR (2.43 ± 0.33), and site D for GI (2.61 ± 0.26).

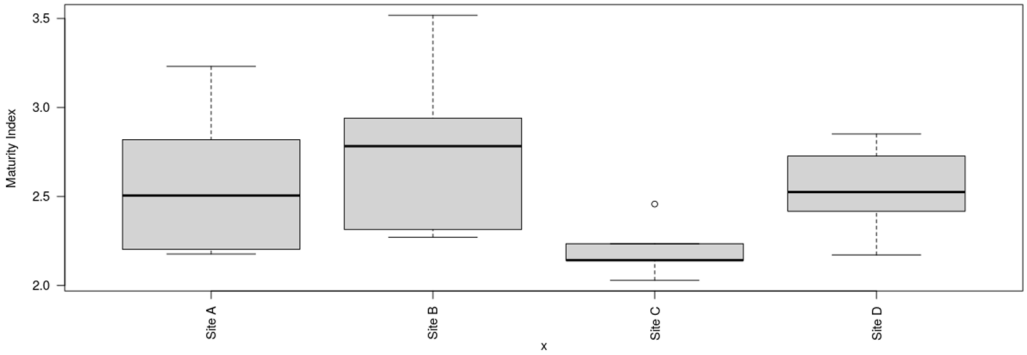

Figure 13. Comparison of the mean (±SD) maturity index values of the four locations for nematodes.

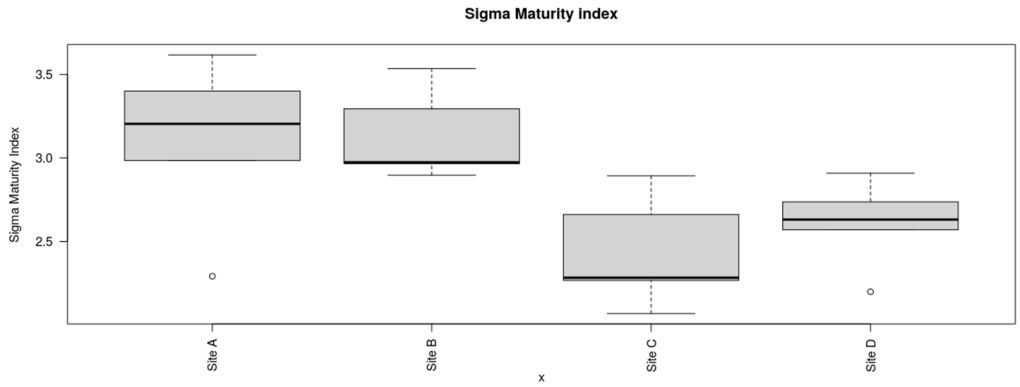

Figure 14. Comparison of the mean sigma maturity index values of the four locations for nematodes.

The analysis of the ecological triangle model depicted in Figure 15 demonstrated that sediment samples from site C—MCR and site A—DHC were closer and placed toward the 100% stressed environment, indicating a stressed state, likely influenced by various factors including temperature, pH, dissolved oxygen levels, heavy metals, pesticides, organic pollutants, and nutrient levels. The sediment samples from site B—MLF and site D—GI, on the other hand, were found to be more stable, despite lower nutrient levels, as denoted by their positioning on the c-p triangle. Overall, the results suggested that the river sediment maintained relative stability and exhibited low levels of stress, implying a good sediment quality suitable for nematodes. However, the presence of MPs in the sediment could perturb the nematode community, leading to alterations in species composition or abundance (Table 4). Notably, the GI samples contained a higher number of MPs, as reported in a previous study [47]. While the positioning of the sediment samples on the nematode c-p triangle at the bottom with the stability and stress axis may signal good sediment health, the presence of MPs could still adversely impact the nematode community within these sediment samples.

