

Article

Mulching with Leaf Litter from Municipal Green Waste Favours Predatory Mononchid Nematodes

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Abstract: Although the incorporation of organic amendments into the soil is widely researched, less attention has been given to the impact of in-situ composting of municipal green wastes (MGW), especially leaf litter, on free-living nematodes. In a four year experiment (2016–2019) of tomato cropping cycles, we tested the hypothesis that leaf litter mulch has a positive effect on mononchid predatory nematodes and influences their species composition. Two treatments were applied every year: plots were either mulched with leaf litter or left unmulched. Soil samples were collected every autumn at the end of the growing season. Active, free-living nematodes were extracted by Baermann funnel from soil samples, the density of mononchid predatory nematodes was determined, and the individuals were identified to genus or species. In the first year (2016), mulching did not affect the density of mononchids. From the second year (2017) on, their number significantly increased in mulched plots, compared to in unmulched ones. During the study period the presence of four mononchid genera: *Clarkus*, *Mylonchulus*, *Prionchulus*, and *Iotonchus*, were detected. Our hypothesis was confirmed, as the number of mononchids increased in the presence of MGW leaf litter mulch layer.

Keywords: organic mulch; sustainable crop protection; organic waste; in-situ composting; circular economy; predatory nematodes; surface mulching

1. Introduction

Attention has been given to finding sustainable procedures and management measures in the past few decades for reducing the populations of plant-parasitic nematodes. Circularity is a key component of sustainability where resources are carefully used and reused. The New Circular Economy Plan of the European Commission acknowledges wastes, including organic waste, as a potential resource for this purpose [1].

The main producers of organic waste are agriculture, industry, and municipalities. It contains biodegradable waste collected from households and public and recreational areas. The organic or biodegradable fraction of municipal wastes is often referred to by different names, so for the purposes of our study we use Municipal Green Wastes (MGW), which includes compostable agricultural, urban, and household waste [2]. The percentage of MGW in total municipal solid waste is estimated from 30–40% to as much as 50–60% [3,4]. MGW is generated every year in massive amounts everywhere in the temperate zone with hard, woody materials including tree and bush cuttings and bark, and soft materials including grass clippings, fallen leaves, and annual and perennial plants [5].

Although this valuable material has potential in agriculture and horticulture, the percentage of bioprocessing (recycling and composting) is somewhat low, but on the rise; landfill and incineration still account for the largest percentage of management of MGW [6]. To support sustainability, decrease the environmental footprint, and enhance the effects of ecosystem services, these organic materials have to be returned to or retained in their site of origin [7]. Physical, chemical, and microbiological characteristics of MGW influence decomposition, and the end-product of a correct compost procedure, mature compost has a definite beneficial impact on soil properties, soil microbiome, and crop health [8]. Composting may take place in larger facilities (compost plants), but also on the location where green waste is generated in special containers or in compost heaps. It may mean the above-noted household options [9,10] and other organic waste that is spread on a surface, creating a slowly degrading and decaying mulch that offers sustainable benefits [11,12]. In-situ composting offers a sustainable and environmentally friendly alternative; benefits in vineyards include lower costs and lower environmental impacts when compared to other composting options [13]. Spreading organic materials (e.g., straw, leaf litter, compost, yard waste, crop residues), as a form of in-situ composting has further positive aspects: mulching increases in-field and soil biodiversity [14,15], protects beneficial organisms [16], enhances soil suppressivity against pests [16–18], and improves soil physical and chemical characteristics [19–21].

