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The Effect of Varying Olive Mill Wastewater Concentrations on Soil Free-Living Nematode Communities and Lettuce Growth

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Abstract: We assessed the impact of increasing olive mill waste (OMW) concentrations (10%, 35%, 70%, and 100% *v*/*v*) on soil free-living nematode communities and *Lactuca sativa* (lettuce) growth, 10 and 45 days after application (DAA). *L. sativa* plants showed a survival threshold at OMW10%, with higher concentrations proving fatal. Contrary to expectations, nematode abundance increased with OMW concentration. OMW10% induced a rapid surge in nematode abundance, stabilizing at 45 DAA, resembling control values. OMW35%, OMW70%, and OMW100% plots exhibited persistent, gradual increases, surpassing control values at 45 DAA. All treatments favored fungal feeders, resulting in the overdominance of the genus *Aphelenchus* both at 10 and 45 DAA. Even though OMW did not exert a toxic effect on nematode populations, this shift in the community structure towards the dominance of a single genus could suggest an imbalance in the soil community, which could have negative implications for soil health and ecosystem functioning. Overall, our study provides insights into the complex interactions between OMW, soil nematode communities, and plant growth, emphasizing the importance of understanding soil ecology for sustainable agricultural management.

Keywords: feeding groups; free-living nematodes; metabolic footprint; nematode indices; organic amendment; soil food web

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1. Introduction

Agricultural practices continually evolve as the demand for sustainable solutions intensifies in the face of environmental challenges. In this context, the utilization of by-products from various industries as soil amendments has gained attention for its potential benefits and drawbacks. One such by-product, olive mill wastewater (OMW), presents a complex mixture of organic compounds, posing both opportunities and challenges in agricultural ecosystems. The olive oil manufacturing process generates significant quantities of olive mill waste which presents a notable problem due to its high acidity and concentrations of chemical oxygen demand (COD) and biological oxygen demand (BOD) [1,2]. Given the impracticality of various tested physical, chemical, and biological technologies for olive mill wastewater treatment, it is often discharged untreated into sewer systems, water streams, or inefficiently stored in evaporation ponds; nevertheless, this practice leads to the degradation of the soil system and greenhouse gas emissions [3,4]. However, repurposing OMW within the waste-to-resource paradigm and recycling it could contribute to a successful strategy for implementing the circular economy model. This approach has the potential to yield significant socioeconomic benefits, particularly for low-income Mediterranean countries [5].

OMW's polyphenols exhibit strong antimicrobial and phytotoxic effects, hindering its biodegradation [1,6]. Preliminary studies suggest that the application of olive mill wastewater can alter soil nutrient availability, affect root development, and influence the overall physiological processes of crops. Even a brief exposure of raw OMW to *Mentha spicata* L. cuttings led to irreversible damage to rhizogenesis and shoot development [7]. The diluted OMW demonstrated a negative impact on the seed germination of maize and tomato. Researchers achieved a significant decrease in monomeric phenols through anaerobic digestion. However, this reduction did not decrease toxicity towards germination, implying that other components may also contribute to phytotoxicity [8].

Despite its toxic aspects, OMW contains organic compounds, sugars, minerals, and growth-promoting substances; the nature of these constituents is influenced by the olive cultivar, maturation degree, climate conditions, and agricultural practices [9]. The richness of compounds present in OMW enables an increase in organic matter content and enhances soil fertility, making it a potential candidate for use as a fertilizer and soil conditioner [10,11]. This conversion into agricultural input stands out as a highly promising valorization approach. When treated with OMW, the soil's water retention capacity increased due to a reduction in bulk density, resulting in increased soil porosity and the formation of stable aggregates [12]. The land application of olive mill wastewater has been reported to increase organic matter content and enrich essential inorganic nutrients (P, K, Mg, Fe), vital for promoting plant growth [13]. Numerous studies reported that OMW contributed to positive metabolic and physiological responses in plants, enhancing their growth [4,10,14]. However, interest has arisen regarding its impact not only on subsequent effects on crop productivity but also on soil organisms.

Several studies demonstrated that OMW addition caused significant shifts in the structure and function of microbial communities, which in turn had an impact on soil fertility [15,16]. Additionally, other studies highlighted that raw OMW and its polar fraction were highly toxic not only to microorganisms but also to invertebrates (*Daphnia magna*, *Thamnocephalus platyurus*, *Chironomus riparius*) and vertebrates (*Danio rerio*, *Cyprinus carpio*), exhibiting toxicity even at low concentrations [17,18]. Furthermore, the use of OMW has also attracted attention as having the potential to control plant pathogens and post-harvest diseases [2,19]. Biopesticide properties were assigned to OMW phenolic extract compounds, which were reported by many as having ecotoxicological effects on soil habitat function [20,21]. Although many studies were conducted on OMW's suppressiveness of plant-parasitic nematodes [2,10], the literature regarding its effect on the soil nematode community is limited [22]. Further investigation is required to comprehensively understand their response to the addition of OMW.

Soil nematodes are considered an essential category of soil organisms and serve as useful indicators of soil health and soil recovery due to their rapid response to changes in soil conditions [23–25]. Their sensitivity to environmental changes, including variations in soil structure, nutrient content, and organic matter [26], positions them as a reliable reflection of soil ecosystem dynamics [27]. Although herbivorous soil nematodes can be harmful for agricultural production, by closely monitoring the abundance and diversity of free-living nematode communities, researchers gain an understanding of the subtle shifts in soil status, offering a real-time assessment of soil health, especially after chemical or biological applications [28].