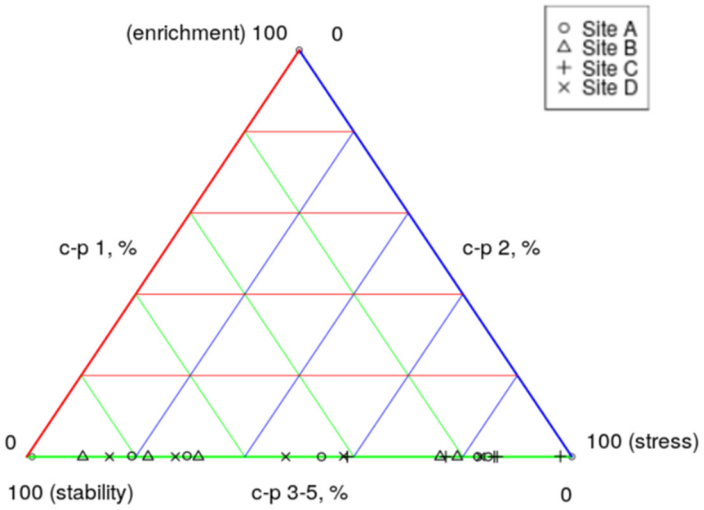

Figure 15. Coloniser–persister triangle depicting river sediment status regarding nematode c-p groups in the River Barrow. The red line represents enrichment opportunists (c-p 1), the blue line indicates stress tolerators (c-p 2), and the green line signifies community stabilisers (c-p 3-5).

The food web analysis depicted in Figure 16 was used for the evaluation of sediment health in the River Barrow (RB). The analysis displayed in Figure 16a revealed indications of poor sediment health, as evidenced by the alignment of all data points along the lower end of the structure index axis. This observed pattern may be attributed to various factors including decreased oxygen levels, elevated pollutant concentrations, or degradation of habitats. Additionally, the alignment of the nematode food web analysis data points along the lower end of the graph suggested the presence of significant stressors impacting the health of sediment and the nematode community within the river sediment. It is important that further investigation and ongoing monitoring of these factors be conducted in order to gain a comprehensive understanding and effectively address potential threats to the ecosystem.

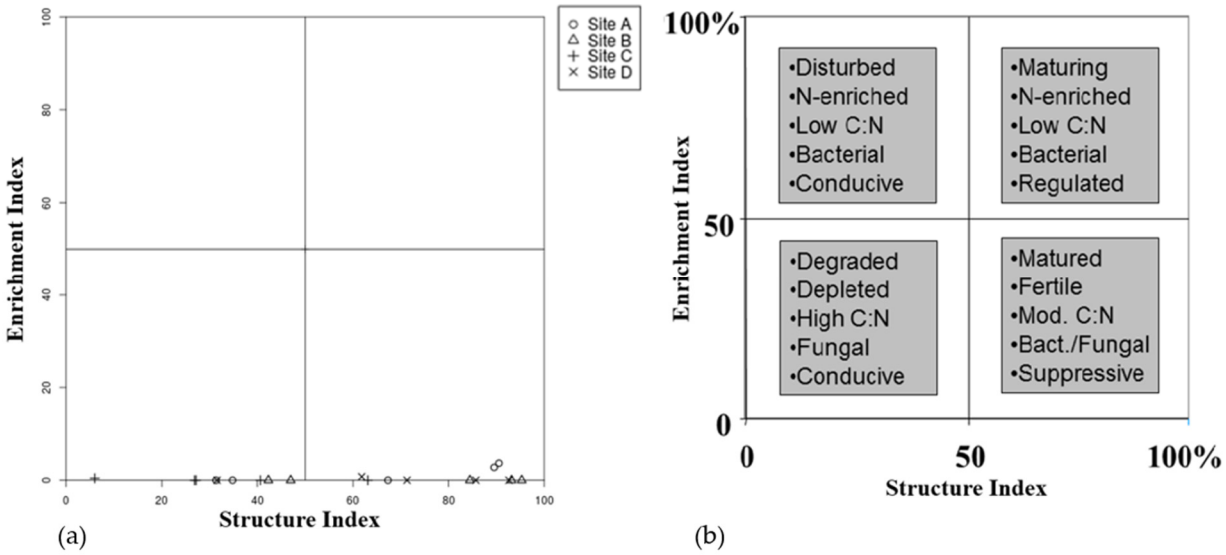

Figure 16. (**a**) Food web analysis of nematode communities at different River Barrow locations sampled, and (**b**) food web analysis interpretation scheme.

