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Assessing the Role of *Melia azedarach* Botanical Nematicide in Enhancing the Structure of the Free-Living Nematode Community

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Abstract: In a greenhouse experiment, we studied the impact of *Melia azedarach* ripe fruit water extract (MWE), Furfural (a key ingredient of M. azedarach), and the commercial nematicide Oxamyl (Vydate® 10 SL) on the soil free-living nematode community. Treatments were applied every 20 days for two months, and soil samples were collected 3 days after the last application (3DAA) and at the end of the cultivation period (34DAA). We assessed short- and long-term effects on nematode community structure, metabolic footprint, genus composition, and interaction networks. Oxamyl and Furfural significantly reduced bacterial and fungal feeder populations. MWE had no impact on free-living nematode populations. Oxamyl and Furfural-treated soil samples were dominated by *Rhabditis* at 3DAA and *Meloidogyne* spp. at 34DAA. On the contrary, MWE-treated soil showed a balanced distribution, with *Rhabditis*, *Panagrolaimus*, *Mesorhabditis*, and *Diploscapter* being equally abundant. MWE treatment exhibited higher diversity indices (Shannon and Simpson) and equitability. Network analysis showed that the Oxamyl network had the highest fragmentation, while the MWE and Furfural networks had higher cohesion compared to the control. *Mesorhabditis* spp. in the MWE network played a crucial role, being directly connected to the omnivore genera *Thonus* and *Aporcelaimellus*. Our results indicated that continuous MWE application, besides controlling *Meloidogyne* spp., could enhance the structure and stability of the soil-free-living nematode community.

Keywords: diversity indices; feeding groups; metabolic footprint; network analysis

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1. Introduction

The transition towards alternative plant protection methods has emerged as a compelling response to the widespread ban and withdrawal of chemical-based products. Agricultural nematologists attempt to mitigate the negative impact of plant-parasitic nematodes (PPN), which are highly damaging agricultural pests globally and cause significant yield reductions and even complete crop loss [1]. The economic impact of PPN infestations is alarming, as these microscopic pests can cause worldwide yield losses of up to 12.3% and annual productivity losses of as much as 173 billion US dollars [2]. In 2013, the scientific community identified the genus *Meloidogyne* (i.e., root-knot nematode) as among the most destructive PPNs [3]. This genus includes about 100 species, and because of its economic importance, it is also one of the most studied ones [4]. While its obligate biotrophic nature presents significant challenges, research on *Meloidogyne* spp. encompasses every aspect

of its existence, including evolution, development, and plant responses to invasion [5–7]. Most of the studies on *Meloidogyne* spp. have primarily focused on mitigating its impact.

For several decades, chemically synthesized products such as Fosthiazate, Fenamiphos, Oxamyl, and Fluopyram were extensively used to control root-knot nematodes. However, the long-term use of these products led to a reduction in the abundance of free-living nematodes and the development of resistance to nematicides [8,9]. The adverse effects of their overuse have driven the development of more eco-friendly crop protection tools and strategies, such as the incorporation of plant materials into the soil to act as biofumigants. In addition to their nematicidal properties, soil bio-amendments may exhibit secondary beneficial effects such as reducing water run-off and soil erosion, preserving soil moisture, regulating temperature, improving soil organic matter, increasing soil microbial activity, suppressing weeds, and enhancing plant growth [10–13]. Organic, plant-based amendments offer a practical solution to address the primary issue associated with synthetic nematicides by enabling effective pest control while preserving the integrity of free-living soil nematodes. These nematodes serve as valuable indicators of soil health and quality as they respond rapidly to changes and possess remarkable capacity for resistance and resilience to overcome disturbances [14].

Soil-free-living nematodes play an integral role in soil ecosystems, contributing to energy transfer and nutrient cycling [15]. The term "free-living nematodes" includes species that exhibit various trophic behaviors, including bacterivorous, fungivorous, omnivorous, and predatory tendencies, as well as plant-parasitic nematodes that undergo at least one phase of their life cycle within the soil. Given the strong connection between soil biodiversity and cropping systems, as most soil processes are mediated by living organisms [11], the nematodes stand out as essential participants that directly contribute to nitrogen mineralization, litter decomposition, and the distribution of biomass within plants [12]. In this context, the intensification of land use, monoculture practices, and primarily the use of nematicides contribute to a reduction in the diversity of free-living nematode communities and structures; subsequently, when one component of the ecosystem is altered, it ultimately affects the overall functioning of the ecosystem. [16]. Soil nematode community richness and connectivity have been shown to enhance system functioning through complementarity and synergistic interactions among species. Conversely, a fragmented network of interactivity between nematode genera can disrupt the structural architecture and microbial dynamics [17]. While assessing all this information is essential for crop system functionality, to our knowledge, many studies overlook crucial elements in assessing the by-side effects of nematicides, such as their impact on the abundance, structure, composition, and interaction network of free-living nematodes. Such considerations hold substantial importance, particularly given the multi-scale nature of biodiversity, which necessitates minimizing disturbances to effectively uphold its ecological functions [18]. Hence, it is necessary to conduct further investigations to address existing gaps on the side effects associated with chemical treatments on non-target nematodes, which are equally affected due to their biological similarity, thus presenting an essential matter given their contribution to soil productivity [19].

In our previous study [20], it was demonstrated that the nematicidal potential of the ripe fruit water extract of *M. azedarach* (MWE) when applied to tomatoes in greenhouses that were inoculated with plant-parasitic *Meloidogyne* spp. We compared the results of MWE to those obtained with the commercial nematicides Oxamyl (Vydate® 10 SL) and Furfural (one of the principal active ingredients of *M. azedarach*). All substances showed a significant positive effect against *Meloidogyne* spp., resulting in a significant decrease in J2 per gram of soil and reduced severity of galling. However, certain findings from the chemical treatments indicated an adverse impact on the structure and composition of the free-living nematode community. Therefore, it was necessary to conduct a supplementary experiment to gain a comprehensive understanding of the effects of these nematicides on the overall functionality of the soil network, including non-target nematodes and their complex interactions. This paper aims to provide complementary results, focusing on the impact

of two chemical treatments (Oxamyl and Furfural) and one botanical treatment (MWE) on the reduction of (i) nematode abundance, (ii) the structural and trophic composition, and (iii) the interaction network. Considering that the application of these nematicides is a continuous disturbance in the soil system, we also aimed to identify the nematode community's recovery capacity.