The aim of our study was to assess the impact of OMW on soil nematode communities. We hypothesized that the application of olive mill waste to soil with low organic matter content would significantly affect the abundance and composition of soil nematode communities. Specifically, we anticipated that increasing concentrations of OMW would reduce their abundance and diversity. Additionally, we sought to evaluate the impact of OMW on plant productivity. Recognizing the intricate relationship between soil ecology and crop performance, our objective was to provide valuable insights for the development of environmentally responsible agricultural practices. Furthermore, we aimed to contribute to the broader discourse on utilizing industrial by-products in agriculture while ensuring soil biodiversity and resilience preservation.

2. Materials and Methods

2.1. Experiment Design

The present study utilized olive mill wastewater (OMW) sourced from a three-phase olive oil mill located in Souroti, proximate to the city of Thessaloniki, Greece. The collected OMW was extracted from settled reservoirs and subsequently preserved at −20 °C, until its application in the experimental procedures. Soil samples for the study were obtained from agricultural fields in Thermi, Thessaloniki. Post-collection, the soil underwent homogenization, the breaking of large aggregates, and sieving through a 6 mm sieve. The soil type was identified as loam using the Bouyoucos method [23]. Furthermore, pH was measured in a soil-distilled water paste (1:1), organic carbon (%) was measured using the titration method [24], and total nitrogen was measured using the Khendjal apparatus [25]. The results are elucidated in Table 1 to provide comprehensive insight into its composition for this investigation.

Table 1. The mean values (±st. error) of the physicochemical parameters of the soil used in our study (n = 5).

Soil Parameter	Value
Sand (%)	47%
Clay (%)	24%
Silt (%)	32%
pH	7.51± 0.01
Soil Organic C (%)	1.82 ± 0.01
Total N (%)	0.15 ± 0.02

2.2. Pot Experiment

Lettuce (*Lactuca sativa*) seeds, obtained from a commercial supplier specializing in plant seeds, were planted in a seed container filled with soil that had been sieved through a 2 mm mesh. The growth period spanned 45 days under open-air conditions. Upon attaining the four-leaf stage, individual lettuce plants were transplanted into 0.5 L pots filled with non-sterilized, sieved soil. Four specific dilutions of olive mill wastewater (OMW) were formulated to establish distinct levels of application: 10% v/v (OMW10%), 35% v/v (OMW35%), 70% v/v (OMW70%), and 100% v/v (OMW100%). A control group that received no OMW application was also included. Each dilution, totaling 80 mL, was carefully administered to its corresponding pot to prevent spillage and ensure containment. OMW application was conducted as a one-time event. Control soils, without OMW, were created by substituting OMW with distilled water. Diverse dilutions were created using water as the diluent substance.

The pots were organized in a completely randomized design, with five replicates per treatment. The pots were consistently watered throughout the experimental duration to maintain a moisture content of 10% w/w, a level that does not hinder plant growth. The daily weighing of pots was conducted to ascertain water loss, following the method outlined by Troelstra et al. [26]. No fertilizers were applied to the pots. To assess the short- and long-term effects of the treatments on both plant development and soil nematodes, two destructive samplings were conducted at 10 and 45 days after the application of olive mill wastewater (referred to as 10 and 45 DAA), with 25 samples collected for each sampling event. The soil was maintained at a cool temperature (4 °C), until processing. After oven-drying plant shoots and roots for 48 h at 70 °C, the total biomass of the plants was determined.

2.3. Nematode Extraction, Identification, and Indices

Nematodes were extracted from 150 mL of each soil sample using the modified Cobb's sieving and decanting method, as detailed by S'Jacob and van Bezooijen [27], culminating in the use of a cotton wool filter in the final step. After quantifying the overall nematode abundance under a stereoscope, nematodes were heat-killed and then preserved in 4% formaldehyde. Approximately 100 randomly selected nematodes were identified at the genus level under a microscope using Bongers' [28] identification key and considering morphological characteristics, including the stomodeum, reproductive organs, and tail. Nematode taxa were then classified into trophic groups following the colonization–persistence gradient (c-p values) by Bongers [29] and Bongers and Bongers [30] and grouped into functional guilds according to Ferris et al. [31] and Bongers and Bongers [30], signifying portions of specific trophic groups exhibiting the same c-p value.

The nematode functional indices employed in our study capture various attributes of the nematode community. The maturity index (MI) for free-living nematodes and the plant parasitic index (PPI) for taxa feeding on plants were computed based on Bongers [29]. Lower MI values indicate greater soil disturbance, as measured by Yeates et al. [32] and Bongers and Ferris [33]. The PPI serves as a maturity index for taxa feeding on plants. The weighted faunal analysis proposed by Ferris et al. [34] was employed to calculate the enrichment index (EI), channel index (CI), and structure index (SI), reflecting the functional structure of the soil food web. The SI provides insights into whether the soil ecosystem is more trophically linked and organized or less trophically linked and degraded, following Ferris et al. [34]. The metabolic footprint (MF), representing carbon utilization by nematodes and encompassing the sum of the lifetime amount of carbon allocated to growth, egg production, and respiration, was computed in accordance with Ferris [34], using the nematode indicator joint analysis online platform (NINJA) [35].

2.4. Statistical Analysis

To assess the influence of time, various treatments, and the interplay between these variables on the soil nematode community and plant characteristics, a two-way analysis of variance (ANOVA) incorporating treatment and time as independent variables was conducted. Initial scrutiny involved testing the data for adherence to ANOVA assumptions, including considerations such as the normality and homogeneity of variance. Subsequently, to discern significant effects, a least square differences (LSD) test was applied, facilitated by STATISTICA 9 Software. Renyi and nonmetric multidimensional scaling (NMDS) graphs, along with a SIMPER test table, were generated using PAST SOFTWARE to visually represent and interpret the observed patterns in the data.