Soil is an extremely diverse habitat [22], in which the group of free-living nematodes represent the largest part of Metazoa [23]. The species composition of the nematode community is an important indicator of soil health condition [24]. Their numbers may be influenced primarily by vegetation and various edaphic factors [23,25]. They represent different life strategies and feeding behaviours, play an important role in the soil food web, and are indicators of the ecosystem and soil organic matter processes [26–28]. They respond with different life cycle and reproduction strategies to environmental disturbances and changes [29]. Among free-living nematodes, predatory nematodes could be a potential part of ecosystem services. They belong to four major orders: Aphelenchida Siddiqi, Diplogasterida Maggenti, Dorylaimida Pearse, and Mononchida Jairajpuri. These groups differ in their feeding apparatus and feeding mechanism. They may consume more than 1000 nematode individuals in their lifetime. They prey on both endo- and ectoparasitic nematodes, but their food preferences can be different [30]. In addition to being natural enemies of harmful nematodes, predatory nematodes also affect nitrogen mineralization by feeding on bacterivore nematodes [31]. Of the predatory nematodes, mononchids are the most fragile group. Their lifespan and reproduction are mainly influenced by natural conditions, especially temperature, in which organic soil amendments could play an important role [32]. Mononchids preferred the following characteristics of soil: pH level of 5.78–6.58, soil temperature 20.6–23.0 °C, soil moisture 17.71–24.53%, and organic matter content of 3.06–4.28% [33].

Organic amendment materials incorporated into the soil may increase the density of predatory nematodes [30,34–38]. The effect of soil surface mulching with organic materials or in-situ composting especially with a mulch of leaf litter from MGW is a less studied topic. Under shredded paper (alone or combined either with biosolids or with MGW compost), the abundance of omnivorous and predatory nematodes (*Aporcelaimellus* Heyns, *Carcharolaimus* Thorne, *Clarkus* Jairajpuri, 1970 *Discolaimus* Cobb, *Paraxonchium* Krall, *Sectonema* Thorne, and *Seinura* Fuchs) was higher than under the unmulched control, alfalfa (*Medicago sativa* L.) hay or plastic mulch in apple (*Malus domestica* Horkh) [39]. Mulching may be a cornerstone to enhance populations of predatory nematodes in open fields [40], and a recent survey [41] investigating the effect of surface leaf litter found that not only the abundance of nematodes increased, but their trophic composition has also changed in favor of bacterivore and omnivore-predatory taxa.

According to a review, only 4% of publications about free-living nematodes between 2000 and 2019 focus on predatory nematodes [32]. Being typical K-strategists, predatory nematodes could be appropriate indicator species of long-term effect of mulching

with leaf litter during an in-situ composting process on predatory nematodes in open field tomato. We tested the hypotheses that mulching with leaf litter (1) has a positive effect on mononchid predatory nematodes; and (2) influences the species composition of Mononchida.

2. Materials and Methods

2.1. Open-Field Experiment

We established our study plots on the Experimental Field of the Plant Protection Institute of Hungarian University of Agriculture and Life Sciences (Magyar Agrár- és Élettudományi Egyetem, MATE, the former Szent István University) Gödöllő, Hungary (47°35'21.97" N 19°22'03.58" E). The dominant soil type is Haplic Luvisol [42] with a coarse sand texture with 2% of organic matter content, pH (H₂O) 7.8, and pH (KCl) 6.8 values. Various common arable crops such as sunflower (*Helianthus annuus* L.), corn (*Zea mays* L.), winter wheat (*Triticum aestivum* L.), and potato (*Solanum tuberosum* L.) were cultivated between 2011 and 2015. The soil was ploughed or rotationally tilled, and no pesticides or fertilizers were applied before the experiment [43].

2.2. The Design of Microplots

Trials were conducted between 2016 and 2019. With the aim to be able to detect any potential long-term effect the treatments may have caused, microplots were located and designed the same way every year (superimposed arrangement). To study the role of mulch, irrigation, and their combinations, the following treatments were set up: mulch only, irrigation only, mulch and irrigation combined, and untreated control. Treatment combinations were arranged in a nested split-split plot design to avoid two adjacent microplots receiving the same treatment. To separate treatments and provide the same size for each microplot, we constructed a pinewood frame and laid it down on the field. It resulted in 24 microplots, each measuring 2 × 2 m on a total area of 96 m². In one microplot, four tomato plants were planted, with 1 m² planting space for every plant. Microplots were either mulched with leaf litter or left unmulched, which served as the control. All in all, 48 replications (plants) were used both in mulched and unmulched control. In the first three years, microplots were also irrigated or not (Figure 1).