2. Materials and Methods

2.1. Experimental Design

This study was conducted within a *Meloidogyne*-infested commercial tomato (*Solanum lycopersicum* cv. Belladonna) greenhouse situated on private land in Volos, Thessaly, Greece. The analysis of soil physicochemical characteristics and soil texture followed the methodology outlined in reference [21]. The soil exhibited a sandy loam texture, comprising 52.4% sand, 36.6% silt, and 11% clay, with a pH (1:2 H_2O) of 7.9 and an organic matter content of 2.4%. The experimental design employed a randomized block structure with four treatments, with each treatment having five replicate plots. Transplantation of 35-day-old nematode-free tomato seedlings was carried out in these plots, with each plot accommodating four plants. The dimensions of each plot were 1.2 m long and 0.8 m wide. The treatments were as follows: (1) untreated control; (2) application of Vydate® 10 SL (Oxamyl) at the recommended dose of 1.5 mL per plant or 15 µL per 100 g of soil; (3) application of Furfural at a rate of 3 mL per plant or 31 µL per 100 g of soil (purchased from Sigma-Aldrich); and (4) application of *M. azedarach* water extract (MWE) at a rate of 430 mL per plant or 1.15 g of dry extract per 100 g of soil. The chosen concentrations for these treatments slightly exceeded the EC 50 values determined in a prior pot trial for MWE and Furfural [18]. The application volume for all treatments was 500 mL, distributed in a circular area with a 26-cm diameter around the base of each plant. Specifically, for MWE, 500 mL represented the actual extract volume applied, whereas for Oxamyl, 500 mL was the volume of water used as a carrier for the diluted Vydate® 10 SL. The first application occurred nine days prior to transplanting, on the 30th of May. Subsequent applications were carried out three more times on the 18th of June, 7th of July, and 25th of July, following a 15–20-day interval schedule, in accordance with the recommended practice for Vydate® 10 SL. Soil sampling was conducted on the 28th of July, three days after the final application (3 DAA), and at the conclusion of the cultivation period, on the 29th of August, which was 34 days after the last application (34 DAA). Within each plot, five soil samples were randomly collected from the upper 15 cm of the soil layer using a 3 cm diameter core sampler. These samples were mixed, resulting in a total of five composite soil samples per treatment and per sampling occasion.

2.2. Nematode Extraction and Analysis

Nematodes were extracted from 150 mL of each soil sample, which underwent a delicate disintegration of soil aggregates manually beforehand. Cobb's sieving and decanting technique, as adapted by S'Jacob and van Bezooijen [22], was employed for nematode extraction. Subsequent to tallying the overall nematode count, they were preserved using 4% formaldehyde. Following preservation, a random selection of 100 nematodes was taken from each sample and taxonomically identified at the genus level using Bongers' identification key [23]. The nematode genera were categorized into trophic groups following the criteria set by Yeates et al. [24], and they were classified along the colonization-persistence gradient (c-p values) [25,26]. Concerning nematode functional indices, the Maturity Index (MI) was computed for free-living nematodes, and the Plant-Parasitic Index (PPI) was determined for plant-parasitic nematodes. Both indices are indicative of the successional stage of the communities [25]. Enrichment Index (EI) and Channel Index (CI), denoting soil enrichment and dominant decomposition pathways, respectively, and the Structure Index (SI) were computed based on Ferris et al. [15]. Lastly, the NINJA (Nematode-Indicator Joint Analysis) online platform was utilized to compute metabolic footprints and generate the soil food web graph [27].

2.3. Statistical Analyses

To assess the impact of treatment, sampling time, and their interaction on soil nematode populations and nematode community indices, we employed a repeated-measures Analysis of Variance (ANOVA). In all statistical examinations, means were compared via the Fisher's Least Significant Difference (LSD) test, with a significance level set at $p < 0.05$. Prior to conducting the analyses, we appropriately transformed the data, as needed, to fulfill the assumptions of ANOVA. Non-metric multidimensional scaling (NMDS) was implemented to explore whether the application of nematicides significantly influenced the structure of the nematode community. NMDS relies on the rank order of dissimilarity within a community [28]. Using the relative abundances of nematode genera, we established the original position of the community within a multidimensional space based on the Bray-Curtis distance coefficient. All statistical analyses were carried out using Statistica 9 for Windows (StatSoft, Tulsa, OK, USA).

To evaluate nematode community diversity, we employed the diversity ordering methodology outlined by Patil and Taillie [29], relying on Rényi's index [30]. Rényi's parametric index of order α exhibits variable sensitivity to both rare and abundant species within a community, contingent on the scale parameter α [31]. This approach provides a diversity index profile widely utilized in community analysis for each community. Specifically, when $\alpha = 0$, the index corresponds to the logarithm of species number; for $\alpha = 1$, it mirrors Shannon's index; and for $\alpha = 2$, it aligns with Simpson's index. As α tends toward infinity, the index becomes highly sensitive to the abundance of species within a community. Consequently, disparities in diversity profiles at small α values are attributed to differences in species numbers, while distinctions at large α values are ascribed to the presence of abundant species. When two diversity profiles intersect, it implies that the two communities may be ordered differently based on different diversity indices. The calculations were executed using PAST 4.03 software [32].

Network analysis, a quantitative approach aimed at representing and evaluating structures, draws upon metrics derived from graph theory, a mathematical field specializing in the analysis of relational patterns among nodes [33]. In the context of this study, nodes correspond to nematode genera, while the connections between these nodes symbolize the co-occurrence of genera within samples.

To construct community matrices based on the co-occurrence of nematode genera, we utilized abundance data pertaining to these genera. The probability of their co-occurrence was computed following the niche overlap index proposed by MacArthur and Levins [34], defined as:

$$\text{Mij} = \frac{\sum_1^n p_{ik} p_{jk}}{\sum_1^n p_{ik}^2}$$

where 'n' represents the number of samples, and 'p_{ik}' and 'p_{jk}' denote the proportional abundances of genera 'i' and 'j' in sample 'k'. Consequently, the matrix element Mij quantifies the extent to which the niche space occupied by genus 'i' overlaps with that of genus 'j'. Notably, due to asymmetry in the influence of one genus on another, the resulting community matrix was not symmetrical. For all networks, we determined threshold values, representing the point at which relations were considered non-negligible, to be up to 20% of the community's maximum value, following a common practice [35]. Thus, if the Mij value fell below 20% of the community's maximum, the impact of genus 'i' on genus 'j' was deemed negligible, and the corresponding matrix entry was set to zero. The network map effectively illustrates the formation of associative spatial relationships. Community matrices were constructed separately for the nematode communities of each treatment and were subsequently subjected to analysis and visualization using UCINET 6 [36]. We calculated various cohesion-related variables for each network, including density, compactness, and fragmentation. Density quantifies the overall strength of connections divided by the number of potential ties, providing insight into the network's level of interconnectedness [37,38]. UCINET employed the binary network version to estimate compactness.

3. Results

All the nematodes observed on the two sampling occasions are presented in Supplementary Table S1. More specifically, it was found the bacterivores *Acrobeles*, *Acrobeloides*, *Chiloplacus*, *Diploscapter*, *Drilocephalobus*, *Heterocephalobus*, *Mesorhabditis*, *Panagrolaimus*, *Plectus*, and *Rhabditis*, the fungivores *Aphelenchoides*, *Aphelenchus*, and *Ditylenchus*, the plant-ectoparasites *Bitylenchus*, *Paratylenchus*, *Quinisulcius*, the sedentary plant-endoparasite *Meloidogyne*, the migratory plant-endoparasite *Pratylenchus*, the epidermal/root hair feeders *Tylenchus*, and the omnivores *Mesodorylaimus*, *Aporcelaimellus*, and *Thonus* (Table S1).