3. Results

Our results on plant characteristics are presented in Figure 1. The OMW10%-treated specimens exhibited greater upper length values (10.48 ± 0.25 cm) at 45 days after application (45 DAA) (Figure 1a). In contrast, the dry mass was lower (0.33 ± 0.01 g) compared to the control (0.44 ± 0.02 g) when OMW10% was applied (Figure 1). Notably, the plants treated with OMW35%, OMW70%, and OMW100% exhibited burn-like symptoms and eventually died.

Our samples contained nematode genera comprising 16 bacterivores, 5 fungivores, 8 herbivores, and 7 omnivores (Table S1). The abundance of distinct nematode trophic groups across different treatments, assessed at 10 days after application (DAA) and 45 DAA, is depicted in Figure 2. Overall, a higher number of nematodes (550 ± 107.85 individuals/100 mL soil) were recorded in the first sampling (10 DAA). Moreover, at 10 DAA, the total nematode population exhibited a significant increase across all treated samples compared to the control, except in the OMW100% treatment, where the increase was not significant. A similar pattern was also evident in bacterivores and fungivores; nevertheless, the OMW35%-treated samples did not show a significant increase in fungivores compared to the control. The populations of the herbivores were solely affected by the Time factor and were significantly lower (19 ± 7.51 individuals/100 mL soil) in the second sampling (45 DAA). At 45 DAA, OMW70% and OMW100% showed significantly elevated values in the total nematode population (735 ± 133.97 and 607 ± 122.47 individuals/100 mL soil, respectively), bacterial feeders (192.1 ± 21.19 and 98.02 ± 18.74 individuals/100 mL soil, respectively), and fungal feeders (496 ± 118.67 and 492.73 ± 101.39 individuals/100 mL soil, respectively). Herbivores exhibited levels similar to the control in treated samples at 45 DAA. Predators and omnivores showed low abundances (10 ± 5.16 and 11 ± 4.72 individuals/100 mL soil) in both sampling instances, consistent with expectations for cultivated soils.

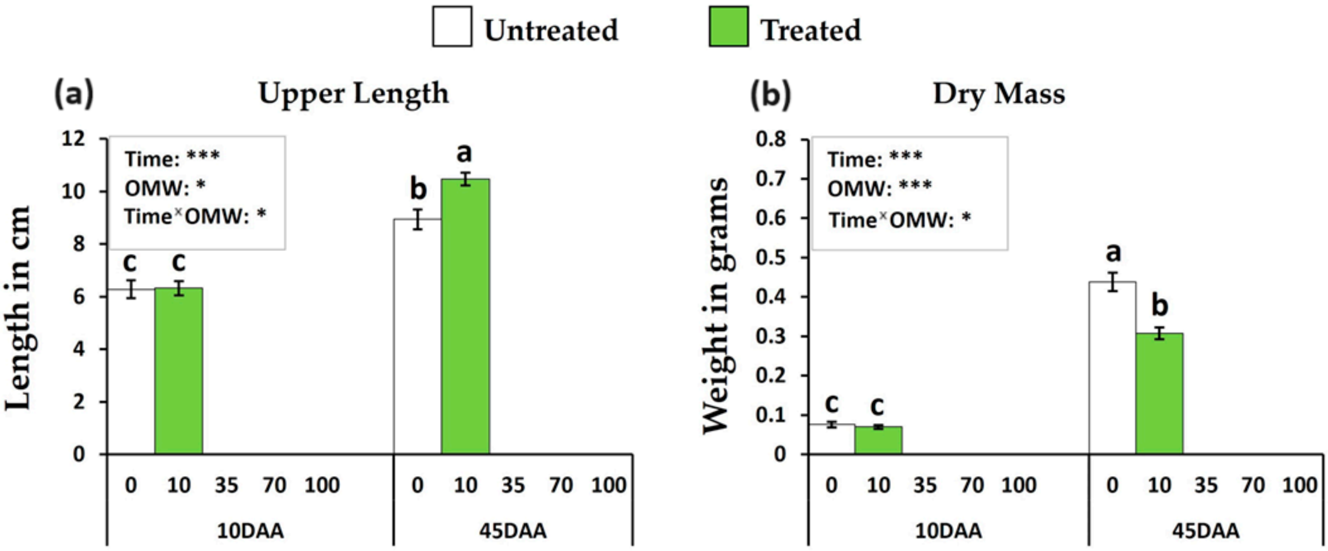

Figure 1. The mean abundance values (±st. error) of plant (**a**) length and (**b**) dry weight under different concentrations of olive mill wastewater (OMW), and the results of the two-way ANOVA 10 DAA and 45 DAA (DAA: days after application). In the case of a significant effect, different letters (a, b, c) indicate significant differences among treatments based on Fisher's LSD post hoc test (*: $p < 0.05$; ***: $p < 0.001$, for all cases $n = 5$). In concentrations exceeding 10%, the plants died, exhibiting burn-like symptoms, and were consequently excluded from the two-way ANOVA. The letter "a" always points out the highest value.

Although an increase in abundance was recorded in some applications, this was not reflected in the relative abundance (Figure 3), suggesting that the composition remained unaffected, with changes observed only in abundance. The percentage contribution noticeably changed only in the OMW70% and OMW100% treatments at 45 DAA, where fungal feeders were more enhanced (Figure 3b). Evidently, at 45 DAA (Figure 3b), the OMW35%, OMW70%, and OMW100% treatments presented a higher relative abundance of fungal feeders (66.2, 67.6, and 76.3%, respectively).

In Figure 4, the disturbance at 10 DAA is not as evident, as both treated and control samples are clustered in the middle of the graph, except for OMW-10%, which exhibited a significant distance from the control. At 45 DAA, on the other hand, the distance between treated and untreated samples becomes clearer, with treated samples observed to be as distant from the control as the concentration of OMW application increases.