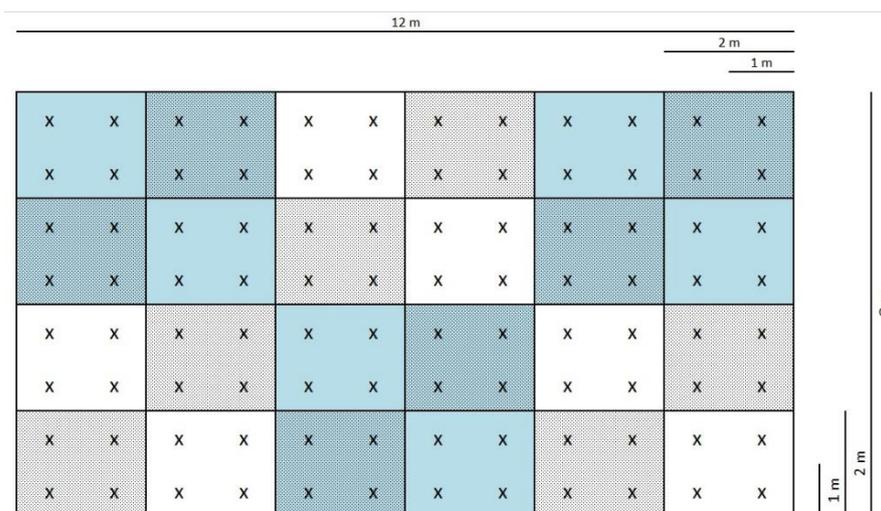


Figure 1. Split-split plot experimental design nested by year for testing the effect of leaf litter mulch on the presence of predatory nematodes (Gödöllő, Hungary, 2016–2019). Coloration means irrigated vs. non-irrigated treatment (blue vs. white); dotted means mulched vs. unmulched treatment (dotted vs. undotted), “X” shows tomato plants.

2.2.1. Plant Materials

The Hungarian heirloom tomato (*Solanum lycopersicum* L.) “Dányi” (gene bank accession RCAT057829) was grown every year. Seeds were provided by the Plant Diversity Center, (Növényi Diverzitás Központ, NöDiK, Tápiószele, Hungary) and the Research Institute of Organic Agriculture (Ökológiai Mezőgazdasági Kutatóintézet, ÖMKi, Budapest, Hungary). Seeds were sown into a general potting soil (Florimo[®], Matécsa Kft., Kecel, Hungary), and seedlings were transplanted into 5 × 5 cm pots. Seedlings received nutrient solution (VitaFlóra[®] for strawberry and vegetables, Vitaflóra Kft., Nemesvámos, Hungary) twice a week until planting. Seedlings were 7–8 weeks old at the time of planting.

2.2.2. Mulching Protocol

Mulching material was evenly spread in a thickness of 15 cm without being incorporated into the soil. When the initial mulch layer was reduced to a thickness of 3–5 cm, additional mulch material was added, but not more than once during a vegetation period. In the first year, our mulch material was leaf litter of various tree species, mostly Norway maple (*Acer platanoides* L.), common oak (*Quercus robur* L.), and sycamore (*Platanus × acerifolia* Mill. ex Münchh.), provided by Zöld Híd B.I.G.G. Non-profit Kft. (Gödöllő, Hungary). For mulching in subsequent years, leaves of various tree species dominated by those noted above were collected from the inner yard and park of MATE (Gödöllő, Hungary). Every spring we found that the leftover mulch layer of the past year was thin (with a maximum thickness of 1 cm), so a fresh layer of mulch was applied.