The abundance of all nematode trophic groups in different treatments at 3 and 34 days after application (DAA) is presented in Figure 1a–e. The sampling time significantly affected the total abundances, primarily due to significantly higher herbivore abundances in all treatments at 34 DAA. The abundances of other free-living nematode groups did not show statistical differentiation, even a month later. Among the nematode trophic groups, bacterial feeders were the most abundant, followed by herbivores, fungal feeders, and omnivores/predators (Figures 1 and 2a,b). Soil samples treated with Oxamyl and Furfural exhibited a significant decrease in total nematode abundance (51% and 66%, respectively), whereas the MWE soil samples showed a comparable abundance to the control group. The decrease observed in the Oxamyl and Furfural soil samples was primarily due to a significantly lower abundance of bacterial and fungal feeders. Herbivore abundances were significantly impacted by all treatments and were lower than the control on both sampling occasions. On the other hand, the abundance of omnivores and predators was not affected by the sampling time, treatment, or their interactive effect, possibly due to the low populations recorded in our samples. Interestingly, several soil samples from the MWE treatment presented a trend of increased population numbers in these groups at 34 DAA.

The percentage contribution of each nematode trophic group within the nematode community is illustrated in Figure 2a,b. In the control samples at 3 DAA, bacterial feeders were the dominant group, while herbivores also had a significant presence. Interestingly, the application of Oxamyl and Furfural did not alter this percentage contribution pattern, despite significantly decreasing the abundances of several free-living nematode groups. Conversely, the MWE treatment resulted in a profound increase in the relative abundance of bacterial feeders compared to the control. At 34 DAA, herbivore nematodes became the dominant group in the control samples, and this pattern was also observed in the Oxamyl and Furfural treatments. However, in the MWE-treated soil samples, the relative abundance of bacterial feeders remained higher compared to the control, although lower compared to the percentages observed in the initial sampling. Additionally, the percentages of omnivores and predators increased in these samples.

At 3 DAA, the rank abundance graphs revealed that genera belonging to the cp-1 bacterial feeder group (e.g., *Rhabditis*, *Panagrolaimus*, and *Diploscapter*) were among the dominant ones in both the control and treated soil samples (Figure 3a). In the control samples, *Rhabditis* and *Meloidogyne* were the dominant genera, while the Oxamyl and Furfural-treated samples were exclusively dominated by *Rhabditis* (Figure 3b). In contrast, the MWE-treated soil exhibited a more balanced distribution, with *Rhabditis*, *Panagrolaimus*, and *Diploscapter* being almost equally abundant (Figure 3a). At 34 DAA, the control samples, as well as the Oxamyl and Furfural-treated samples, displayed a completely different pattern, with *Meloidogyne* spp. dominating the communities (Figure 3b). However, the MWE-treated samples continued to exhibit a distinct community structure, similar to what was observed at 3 DAA, with multiple cp-1 bacterial feeders comprising the highest populations (Figure 3b). Notably, *Mesorhabditis* spp. significantly increased its population numbers compared to the initial sampling.

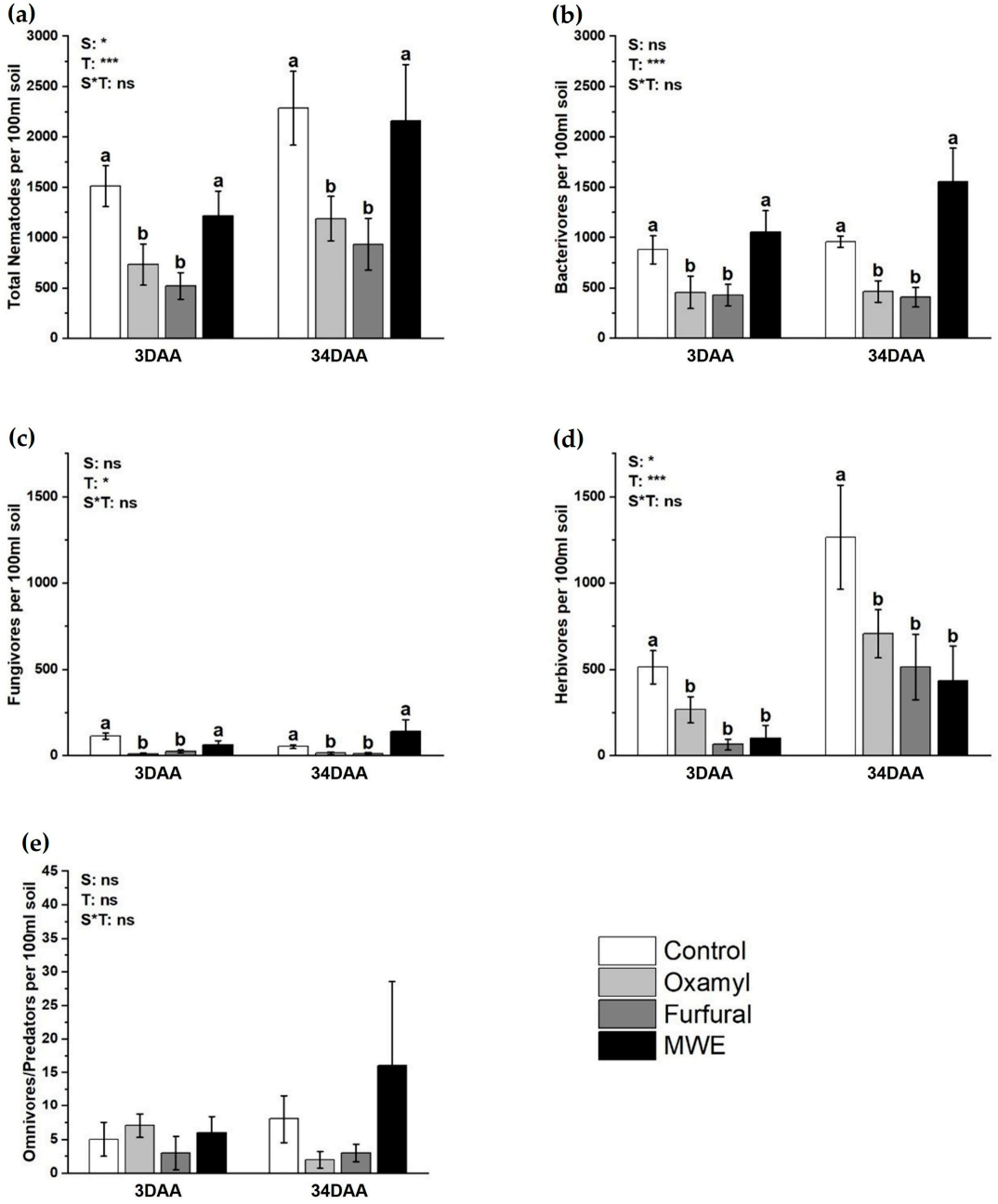

Figure 1. Mean abundance values (±st. error) of nematode trophic groups [total nematode abundance (**a**), bacterial feeders (**b**), fungal feeders (**c**), herbivores (**d**), and omnivores/predators (**e**)] under different treatments and results of repeated-measures ANOVA regarding Sampling (S), Treatment (T), and their interactive effect (SxT) in both sampling occasions (3 and 34 DAA). Different letters (a,b) indicate significant differences among treatments based on Fisher's LSD post hoc test (*: $p < 0.05$; ***: $p < 0{,}001$; ns: non-significant, for all cases $n = 5$).