At 10 DAA, there is a notable enhancement in *Aphelenchus* abundance across all applications (Figure 5). Additionally, the abundance of two bacterivores (*Acrobeloides* and *Chiloplacus*) shows a substantial increase in each application, resulting in minimal observable changes in the overall percentage contribution. At 45 DAA, a distinct trend emerges where the OMW70% and OMW100% treatments were characterized by a greater dominance of *Aphelenchus* (419 ± 118.87 and 386 ± 88.76 individuals/100 mL soil, respectively). This observed dominance suggests a disturbance within the system, indicating that a significant and pronounced impact has occurred. This enhancement in *Aphelenchus* abundance was also evident through the SIMPER test (Table S2). This enhancement closely correlated with the corresponding OMW concentration applied. Although the overall structure remained consistent, the difference between treatments was primarily attributed to the increase in *Aphelenchus* population.

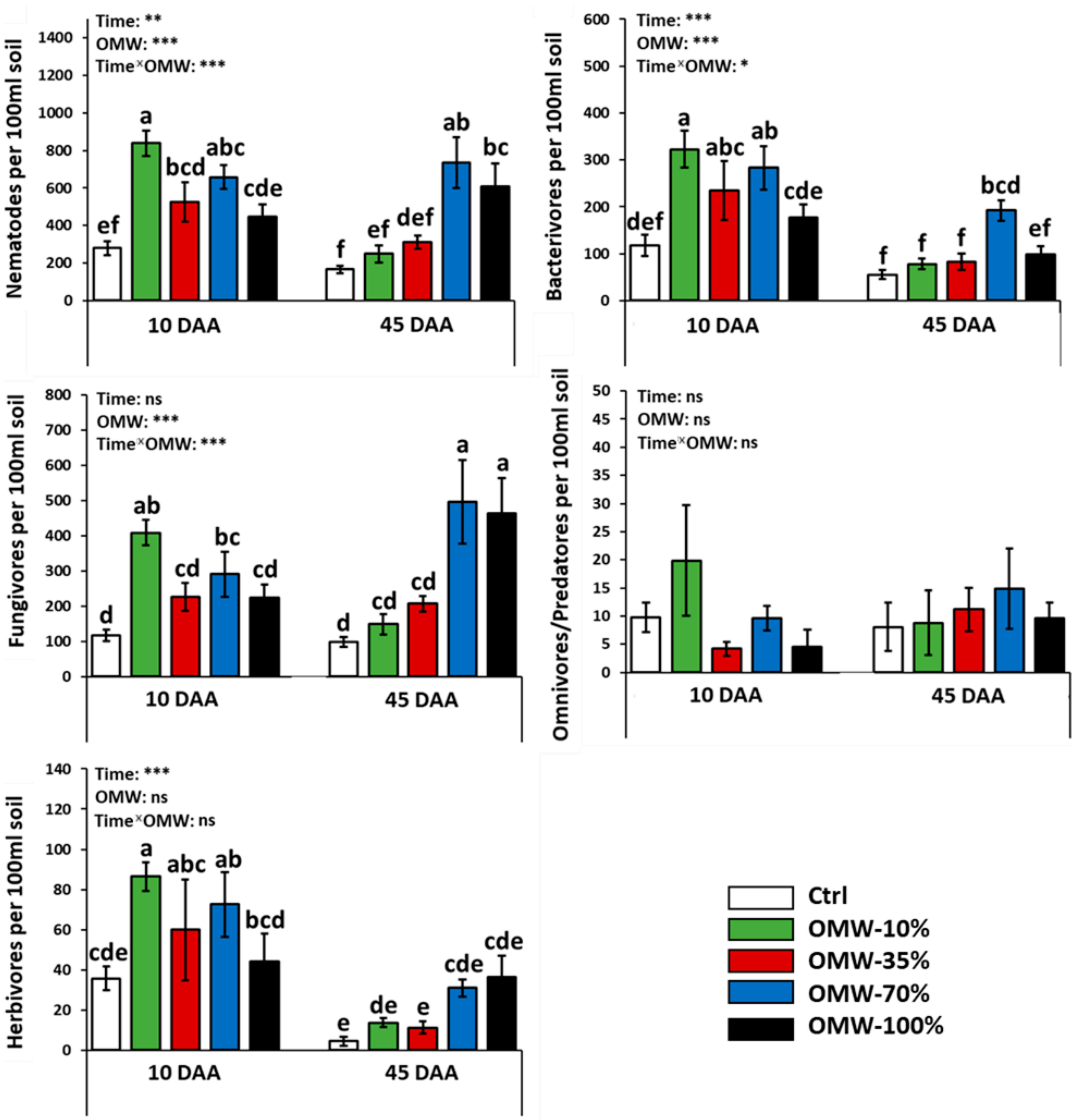

Figure 2. The mean abundance values (±st. error) of nematode trophic groups under different treatments and the results of the two-way ANOVA regarding "olive mill wastewater" (OMW) 10 DAA and 45 DAA (DAA: days after application). In the case of a significant effect, different letters (a, b, c, d, e, f) indicate significant differences among treatments based on Fisher's LSD post hoc test (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: $p > 0.05$, for all cases $n = 5$). The letter "a" always points out the highest value.