2.2.3. Plot Maintenance

Tomatoes were planted into holes made to undisturbed soil (one crop per year), and no disturbance occurred throughout the year. Mulched plots were hand-weeded until the termination of the experiment in 2019. Unmulched plots received a mechanical weeding protocol comprising of hoeing and hand-weeding as described in Petrikovszki et al. (2020) [43]. No other soil disturbances occurred.

2.2.4. Irrigation Protocol

In the first three years, water was supplied by a drip irrigation system consisting of lines and individual drippers at each plant. Following the method of Helyes and Varga (1994) [44], the amount of supplied irrigation water was based on the daily weather dataset corrected for the amount of rainfall. The calculated optimal amount of water was supplied to the plots three times a week. Because irrigation had no effect on the number of free-living nematodes, or on yield, we discontinued irrigation in 2019 (Table 1).

Table 1. Timeline of actions and weather conditions during the growing seasons of our study years (2016–2019, Gödöllő, Hungary).

Year	2016	2017	2018	2019
Planting (Beginning of season)	2 June	12 May	9 May	30 May
End of growing season	30 August	19 September	26 September	4 October
Mulching	18 March	17 March and 18 July	9 May	3 April and 23 August
Collection of soil samples	30 August	18 September	25 September	5 September
Rainfall	213 mm	299.5 mm	370.5 mm	166 mm
Irrigation water	153 mm	303.2 mm	193.4 mm	-
Average temperature	21.0 °C	21.1 °C	21.6 °C	20.8 °C
Minimum temperature	8.6 °C	7.0 °C	0.0 °C	3.0 °C
Maximum temperature	35.0 °C	38.0 °C	35.0 °C	36.0 °C

2.3. Soil Sample Collection

At the end of the growing season, once per year, approximately 200 g of soil was collected with a T-shape soil sampler with 2 cm of diameter (LD-Agro Technologies Ltd., Székesfehérvár, Hungary) (Table 1), when the plants were still in the field. From each of the 96 plants, four subsamples were taken from 20–30 cm depth, from an imaginary circle drawn around every plant (15–20 cm away from the base of the plant). Subsamples

then were mixed to have one sample per plant per year. Samples were stored at 5 °C until further examination. Active, free-living nematodes were extracted from the soil by the Baermann funnel technique [45]. The following modification of this technique was made: a paper handkerchief disc was placed on a 9 cm diameter sieve and 25 g of soil (instead of the original 20 g) was weighed and placed into the sieve. The bottom of the funnels ended in a rubber tube closed with a clamp. Funnels were filled with water and sieves were placed on top. After 48 hours, the clamp was opened and approximately 10–15 mL of water, together with the extracted nematodes, was introduced into a 15 mL centrifuge tube [46]. For counting nematodes, counting dishes of diameter 6.3 cm were used, and the grids at the bottom of the dish were 7.5 × 3.5 mm. Suspensions were filled in counting dishes, and after 5–10 min of sedimentation, nematodes were counted with a stereomicroscope (Olympus SZH 10, Olympus Optical Co. (Europa) GmbH., Hamburg, Germany) in transmitted light at 30× magnification.

2.4. Identification of Mononchids

During the enumeration of free-living nematodes, mononchid predatory nematodes were removed from the soil sample extracts with an automatic pipette (20–200 µL capacity) and collected separately into Eppendorf tubes per sample. Individuals were fixed in a formalin solution of 4% (± 80 °C). After 48 hours, the fixed specimens were moved into a mix of alcohol and glycerine (9:1). When alcohol evaporated, nematodes were placed into a drop of glycerine (87%) on a slide, which was then closed with a coverslip. Slides prepared this way were examined under a light microscope (Euromex Delphi-X Observer DX.1153-PLPHi, Euromex microscopen B.V., Arnhem, The Netherlands) at 200× and 400× magnification. Juvenile mononchids were identified to the genus level, whereas adult mononchids were identified to the species level. Identification was based on the descriptions by Andr assy (2009) [47] and Ahmad and Jairajpuri (2010) [48]. Predatory mononchids were counted in each year but identified only from the samples taken in 2018 and 2019.