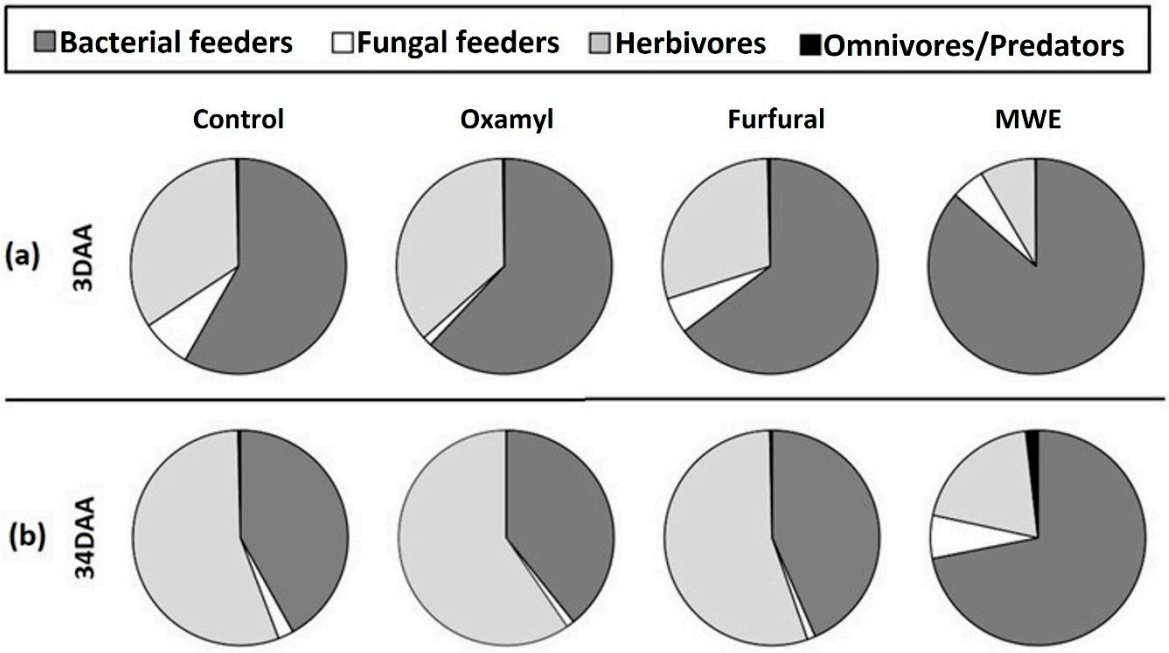

Figure 2. The relative abundance of trophic groups at different treatments in both sampling occasions 3 DAA (**a**) and 3 DAA (**b**). For all cases $n = 5$.

The diversity profiles showed slight differences in the number of taxa (corresponding to a = 0) on both sampling occasions, with the MWE treatment consistently exhibiting the highest genus richness (Figure 4). The Shannon (a = 1) and Simpson (a = 2) diversity indices (Figure 4 and Table S2a,b), as well as the equitability (indicated by a > 2), were higher in the MWE treatment on both sampling occasions. Our results indicated that MWE samples were the only ones where diversity indices increased at 34 DAA (Table S2a,b). The Oxamyl treatment exhibited lower diversity indices compared to the reference control community. Similarly, the Fur-treated soils followed the same pattern; however, in the second sampling, this difference was no longer observed as their diversity became comparable to that of the control reference site. NMDS indicated that there were significant differences in the nematode communities from different treatments; Oxamyl and Furfural samples were the ones that differed more compared to the control. This pattern was obtained on both sampling occasions (Figure 5).

At the end of the cultivation period (34 DAA), the networks of the MWE, Fur treatments, and control demonstrated greater cohesion compared to the Oxamyl treatment network, which appeared fragmented (Figure 6). This observation was further supported by network analysis parameters (Table 1). The Oxamyl treatment exhibited the highest fragmentation value, the lowest connectedness value, a lower average degree, and fewer ties between nematode genera compared to the other treatments and the control. In contrast, the network analysis values indicated that the MWE and Fur treatments displayed higher cohesion compared to the control. Notably, the MWE treatment exhibited the most cohesive network, with the lowest fragmentation value, the highest connectedness value, the highest average distance value, as well as the greatest number of ties and nodes. Additionally, *Mesorhabditis* spp. was identified as a highly impactful genus in the community, with direct connections to the omnivore genera *Thonus* and *Aporcelaimellus.*