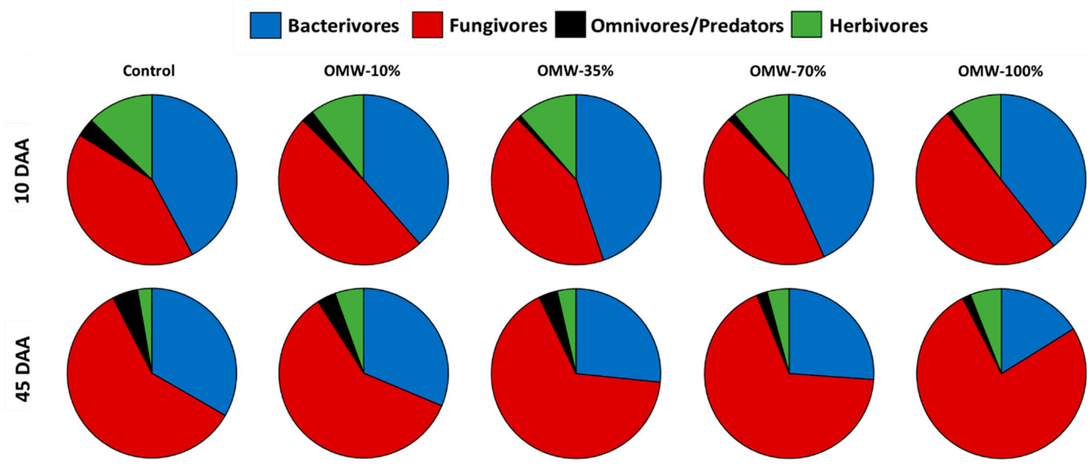

Figure 3. The percentage contribution of the trophic groups at different treatments 10 DAA and 45 DAA (DAA: days after application). For all cases, $n = 5$.

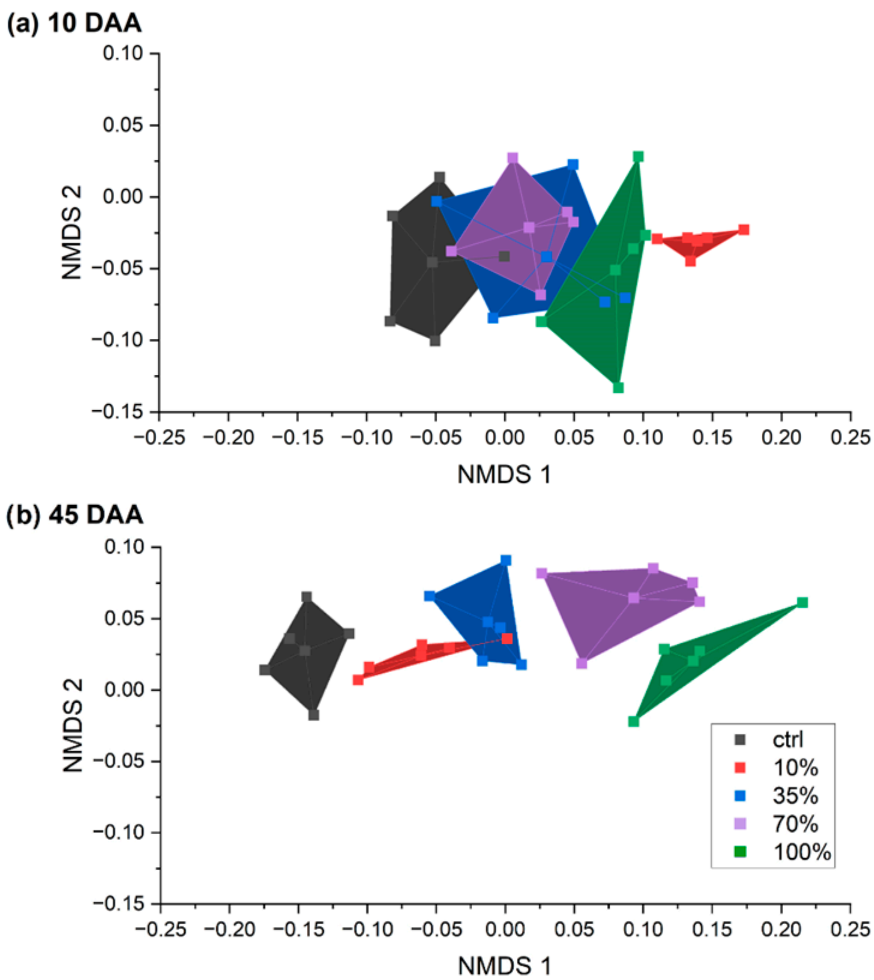

Figure 4. A nonmetric multidimensional scaling (NMDS) plot showing the diversity of soil ecosystems. A Bray–Curtis distance similarity matrix was calculated based on the pairwise taxonomic profiles of 40 soil samples and used to generate NMDS coordinates of each sample. The distance linking two samples is shorter, indicating higher similarity between these samples. Samples from the five different treatments (control, 10%, 35%, 70%, and 100%) are illustrated by different colors in two different time intervals ((**a**): 10 DAA and (**b**): 45 DAA) (DAA: days after application). For every treatment, $n = 5$. The central symbol of each polygon is the mean value.