4. Discussion

To the best of our knowledge, this is the first study to evaluate possible toxic effects of MP pollutants on EPNs. Previous studies [41,62,63] in relation to MP toxicity have used

C. elegans mostly and the freshwater amphipod *Gammarus duebeni* as sentinel organisms, while focusing more on the impacts of MPs on ingestion, reproduction, and oxidative stress, with less emphasis on assessing mortality. The absorption of contaminants by MPs primarily depends on factors such as particle size hydrophobicity [64,65]. In this study, it was observed that contaminated MPs were able to take up toxicants such as atrazine, resulting in the mortality of nematodes. Similar studies in relation to MPs as carriers of toxicants have also shown that MPs act as vectors of various environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs), or hydrophobic persistent organic pollutants (POPs), pesticides, and heavy metals, thus affecting test organisms, including nematodes [15,39]. Furthermore, refs. [43,46] have also found that MPs cause mortality of *C. elegans*, as well as inhibiting their growth and reproduction [43,46]. This study is the first to evaluate the effects of MPs on the three nematode species tested. The findings can now be compared to other studies that have investigated the impact of MPs on nematodes. Previous research also examined how MPs affect *C. elegans*, concluding that MPs are causing significant issues in this particular nematode species [19,66–70]. By comparing the results of this study to these previous findings, a more comprehensive understanding of the impact of MPs on nematodes can be achieved.

This study demonstrated that increasing the MP exposure time from 72 to 96 h increased the mortality of nematodes exposed to the various treatments. In the case of naphthalene, these increases were from 26 to 30% (*S. feltiae* SB12(1) IJs), 29 to 40% (*S. carpocapsae* IJs), and 26 to 35% (*C. elegans* J3). Nematode mortality was shown to increase more so in the presence of MPs treated with atrazine and 1,3-dichloropropene than in the presence of MPs treated with naphthalene and fluorene. Refs. [36,40] have also reported the impacts of MPs on *C. elegans*. When comparing the three species, the results of this paper clearly showed that *S. carpocapsae* IJs and *C. elegans* J3 responded differently to all the treatments containing MPs compared to *S. feltiae* SB12(1) IJs. In addition, the *S. carpocapsae* IJs and *C. elegans* J3 mean percentage mortality levels were significantly higher ($p < 0.05$) compared to that in *S. feltiae* SB12(1) IJs. From the three species used, *C. elegans* is usually the most sensitive test organism [40]. However, *C. elegans* J3 mortality was lower compared to that in *S. carpocapsae* IJs. This presents a possible justification for the utility of *S. carpocapsae* IJs as sentinel organisms in future toxicology assays, and research-based evidence suggests that *S. carpocapsae* can also be used as a sentinel organism alongside *C. elegans*. This study demonstrated that the toxicity of MPs on the three nematode species differed depending on the treatments and exposure time. The cause of mortality in nematodes could be the leaching of MP additives, as was observed by [40]. Similar findings indicated that toxic chemicals absorbed by MPs may potentially have a very high impact on *C. elegans* tissues, inducing toxic effects [34,39,41].

This study is also the first to fill the gap in knowledge on MP pollution and its environmental impact on the RB sediment nematode communities. The majority of similar studies focused on the impacts of MPs on nematode (namely *C. elegans*) growth, reproduction, and mortality [19,71], as well as MP ingestion by benthic organisms such as freshwater amphipod *Gammarus duebeni* [63]. Studies [20,25] showed that nematodes found in soil and river sediment can act as bioindicators of MP environmental pollution. Recently, the authors of this study confirmed the presence of MPs in RB sediment samples [47]. A similar study [68,71,72] made similar observation confirming plastic microfibres widely present in freshwater sediments.

The higher number of reads obtained in the MCR samples is likely due to the abundance of Monhysterida in this particular location (57 $\pm$ 22%) (Figure 6a). The average read length was 301 bp, which was larger than 99% of Good's coverage, as defined in the scikit-bio documentation for the 18 S rRNA gene region, with an average GC content of 46%. According to rarefaction curves, a common method for assessing the biodiversity of samples, OTU saturation was achieved at approximately 60,000 reads per sample.