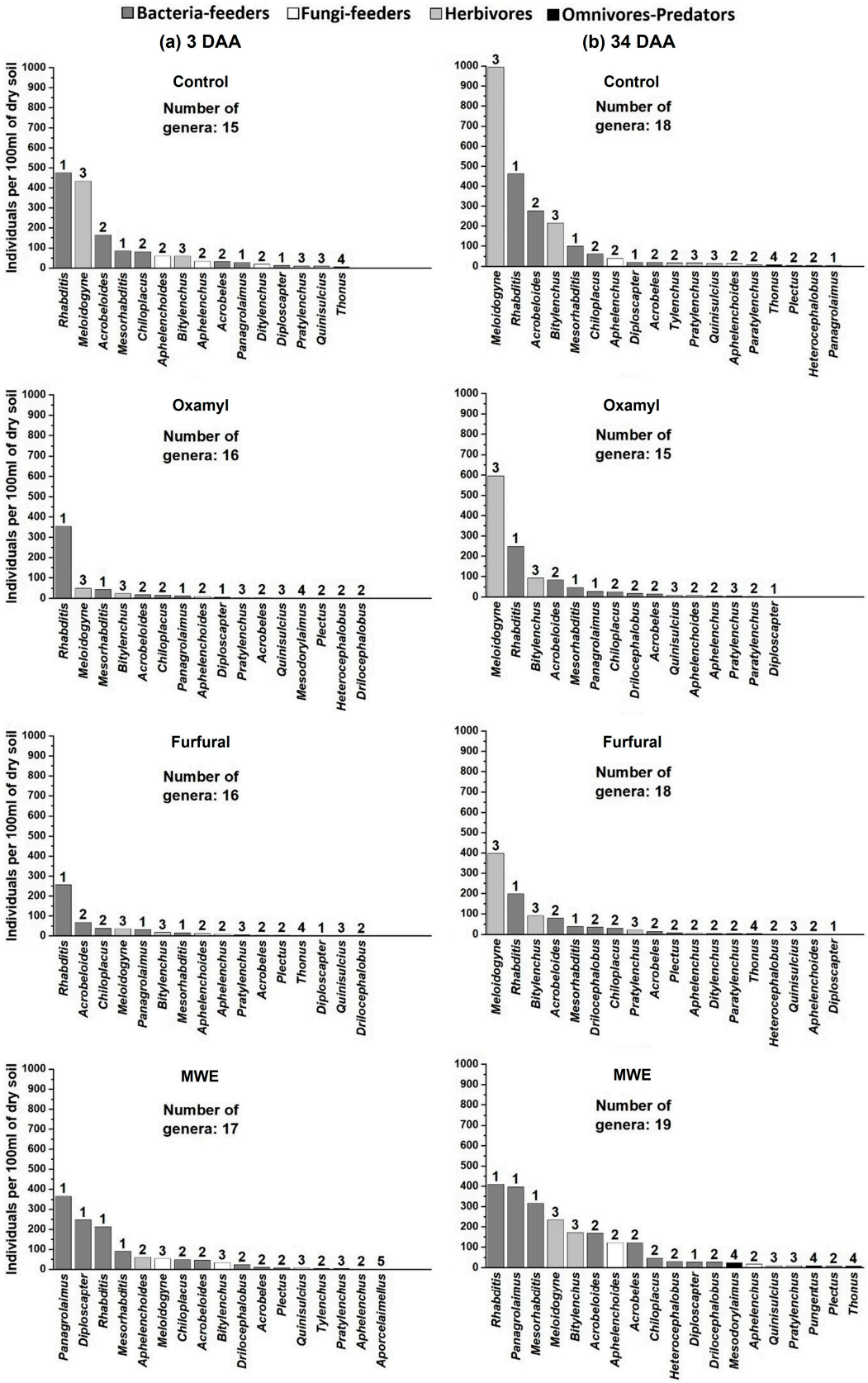

Figure 3. Rank abundance graphs for nematode genera at different treatments (**a**) 3 days after the last application (3 DAA) and (**b**) 34 days after the last application (34 DAA). Genera are ranked from the most to the least abundant. The numbers above bars indicate the c-p value of each genus. For all cases, $n = 5$.

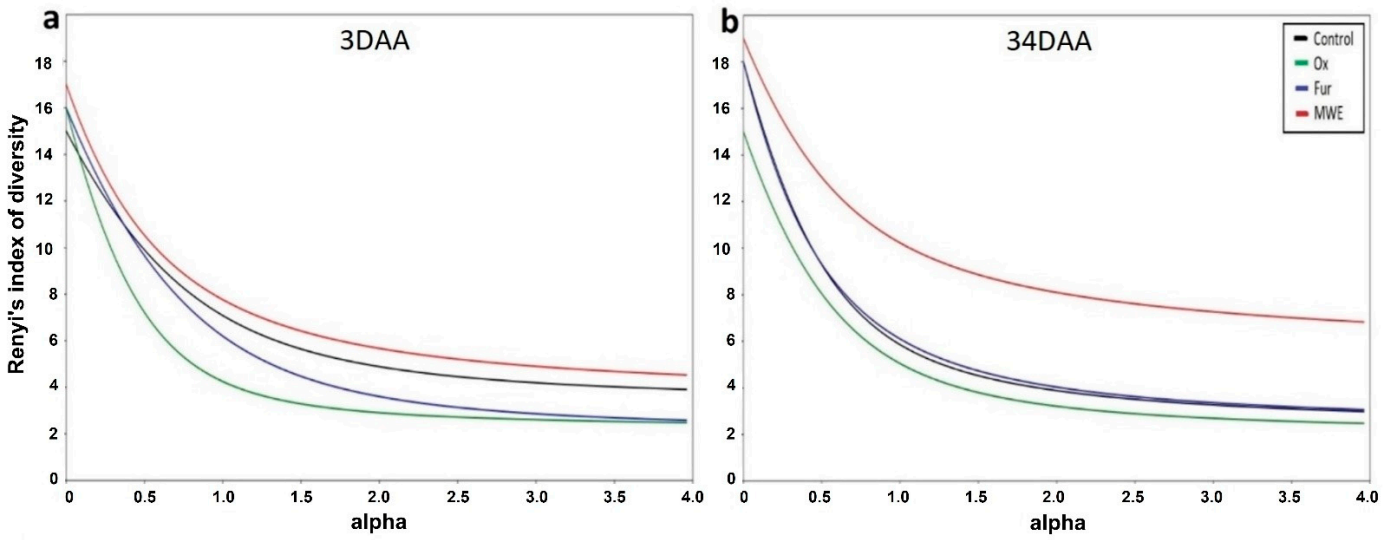

Figure 4. Diversity profiles of nematode communities in the three treated sites (Ox: Oxamyl; Fur: Furfural; and MWE: *M. azedarach* water extract) and the control, 3 and 34 days after last application ((**a**) and (**b**), respectively). For a = 0, 1, and 2, the index is equal to the genus richness, the Shannon index, and the Simpson index, respectively.