2.5. Data Analysis

Mulched and unmulched plots were compared by two sample T test by years in case of the following nematode groups: non-predators (2016–2019), total predators (2016–2019), *Clarkus* (2018 and 2019), *Mylonchulus* Cobb (2018 and 2019), and *Prionchulus* Wu & Hoeppli genus (2018 and 2019). The effect of seasons (years) was tested by ANOVA and LSD (Least Significant Difference) post hoc test separated by mulched and unmulched plots in case of non-predators and total predators.

3. Results

Free-living nematodes extracted and counted from soil samples were divided into two functional groups during the evaluation: non-predators and predators.

Comparing the numbers of individuals per year according to mulching treatments, the number of non-predators fluctuated strongly in unmulched plots, whereas it stagnated in mulched ones. In the case of predatory nematodes, low numbers of individuals without significant changes were observed in the unmulched plots every year, but in the mulched plots, their numbers increased (Figure 2).

The presence of four mononchid genera—*Clarkus* Jairajpuri, *Mylonchulus* Cobb, *Prionchulus* Cobb, and *Iotonchus* Cobb—was detected in our experimental plots and various tendencies were noticed in the distribution of mononchid nematodes in the years 2018–2019 according to mulching treatments.

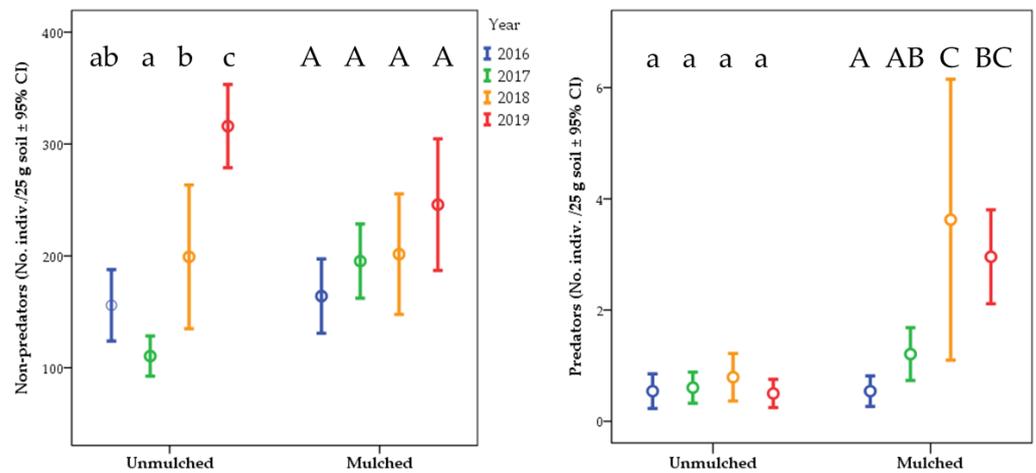


Figure 2. Numbers of predatory and non-predatory nematodes in mulched vs. unmulched open-field tomato plots in four consecutive experimental years. Gödöllő, Hungary. (One-way ANOVA, LSD (Least Significant Difference) post hoc test. Different letters indicate significant difference at $p < 0.05$ level. Small letters refer to the differences within unmulched, uppercase letters refer to the differences within mulched treatment. CI 95% means 95% of Confidence Interval).

Among the identified genera, the density of *Clarkus* was influenced positively ($p = 0.030$) by mulching in 2018. In addition, a similar tendency ($p = 1.148 \times 10^{-4}$) was observed in their number in the next year (2019). There was no difference ($p = 0.277$) in the number of *Mylonchulus* under mulch treatment and in the unmulched plots in 2018. In contrast, their number significantly increased ($p = 1.460 \times 10^{-4}$) in mulched plots as compared to the unmulched control. It is remarkable that individuals of *Prionchulus* were detected only in mulched plots both years, and no member of this genus was found under unmulched conditions (Figure 3).