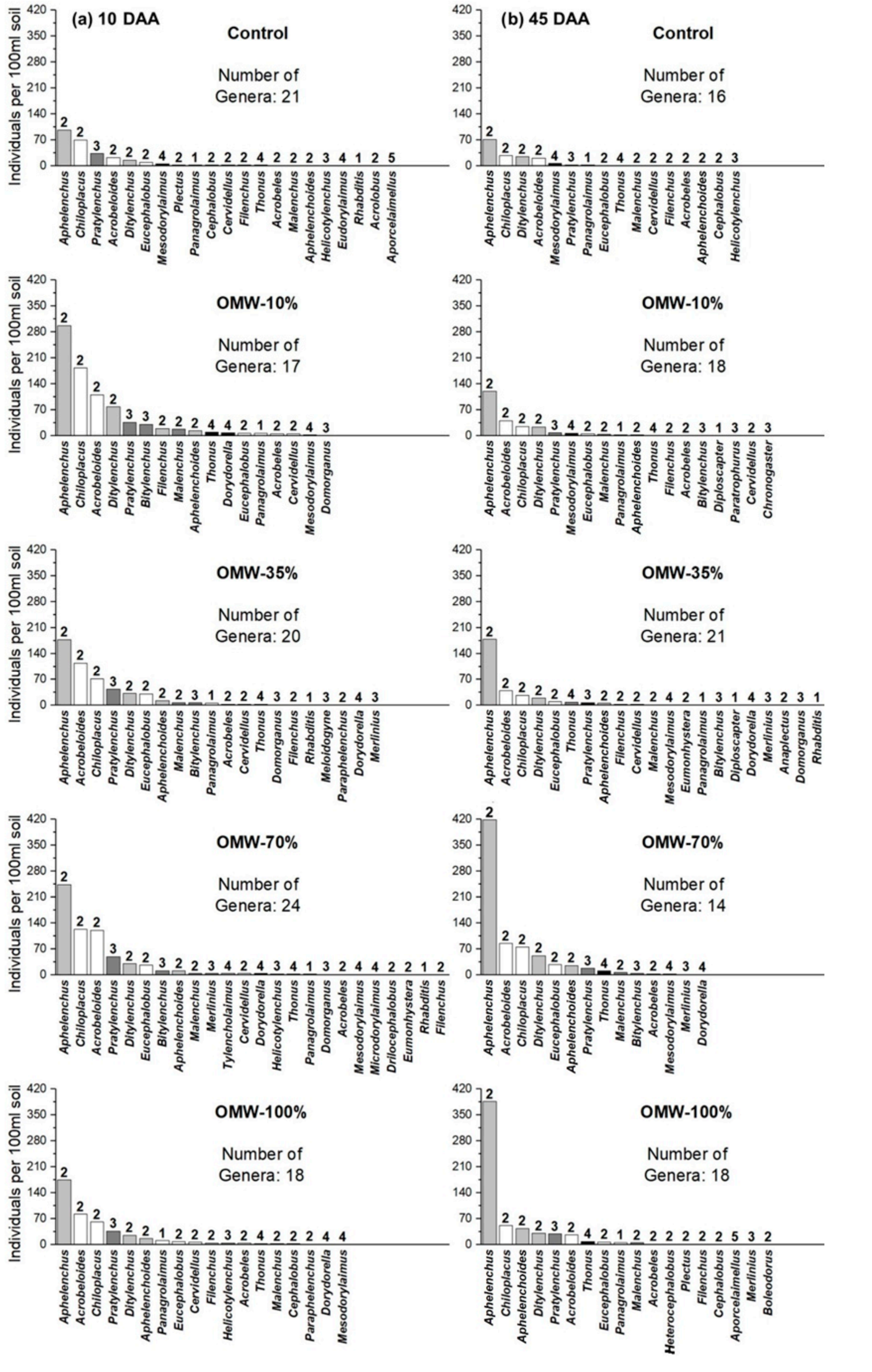

Figure 5. Rank abundance graphs for nematode genera at different treatments (**a**) 10 and (**b**) 45 DAA (DAA: days after application). Genera are ranked from the most to the least abundant. The numbers above bars indicate the c–p value of each genus. Light gray columns indicate the fungal feeders, white columns indicate the bacterial feeders, black columns indicate the omnivores/predators, and dark gray indicate the herbivores. For all cases, n = 5.

The EI, CI, MI, bacterivore, and fungivore footprint values were estimated at 10 and 45 DAA and are presented in Table 2. OMW application affected only bacterivore and fungivore footprints. The interaction between OMW and Time affected only the fungivore footprint; interestingly, the OMW70% and OMW100% treatments had low values (31.16 ± 13.93 and 23.64 ± 10.57, respectively) at 10 DAA but appeared to be the highest (52.72 ± 10.97 and 46.94 ± 9.57, respectively) at 45 DAA. Conversely, the Time factor significantly affected the CI and EI with values higher (94.22 ± 3.71 and 43.30 ± 1.49, respectively) at 45 DAA. On the contrary, the bacterivore footprint was significantly higher (10.98 ± 1.73) in the first sampling (10 DAA).

Table 2. The mean values of the channel index (CI), enrichment index (EI), bacterivore footprint (BF), and fungivore footprint (FF), under the different treatments 10 DAA and 45 DAA (DAA: days after application). For each sampling occasion, within columns, means followed by the same letter are not significantly different (two-way ANOVA and Fisher's LSD post hoc comparisons; $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: non significant; for all cases, $n = 5$).

Time	OMW	CI	EI	MI	FF	BF
10 DAA	Control	89.00 ± 3.41	36.40 ± 1.89	2.08 ± 0.03	12.7 ± 5.68 d	8.60 ± 0.60
	OMW10	93.00 ± 3.12	33.50 ± 3.07	2.05 ± 0.03	45.76 ± 20.46 ab	12.20 ± 2.05
	OMW35	83.60 ± 6.98	37.00 ± 2.79	2.01 ± 0.01	24.69 ± 11.04 cd	11.70 ± 2.92
	OMW70	94.30 ± 2.50	37.60 ± 0.35	2.04 ± 0.01	31.16 ± 13.93 bc	10.90 ± 0.56
	OMW100	85.00 ± 6.16	40.20 ± 2.92	2.01 ± 0.03	23.64 ± 10.57 cd	11.50 ± 1.56
45 DAA	Control	92.30 ± 3.88	43.10 ± 1.29	2.08 ± 0.04	11.74 ± 2.37 d	7.90 ± 0.38
	OMW10	89.80 ± 5.42	42.60 ± 1.32	2.04 ± 0.03	16.59 ± 3.39 cd	8.70 ± 0.31
	OMW35	94.60 ± 4.08	43.50 ± 1.50	2.06 ± 0.02	21.68 ± 3.00 cd	7.30 ± 1.20
	OMW70	100.00 ± 0.00	40.90 ± 1.71	2.04 ± 0.02	52.72 ± 10.97 a	8.20 ± 0.86
	OMW100	94.40 ± 2.70	46.40 ± 1.13	2.03 ± 0.01	46.94 ± 9.57 ab	4.70 ± 1.45
Time		*	***	ns	ns	***
OMW		ns	ns	ns	***	**
Time*OMW		ns	ns	ns	***	ns