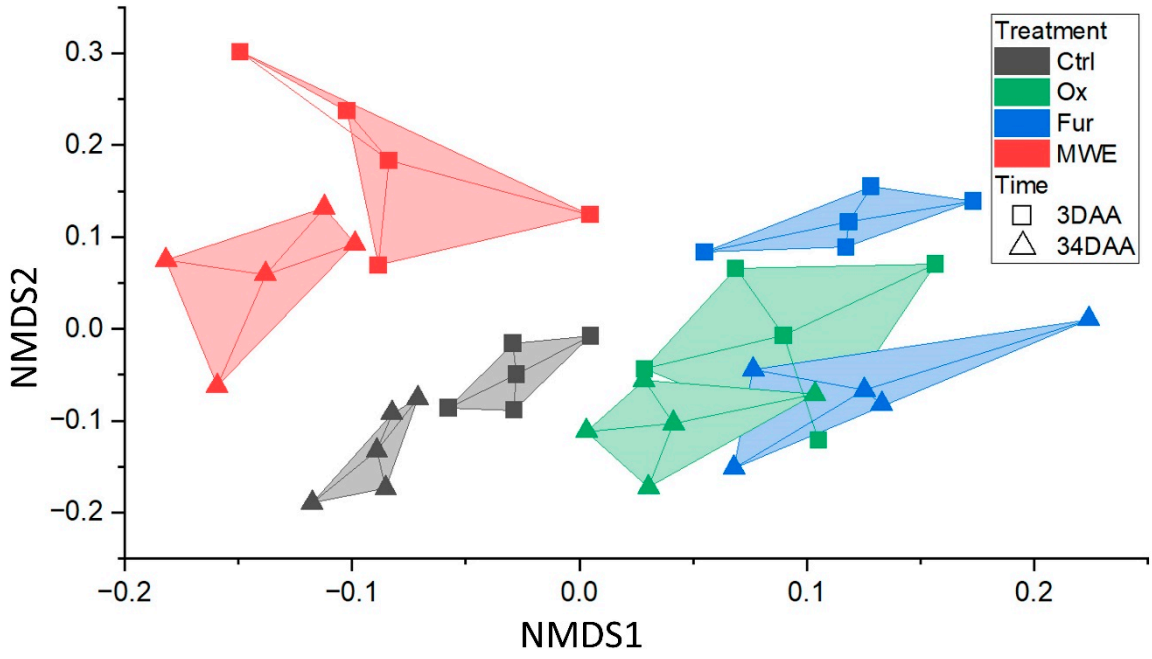

Figure 5. Non-metric multidimensional scaling (NMDS) ordination (Bray-Curtis dissimilarity) of nematode communities based on relative abundances of nematode genera, 3 and 34 days after application. Each point reflects the community found in an individual sample (n = 5 per treatment × 2 samplings). Points that are close together have more similar communities than points that are far apart. (Ox: Oxamyl; Fur: Furfural; and MWE: *M. azedarach* water extract).

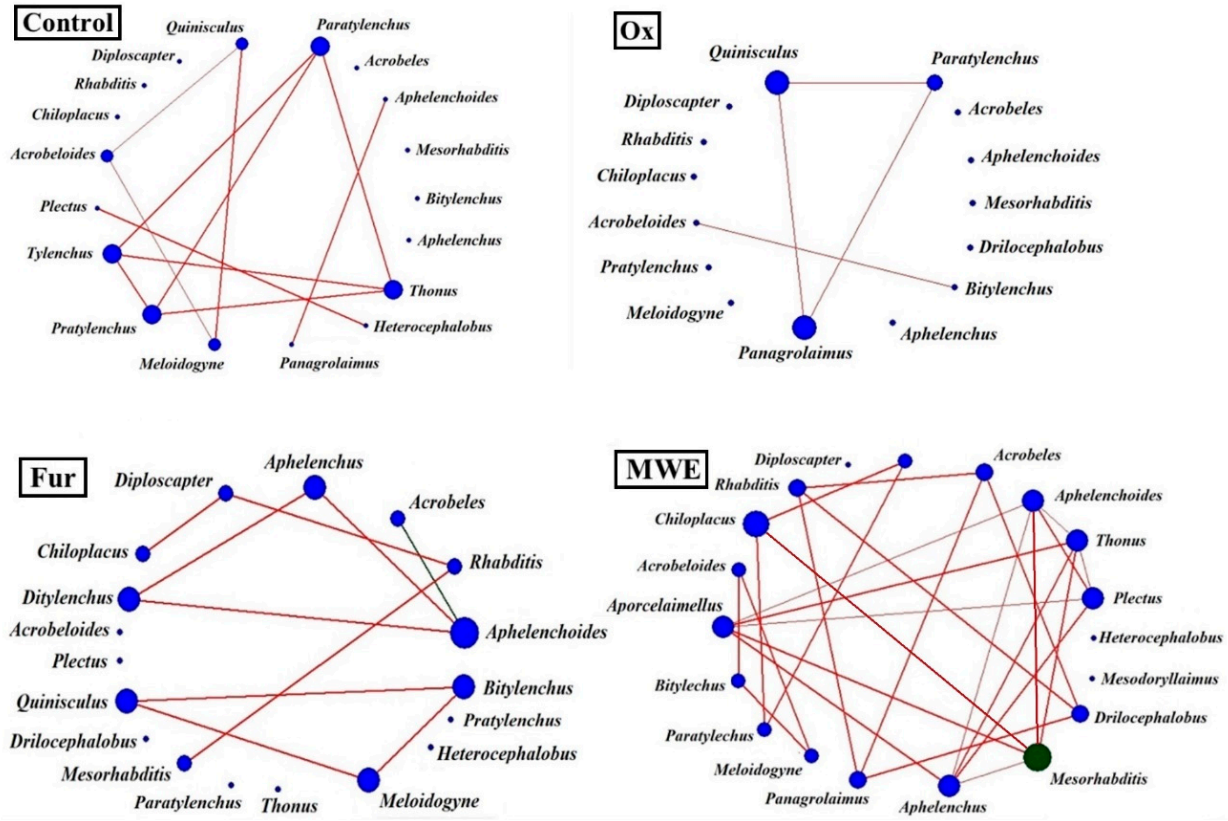

Figure 6. The network of interactions between nematode genera in Control, Ox, Fur, and MWE treatments (Ox: Oxamyl; Fur: Furfural; and MWE: *M. azedarach* water extract). The larger the node, the higher its degree of centrality values. (black circles: influencers; red lines: high significance interaction; grey lines: low significance interaction).

Table 1. List of network analysis parameters that showed the most significant differences between the treatments Control, Ox, Fur, and MWE (Ox: Oxamyl; Fur: Furfural; and MWE: *M. azedarach* water extract).

Treatment	Number of Ties	Connectedness	Fragmentation	Average Distance
Control	22	0.071	0.928	1.222
Ox	8	0.038	0.961	0.533
Fur	28	0.091	0.908	1.555
MWE	54	0.263	0.736	2.842

The SI values were not significantly affected by sampling, treatment, or their interactive effect (Table 2). The MI values were higher at 34 DAA compared to those recorded at 3 DAA, while the opposite pattern was observed for the EI values, which were significantly higher at 3 DAA. The control and Fur-treated samples exhibited the highest MI values, while the Oxamyl samples had the lowest values. On the other hand, the MWE-treated samples displayed the highest EI values. Among the nematode indices, the CI was the only one significantly affected by the interaction of sampling time and treatment. The control CI values at 3 DAA were significantly higher compared to all other treatments on both sampling occasions (Table 2). For soil nematodes, NMDS showed clear separation of MWE from the untreated ones (Ctrl), with the other two chemical treatments clustering together (Figure 5).

Table 2. Mean values ± standard error of the Maturity Index (MI), Enrichment Index (EI), Channel Index (CI), and Structure Index (SI) under the different treatments in both samplings were 3 and 34 DAA (Ox: Oxamyl; Fur: Furfural; and MWE: *M. azedarach* water extract). The same letters at the top of the columns show the mean values that are not significantly different (repeated measures ANOVA and Fisher's LSD post-hoc comparisons; *: $p < 0.05$; **: $p < 0.01$; ns: not significant, for all cases $n = 5$).