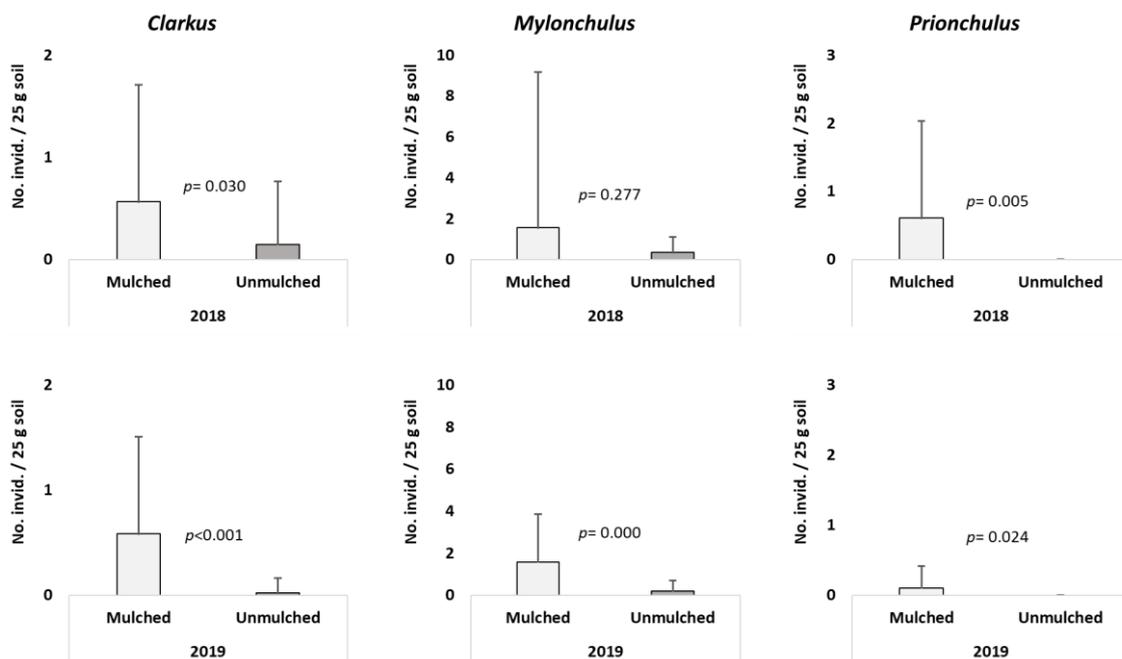


Figure 3. Numbers of mononchid individuals in mulched vs. unmulched open-field tomato plots in four consecutive experimental years. Gödöllő, Hungary. (Two sample T test).

Within the above-noted taxa, three species were identified: *Clarkus papillatus* Jairajpuri (Figure A1), *Mylonchulus brachyuris* Butschli (Figure A2), and *Prionchulus punctatus*

Cobb (Figure A3) (Table 2). The only *Iotonchus* individual found was a juvenile, so its identification to species level was not possible.

Table 2. Morphometric data of *Clarkus papillatus*, *Mylonchulus brachyuris*, and *Prionchulus punctatus* specimens from open-field tomato plots. Gödöllő, Hungary (mean \pm CI 95% and range of the measured values. CI 95% means 95% of Confidence Interval).