The food web analysis based on the EI/SI ratio of the OMW treatments and the control for both samplings is presented in Figure 6. All samples from both samplings were aligned in the lower left quadrant, indicating that all soil samples were experiencing stress due to low nitrogen concentration.

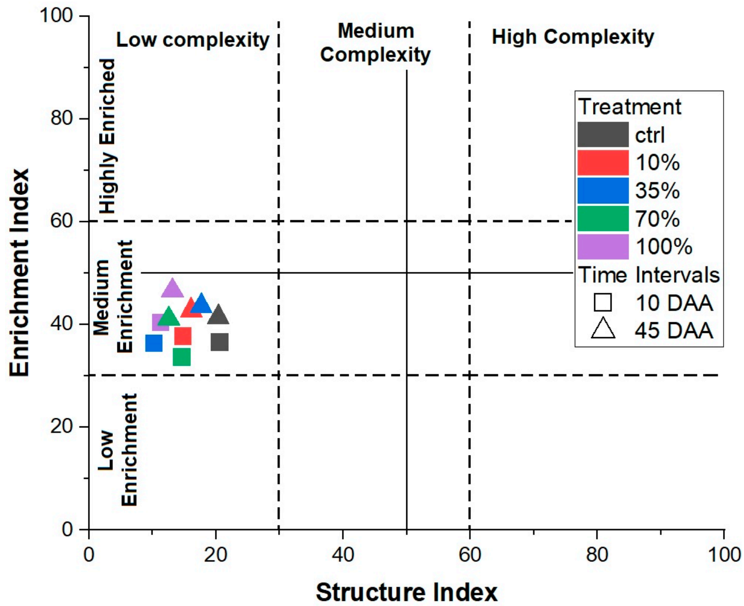

Figure 6. Food web analysis according to the ordination of the samples based on the EI and SI mean values of different treatments at 10 DAA and at 45 DAA (DAA: days after application). Different colors show a different treatment. For all cases, $n = 5$.

4. Discussion

4.1. Impact of OMW on Lactuca sativa

We studied the impact of OMW on *Lactuca sativa* plants and determined a survival threshold at OMW10%, with higher dosages proving fatal. Dilution with water, a proposed mitigation strategy, has been shown to decrease OMW phytotoxicity [36,37], which clarifies the observed phytotoxic effects (e.g., acidity, salinity) on plants with increasing concentrations of OMW (above 10%). Presumably, the boundary between beneficial and harmful concentrations is narrow and likely crop-specific, consistent with findings by Rusan and Malkawi [4] and Elayadi et al. [38].

4.2. Effect on Nematode Abundance, Community Composition, and Genera Diversity

Due to the observed plant demise in all treatments exceeding OMW10%, exploring the relationship between plant growth and nematode abundance, diversity, and plant interaction was not feasible. Our initial hypothesis was that nematode abundance and diversity would decrease with increasing waste concentration; however, our results proved the opposite. At 10 DAA, OMW10% induced a rapid surge in nematode abundance, stabilizing at 45 DAA resembling control values, indicating that the organic material was decomposed by the time of the second sampling. In contrast, OMW35%, OMW70%, and OMW100% plots showed persistent, gradual increases, significantly surpassing the control group at 45 DAA. The presence of phenolics in OMW did not exhibit toxicity effects on soil nematode populations, suggesting a collaborative degradation of lignin-derived aromatic compounds by fungi and bacteria [39,40]. At 10 DAA, in all treated plots, even though fungal feeders increased their abundances, this was also accompanied by an increase in bacterivorous numbers; however, by 45 DAA, fungal feeders dominated in OMW70% and OMW100% plots, highlighting their interconnection with OMW-rich environments. The delayed increase in the fungivore population numbers in the plots treated with higher OMW concentrations (over 35%) was likely due to the higher OMW concentrations containing larger amounts of less labile organic compounds. This resulted in slower decomposition by microorganisms and an increased dominance of fungivores in the long term. These findings differ from those recorded after the incorporation of other types of organic amendments with high C/N ratios in the soil system, which typically increase bacterial populations, causing a spike in bacterivorous nematodes [41,42]. Several studies reported increased fungal populations and fungal/bacterial ratios in OMW-amended soils, suggesting that OMW serves as a suitable substrate for fungi [36,43–46] and thus explaining the dominance of fungivores post-OMW addition. Presumably, OMW phenolic compounds stimulate yeasts, fungi, and consequently enhance the population of their predators, i.e., fungivorous nematodes [47].

A contrasting pattern was observed for herbivores compared to the increase in non-herbivorous nematodes. By 45 DAA, there was a visible decline in their abundance across all plots. Several studies have indicated that plant-derived phenolics have a detrimental effect on plant-parasitic nematodes but exert minimal or no negative impact on free-living nematodes [48,49]. Dutta et al. [50] explain this differential response due to variations in chemosensory gene sequences, which make PPNs more responsive to plant-derived metabolites. Lastly, the decline in PPNs by 45 DAA can be explained by the subsequent demise of plants in treatment groups with increased concentrations, leaving herbivorous nematodes without a viable food source. This scarcity accounts for the absence of a notable increase in their abundance.