	Treatment	MI	CI	EI	SI
3 DAA	Control	1.36 ± 0.09 ab	5.11 ± 1.2 a	84.89 ± 4.02 b	3.23 ± 1.23
	Ox	1.15 ± 0.03 c	0.76 ± 0.37 c	89.76 ± 1.21 ab	11.11 ± 5.11
	Fur	1.42 ± 0.04 a	1.75 ± 0.48 bc	88.77 ± 1.95 b	10.27 ± 5.55
	MWE	1.21 ± 0.05 bc	1.74 ± 0.5 bc	94.33 ± 1.24 a	5.17 ± 2.17
34 DAA	Control	1.43 ± 0.10 ab	2.2 ± 0.34 bc	83.71 ± 4.88 b	4.55 ± 2.55
	Ox	1.37 ± 0.10 c	1.47 ± 0.54 bc	85.66 ± 5.62 ab	0 ± 0
	Fur	1.50 ± 0.10 a	1.66 ± 0.66 bc	78.43 ± 6.60 b	5.24 ± 3.22
	MWE	1.39 ± 0.05 bc	2.68 ± 0.98 bc	90.39 ± 1.15 a	20.33 ± 10.37
	S	**	ns	**	ns
	T	*	ns	*	ns
	SxT	ns	*	ns	ns

Soil food web analysis, based on the values of the enrichment and structure indices, showed that the soil samples from all treatments and the control were in an enrichment stage in both sampling times, as they were ordinated in the upper left quadrant (Figure 7a,b). These results also indicated that the bacterial metabolic pathway was the dominant one. Both bacterivore and fungivore footprint values were significantly lower in the Oxamyl and Furfural treatments compared to the control, and the MWE treatment (Table 3).

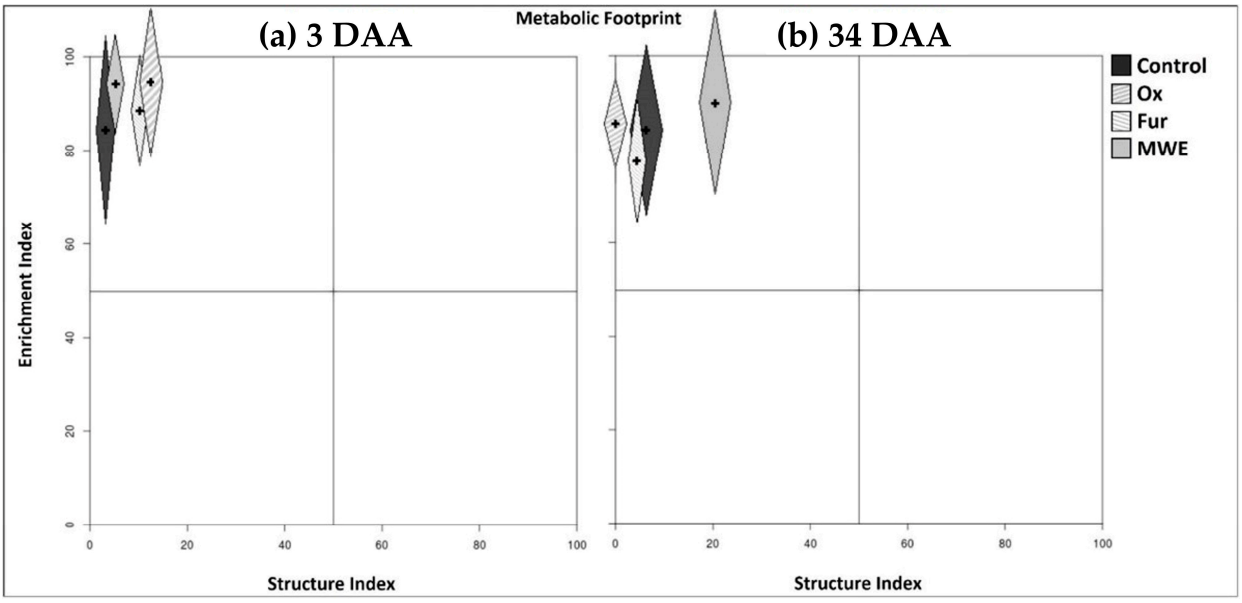

Figure 7. Food web analysis according to the EI/SI ratio of different treatments (Ox: Oxamyl; Fur: Furfural; and MWE: *M. azedarach* water extract) (**a**) 3 days after the last application (3 DAA) and (**b**) 34 days after the last application (34 DAA). The crosses point to the mean value of the ratio, and the colored areas show the respective standard deviation. Different colors show a different treatment. For all cases, $n = 5$.

Table 3. Mean values ± standard error of the metabolic footprints under treatments 3 and 34 DAA (Ox: Oxamyl; Fur: Furfural; and MWE: *M. azedarach* water extract) For each sampling occasion, within columns, means followed by the same letter are not significantly different (repeated measures ANOVA and Fisher's LSD post-hoc comparisons; *: $p < 0.05$; ns: not significant, for all cases $n = 5$).

	Treatment	Fungivore Footprint	Bacterivore Footprint
3 DAA	Control	18.21 ± 6.91 a	1080.61 ± 265.15 a
	Ox	0.77 ± 0.43 b	771.78 ± 331.76 b
	Fur	3.58 ± 1.25 b	568.86 ± 199.41 b
	MWE	4.71 ± 1.73 ab	871.43 ± 123.13 ab
34 DAA	Control	11.12 ± 2.53 a	1067.18 ± 147.72 a
	Ox	2.15 ± 0.76 b	568.83 ± 204.72 b
	Fur	2.87 ± 1.42 b	461.03 ± 169.61 b
	MWE	13.29 ± 7.26 ab	1188.17 ± 199.47 ab
	S	ns	ns
	T	*	*
	SxT	ns	ns

4. Discussion

The application of Oxamyl and Furfural treatments resulted in a significant decrease in the total nematode abundance on both sampling occasions. At 3 DAA, this reduction was accompanied by detrimental effects that extended beyond root-knot nematodes to free-living nematodes, including beneficial bacterial and fungal feeders, as well as other herbivore nematode genera. These negative impacts persisted even until the end of the cultivation period, at 34 DAA, highlighting the long-term effects of these treatments. Khanal et al. [8] similarly reported the toxic effects of Oxamyl on the aforementioned trophic groups, suggesting a non-selective mode of action that had a stronger negative impact on bacterivorous and secondarily on fungivore nematodes. Similarly, Grabau et al. [39] attributed damaging effects on bacterial and fungal feeding nematodes to Oxamyl, particularly at concentrations exceeding 0.5 mL/kg [40]. Interestingly, although *M. azedarach* contains Furfural as an active substance, the MWE application did not demonstrate a similarly harmful effect on free-living nematodes. This could be either due to the presence of other components in *M. azedarach* water extract that mitigate Furfural's toxicity [41] or due to lower concentrations of Furfural found in MWE, as botanical products typically contain lower concentrations of Furfural (<0.31 mL/kg). Nevertheless, our experiment revealed the toxicity of Furfural to bacterial and fungal feeders, even at lower dosages administered (0.31 mL/kg), compared to previous findings [40].