The four locations selected in this study revealed significant differences in nematode communities. For example, the DHC location was sandy/gravelly, the MLF location

was gravelly, the MCR location was muddy/fine grain sized, and the GI location was muddy/fine grain sized, which were the notable differences across the study locations. The smaller the grain size, the less diverse a nematode assemblage [69]. For instance, compared to the sediment samples from the other three locations, the GI location's sediment samples had higher abundances of only three nematode orders. The three main orders observed from the GI sediment samples were Monhysterida, followed by Chromadorida and Dorylamida. Monhesterida were among the most numerous groups of nematodes in the sediment samples according to the data of this study. According to [40], MPs and their associated toxicants have the potential to adversely impact soil nematodes. As many of the harmful chemicals introduced into waters bind to settling particles, soft sediments are frequently very polluted [69]. This could explain the difference in nematode feeding types and communities observed across the four locations. Ref. [70] similarly observed that sediment grain size and organic matter content have strong correlations with nematode communities.

In this study, it was observed that the coarser the sediments, the more diverse the communities. This was particularly observed in how the diversity and abundance of sediment nematodes significantly decreased in MCR and GI samples compared to DHC and MLF. It has been also shown via the assessment of MPs in Irish river sediment that GI was the most polluted location with MPs compared to the rest of the locations used in the current work [47]. There is a clear evidence that MPs have significantly affected nematode communities, as the numbers of nematode orders in MCR and GI were lower compared to those in the DHC and MLF samples (Figure 8b). Similar findings were observed in [71].

The increasing MP pollutants have been considered an emerging threat to biodiversity and ecosystem functioning while exerting significant negative effects on the abundance of nematodes [67,73]. Furthermore, the authors' recent paper [47] revealed that finer sediments act as a sink for MP deposition, which clearly explained the decline in the species abundance and nematode feeding types in the MCR and GI locations. These two particular sites were found to have fine sediments with more deposited MPs compared to sediment samples from the DHC and MLF locations. Refs. [67,73] confirmed that the presence of various MPs in sediments can reduce nematode feeding types and alter the sediment pH. Despite all this evidence, however, the impacts of MP pollution on nematode communities of sediments in the RB in other counties in Ireland remain largely unknown.

The nematode community structure is indicative of the condition or health of the soil/sediment in which nematodes live, as they respond rapidly to disturbance and enrichment [43]. The impact of MP contamination in river sediment, causing the disruption of nematode communities, was evaluated using a number of indices. For free-living nematodes, the maturity and sigma maturity indices were used to assess the environmental disruption caused by MPs. Nematodes were assigned c-p values according to [60]. The most resistant nematode taxa to MP pollution were found to be those in class c-p2. Ref. [74] also reported similar results, but regarding heavy metals rather than MPs. Among the nematode feeding type composition, there was more variation at sites A—DHC and D—MLF compared to sites C—MCR and D—GI. Bacterivores were the most dominant, followed by predators and herbivores. The abundance of bacterivores was high at sites C (MCR) and D (GI). A plausible explanation is that the presence of environmental factors, including organic/inorganic carbon, nitrogen, phosphorus, and other minerals in the environment, may modify the direct negative impact of MPs. Refs. [2,67] also made similar observations. On the other hand, omnivores were the lowest in abundance. Sites C—MCR and D—GI had the highest levels of MPs compared to the two other locations. The disturbance in nematode feeding types could be caused by the presence of MPs. Therefore, these indices can be used as guidelines by the EU Water Framework Directive (WFD) when monitoring MP pollution in RB sediments. Lower maturity and sigma maturity indices in river sediment have also been observed at the four locations, particularly at the locations with high numbers of MPs (MCR and GI), indicating sediment-dwelling nematode communities disturbed by MPs. The findings reported here support the hypothesis that perturbation

generally leads to a lower maturity indices [60]. The disturbance caused by MPs likely disrupts the natural habitat and food sources of sediment nematodes, ultimately impacting their development and maturity levels. Thus, the findings from this study provide further evidence linking perturbation, specifically from MPs, to lower maturity indices in sediment nematode communities.