While we observed differences in nematode abundances among all treatments at 10 DAA, the percentage contribution of trophic groups did not differ compared to the control, indicating an unaltered community composition. The incorporation of OMW resulted in increased numbers of fungal and bacterial feeders, proportionally. The genus *Aphelenchus*, a cp2 fungal feeder belonging to the family Aphelenchidae, was the most affected and increased in numbers. Bacterivorous genera such as *Chiloplacus* (cp-2) and *Acrobeloides* (cp-2), members of the Cephalobidae family, were also positively affected by

OMW additions. However, at 45 DAA, the OMW70% and OMW100% treatments exhibited a completely different community composition, with the overdominance of *Aphelenchus*. This overdominance of the *Aphelenchus* genus in response to increasing organic waste concentrations can be attributed to the adaptive capabilities of *Aphelenchus* nematodes to tolerate a wide range of pH levels (including highly acidic) and maintain osmotic balance in response to changing environmental conditions, contributing to their resilience in the presence of high concentrations of OMW [51].

The increased abundance of these cp-2 nematodes indicates stress in the soil system [31,52]. The MI values remained near 2 across all treatments and the control, indicating a stressful environment due to a disturbance unrelated to nutrient enrichment. The low MI values in the control suggest that the soil from the field site may have been disturbed even before the application of the treatments. Notably, although an increase in cp-1 nematodes, indicative of an enriched nutrient status, was expected with the addition of OMW, their rapid population expansion was not observed. The incorporation of OMW did not significantly alter the food web condition. The EI and CI values, observed across all treatments including the control, remained low (<50), indicating limited nutrient availability and a predominant fungal decomposition pathway [33,53]. Additionally, the low SI values suggest that OMW application resulted in the increase in the abundance of soil nematodes belonging to lower c-p guilds rather than omnivores/predators [54]. The impact of stress factors (i.e., low nitrogen concentrations resulting in high C/N values) on community functionality and stability could be significant, potentially leading to a decline in diversity due to the disappearance of the most sensitive species (cp-4 and cp-5) [30,55]. Thus, no significant increase in cp-4 nematodes was recorded, indicating a further decline in the soil food web condition along with the depleted stage (Figure 6).

4.3. Assessing Levels of Disturbance

At 10 DAA, OMW10% caused the highest level of disturbance relative to the control group, as evident in the NMDS plot. However, this change was reflected in a proportional increase in both fungivores and bacterivores, suggesting a balanced response within the community. By 45 DAA, the community structure had reverted to its predisturbance state in the OMW10% plot, exhibiting no significant deviations from the control. This may indicate the resilience of the nematode community and its capacity to restore equilibrium following transient perturbations. A contrasting scenario was observed at 45 DAA for plots OMW75% and OMW100%, with a pronounced overdominance of a single genus—Aphelenchus—marking a substantial deviation from the control. This, in turn, indicates a persistent disturbance and suggests a long-term alteration of the nematode community dynamics, as is also evident from the SIMPER test (Table S2). Given the correlation between diversity and ecological stability, defined by the resistance and resilience of the soil's ability and speed to recover after disturbances [56], the overdominance of one specific genus may weaken the diversity of ecosystems, which are better equipped to withstand environmental perturbations when there is the presence of species with varying traits [57]. Hence, despite the absence of reductions in abundance or genera numbers, the community is evidently under increasing pressure due to the disturbance from OMW, and subsequently, the stability of the community is compromised and could potentially lead to problematic outcomes if another disturbance were to occur within this system.

5. Conclusions

We conducted a pot experiment using varying concentrations of olive mill waste diluted with water (10%, 35%, 70%, and 100%). All amendments increased nematode abundance, particularly favoring fungal feeders, whereas higher concentrations (35%, 70%, 100%) resulted in a permanent shift in the community towards the dominance of the genus *Aphelenchus*, decreasing species diversity. OMW did not promote plant growth and had detrimental effects, leading to plant mortality at concentrations exceeding 10%. Our findings suggest that the application of OMW10% has the potential to be utilized as an organic amendment without eliciting negative impacts on the soil nematode community, structure, or soil stability. Hence, we attempted to investigate the complex interactions between OMW, plants, and soil nematodes. Despite the challenges posed by OMW's phytotoxicity, implementing careful management practices, such as dilution and monitoring application concentrations, may offer sustainable solutions. Redirecting research efforts toward exploring alternative wastewater treatment techniques, particularly those that would be, most importantly, techno-economical viable options, is crucial. Additionally, it is required to assess the long-term consequences of these practices on soil ecosystems. Ultimately, this 45-day experiment provided valuable insights into the immediate effects of these practices at a small scale, and it is further required to assess the long-term consequences of such practices on soil ecosystems. Moreover, studying the effects of OMW on other types of soils is necessary, which potentially could lead to different outcomes. Further research should prioritize the implementation of proper nematode sampling schemes in large-scale experiments to define the exact distribution patterns and co-occurrence of soil nematode communities under real field conditions [58].

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/su16093848/s1, Table S1: The genera observed in both samplings (10 DAA and 45 DAA) with their cp values and respective trophic group (10 DAA: ten days after application; 45 DAA: forty-five days after application); Table S2: Dissimilarity percentages between treatments and results of Similarity Percentage analysis (SIMPER) based on Bray-Curtis. Genera accounting for ∼70% of overall dissimilarity are ranked in order of importance of their contribution. The upper right half of the table refers to the first sampling (10 DAA) and the lower left half of the table refers to the second sampling (45 DAA) (DAA: days after application). For every treatment $n = 5$.

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