The application of MWE resulted in a significant increase in nematode abundances, even at 3 DAA. This positive and promising outcome suggests that MWE not only acts as a nematicide but also as a soil improver, as its application promotes the proliferation of beneficial nematode populations over the long term. *M. azedarach* botanical products contain a combination of easily and slowly degradable compounds, such as cellulose, hemicellulose, and lignin [42], which can serve as food sources for the microbial community [20,43], thereby increasing the abundance of microbial-feeding nematodes [18]. The sustained increase in free-living nematodes indicated that the labile organic matter introduced by MWE remained available for decomposition. Generally, the incorporation of organic inputs into the soil system has been found to increase the total number of nematode populations compared to plots that received chemical inputs [44]. Similarly, to our findings, Ntalli et al. [45] also reported significantly higher abundances of free-living nematodes in samples treated with botanicals as nematicides (such as anise, parsley, and rocket), while the application of fluopyram had a strong negative impact on their populations.

Despite the observed decrease in free-living nematode abundances from Oxamyl and Furfural, their incorporation did not alter the percentage composition of each group and appeared to be similar to that of the control, indicating a uniform effect against all nematode trophic groups. This negative and universal impact on the community

composition suggests an overall disturbance in the system. This pattern was also recorded at 34 DAA. Our findings are consistent with the results of [46], who also reported a decline in all nematode trophic groups, highlighting the equal impact of chemical nematicides on the faunal profile. Contrary to the Oxamyl and Furfural patterns, the percentage composition of the nematode community in the MWE treatment showed a greater abundance of nematode trophic groups, with significantly higher percentages of bacterial feeders in both samplings. Furthermore, the control samples, as well as the Oxamyl and Furfural-treated ones, were characterized by the predominance of the *Rhabditis* genus in both samplings. The low values of our MI and SI indices indicate that our field site was already highly disturbed prior to the application of our treatment, likely due to intensive greenhouse soil management. Hence, the dominance of a single nematode genus is typical in agricultural ecosystems that experience constant disturbance [18], and any further disturbance could enhance this predominance of tolerant genera over sensitive organisms. On the contrary, the rank abundance graphs revealed that in the MWE samples, the nematode richness and evenness were significantly increased, even three days after the last application. Three cp-1 bacterial feeders (*Rhabditis*, *Diploscapter*, and *Panagrolaimus*) had equal representation in the nematode community. This shift towards a more balanced distribution of genera implies a potential compensatory mechanism in response to the treatment and is an indication of soil quality improvement. At 34 DAA, the nematode community was even more evenly distributed compared to the previous sampling, and the bacterial feeding genera *Rhabditis*, *Panagrolaimus*, and *Mesorhabditis* were the most abundant ones. This indicates that MWE has the capacity to alter the nematode structure without causing widespread disturbances, suggesting a more targeted effect.

In continuation of the aforementioned observations, the diversity profile exhibited the lowest indices under the Oxamyl and Furfural plots. This is concerning, considering that biodiversity is crucial for functional complementarity and continuity [47]. This outcome is significant as a decline in the structural stability of an ecosystem can be observed through changes in community composition and richness indices over time, indicating reduced resilience and adaptability to disturbances [48]. In contrast, the MWE treatment displayed a more evenly distributed set of genera (Figure 3). This outcome was also visible on NMDS analysis, where the bigger distance between the chemical treatments and untreated samples is visible (Figure 5); moreover, MWE appeared to have a much smaller distance from the control. The presence of highly diverse nematode genera and the co-dominance of three cp-1 bacterial feeders suggest that botanical applications may lead to a healthier and more stable soil system [49]. Therefore, the equal dispersion of genera serves as a positive indicator of a balanced habitat and environment.

A healthy and functioning soil ecosystem is characterized by a well-structured food web that facilitates efficient energy flow and is critical for delivering essential services such as water storage, erosion control, and food and fiber production [50]. A fragmented network, on the other hand, exhibits a bottom-heavy structure, hinders population growth of predators, disrupts biological interactions, and limits mobility of soil organisms due to physiochemical constraints in the soil matrix [51]. In our network analysis assessment of nematode interaction structure, specifically focusing on density, compactness, and fragmentation of associative spatial relationships [52], we found the lowest interactivity in the Oxamyl and Furfural treatments, resulting in simplified and less structured communities. Moreover, this fragmentation further disrupts the intricate network and its constituent elements, resulting in adverse effects on agricultural fields, such as low soil fertility due to erosion and intensive agricultural practices, and contributing to biodiversity loss. Our results clearly indicated the association between Oxamyl application and strong fragmentation of the structural network. In contrast, the MWE treatment network exhibited the highest coherence, surpassing even the control, indicating not only an increase in nematode populations but also enhanced connectivity among genera (Figure 6). Highly connected networks, such as the one observed in the MWE-treated plot, are considered to be more resilient in the face of disturbances [53]. In agreement with our observations, the findings

of [38] highlighted larger and more complex networks after organic amendment, with increased interactions between genera, which indicates stability within the community. This is particularly significant in the system, as any changes in diversity within a specific functional guild or trophic group can have implications for the abundance, diversity, and functioning of other associated groups [54]. In our case, the increased numbers of *Mesorhabditis* recorded at 34 DAA could play an important role in this improvement. The MWE network clearly highlighted their influential role and their relationship with the omnivorous genera *Aporcelaimellus* and *Thonus*. There appears to be a trophic specialization of the omnivore genera found in our study towards *Mesorhabditis*. Achieving increased populations of this specific genus could potentially lead to more balanced communities. These results were further supported by the SI values in the MWE samples at 3 DAA, mostly due to the increased numbers of cp-4 (*Thonus*, *Mesodorylaimus*) and cp-5 (*Aporcelaimellus*) omnivorous nematodes, indicating a tendency towards a more stable and mature community [55]. Gupta et al. [56] also reported a higher accumulation of nematodes with higher c-p (3–5) values in plots treated with botanical pesticides, resulting in higher SI values and indicating more structured food webs.

5. Conclusions

Our study revealed important insights into the effects of Oxamyl, Furfural, and *M. azedarach* water extract (MWE) treatments on nematode communities. Oxamyl and Furfural treatments led to notable declines in overall nematode abundance, affecting both plant-parasitic and free-living nematodes across sampling intervals. Contrasting this, the MWE application displayed a dual benefit by increasing nematode populations and enhancing soil quality. The uniform impact of chemical treatments on community composition and the targeted positive influence of MWE emphasize the potential of botanical treatments for sustainable pest management and ecosystem health. These findings contribute to our comprehension of agricultural practices that harmonize pest control with ecosystem vitality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/soilsystems7040080/s1. Table S1: The genera that were recorded in this study, along with their cp categorization and their feeding type. Table S2(a,b): Mean values (±st. error) of Shannon and Simpson indices for every treatment and results, of ANOVA in both sampling occasions (3 and 34 DAA). Different letters indicate significant differences among treatments based on Fisher's LSD post hoc test (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). For each treatment, $n = 5$.

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