Analysis of river sediment samples from the four locations along the RB shows that MPs affect nematode communities. Therefore, based on the findings of this study, it can be inferred that MP pollution of river sediment in the four locations along the RB is evident. This statement is supported by the observed decrease in nematode diversities, changes in feeding type composition, and lower maturity indices in sediment nematode communities. The disruption caused by MP contamination likely alters the natural habitat and food sources of sediment-dwelling nematodes, ultimately influencing their development and maturity levels. The results from this research provide further evidence of the relationship between disturbance, particularly from MPs, and reduced maturity indices in sediment nematode communities. This in turn indicates that the health and condition of the river sediment in the four locations are indeed affected by MP pollution, as suggested by the observed changes in nematode communities. Nematode communities are at risk as a result of the accumulation of MP contaminants in river sediments. Nematode communities were mostly affected by the presence of MPs in samples from the MCR and GI locations. Decreases in the number of nematode feeding types and maturity indices were also observed in the MCR and GI locations, potentially associated with the high numbers of MPs. Furthermore, sediment MP contamination can modify the composition of feeding types in nematode communities, both directly and indirectly, due to differences in the sensitivity of various nematode species [75,76].

The low sediment web maturity indices observed can be attributed to the ongoing rise in MPs within RB sediments [47]. Therefore, it is important for ecologists to use these indices as means to improve the sediment quality of the RB by reducing the use of non-biodegradable plastics and thereby mitigating the environmental effects of MPs. Stakeholders such as policymakers, industry, government agencies, and the general public can also play a role in reducing non-biodegradable inputs and mitigating the environmental impacts of MPs. Implementing regulations on the use of plastics, promoting recyclable and biodegradable materials, and educating the public on the importance of reducing plastic use and pollution can all contribute to improving the sediment quality of the RB. Collaborative efforts between environmental activists, policymakers, industry, and the public are essential in addressing the issue of MP contamination and promoting environmental sustainability. The impact of this research is significant, as it highlights the negative effects of MPs on beneficial nematodes and nematode biodiversity in river sediments, ultimately affecting the overall ecological health of aquatic ecosystems.

The findings of this research can be implemented in several ways: (1) Environmental policy and regulation: The data on the impacts of MPs on nematode communities can inform policymakers and regulators about the potential ecological risks posed by MPs in aquatic environments. This information can be used to develop strategies to reduce MP pollution and protect benthic ecosystems. (2) Eco-friendly product development: The identification of commercial cosmetic products as a potential source of MPs underscores the importance of developing eco-friendly alternatives that do not contain harmful pollutants. Relevant industries can use this information to reformulate their products and reduce their environmental impact. (3) Further research: This study highlights the need for more research on the pathways of nanoparticles in organisms and the effects of MPs on different types of organisms. Future studies can build upon these findings to deepen our understanding of the ecological implications of MP pollution. By addressing the issue of MP pollution and its effects on benthic organisms, such as nematodes, steps can be taken to protect and preserve aquatic ecosystems for future generations. It is therefore hoped that this study will inspire others to contribute to the limited work on the impacts of MP pollutants in RB sediments.

5. Conclusions

This paper has presented for the first time the toxic effects of MP pollutants on EPNs, demonstrating that MPs can act as vectors for chemical pollutants, significantly impacting nematode mortality. Using ATR-FTIR spectroscopy, we observed that atrazine accumulated on the surface of polyethylene MPs. Additionally, there was evidence of interaction between these MPs and both 1,3-dichloropropene and naphthalene; however, fluorene did not appear to be retained on the MP surface, which notably affected the mortality rates of *S. carpocapsae* and *C. elegans*. Our findings indicated that continuous exposure to MPs significantly increased nematode mortality rates of *S. carpocapsae* and *C. elegans*, particularly when compared to the less affected species, *S. feltiae*. Moreover, in this study, the highest nematode richness and abundance were observed at the DHC location, followed by MLF, while the lowest richness and abundance were found at the MCR and GI locations. Furthermore, the community composition of nematodes in river sediments was influenced negatively by the presence of MPs, with Monhysterida emerging as the predominant order across all samples, especially in those from the MCR location. This research suggests that nematodes, particularly *S. carpocapsae*, could serve as sentinel organisms, allowing us to observe the effects of MP pollution in aquatic ecosystems. Our results highlight the necessity of further investigation into the ecological implications of MP pollution and emphasise the importance of mitigating MP release, to protect nematode communities and overall ecosystem health.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/applbiosci3040034/s1, Text S1: Microplastics' containment in the laboratory.

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