Character	<i>Clarkus papillatus</i>	<i>Mylonchulus brachyuris</i>	<i>Prionchulus punctatus</i>
n	20 ♀	17 ♀	1 ♂
L (μm)	1212.2 \pm 43.4 1012.8–1395.2	1089.2 \pm 44.4 948–1288.2	1198.2 –
a	24.3 \pm 2.2 18.6–40.3	28.7 \pm 1.8 22.1–34.7	31.6 –
b	3.7 \pm 0.1 3.4–4	3.7 \pm 0.1 3.4–3.9	3.8 –
c	16.6 \pm 0.5 15.1–18.4	31.7 \pm 1 28.1–35.5	31.9 –
V (%)	62 \pm 0.5 60.5–65.8	60.2 \pm 0.6 57.1–62.2	– –
Buccal cavity length (μm)	24.9 \pm 0.5 22.5–26.3	20.6 \pm 0.7 16.4–22.6	21.1 –
Buccal cavity width (μm)	12.9 \pm 0.5 10.1–15.2	13.7 \pm 0.9 12.1–16.3	12.4 –
Maximum body width (μm)	51.2 \pm 3.6 30.7–68.7	38.4 \pm 2.3 29.6–47.3	38 –
Anal body width (μm)	28.8 \pm 1.1 23.7–33.3	25 \pm 1.4 19.5–31	31.3 –
Tail length (μm)	73.1 \pm 2.8 63.6–85.8	34.5 \pm 1.6 28.6–41.2	37.5 –
Supplements	–	–	12

4. Discussion

At the end of the first year of our study (fall 2016), we recorded a similar number of mononchid nematodes in mulched and unmulched plots. However, by the end of the fourth year (2019), the number of mononchids significantly increased in mulched plots, whereas it stalled in unmulched plots that were hoed regularly. As noted by Stubbs et al. (2004) [49], the presence or lack of soil disturbance shapes soil biota, in terms of population characteristics and community structure. As mulching corresponds to a managed regime without disturbance, we assume that the fact that no tillage work was performed on mulched plots contributed to the increasing density of mononchid nematodes in mulched microplots, as members of this group are known to be sensitive to long-term cultivation [50]. This is also in line with the finding of Steel and Ferris (2016) [51], who recorded significantly higher relative abundance figures of predators in undisturbed samples when comparing the soil of agricultural and set-aside areas. In our unmulched microplots, however, it was hoeing that possibly created some level of disturbance for mononchids. Studying unmulched, regularly cultivated arable areas (sugar beet fields), Thorne (1927) [52] found that the density of mononchids is not stable: not only may their number drastically decrease, but they may even completely disappear from the fields.

The fact that the number of non-predators highly varied between years in uncovered plots may be explained by the fact that the samples were dominated by more or less r-strategist groups (such as cephalobid nematodes) that are quicker to adapt to the climatic conditions. In contrast, the predominantly K-strategist predators were unable to accommodate to the extreme conditions of uncovered plots, so their numbers remained low [29,53].

Changes to the number of predator taxa may also be explained by the presence of a mulch layer. Mulching seemed to have changed the microenvironment for the benefit of this group by creating more stable ecological conditions, thus affecting this important group of predatory nematodes to a larger extent than the natural population of other free-living nematodes. Mulch increases soil moisture and balances soil temperature [19], and that may provide favourable conditions for mononchids. Arpin (1969, 1975) [54,55] discovered that mononchids are more sensitive to the reduction of soil moisture than plant-parasitic and bacterivorous nematodes. Because the results of releasing artificially reared *Mononchus Bastian* populations into fields for the control of plant-parasitic nematodes proved to be short-lived [56], helping and encouraging the reproduction of indigenous populations

of mononchids is an important task. Bilgrami and Brey (2005) [57], highlighting the importance of organic soil amendments and nitrogenous compounds in the conservation of predatory nematodes, note the lack of information on this subject. As the addition of organic material triggers changes in soil properties, and altered conditions may either promote a higher general health status of crops or establish and provide favorable conditions for populations of natural biological control organisms [58]. Organic materials may be added in the form of a compost mulch or materials intended for composting spread on the surface in the form of in-situ composting. Leaf litter, an organic material that is considered waste in the conventional municipal management, but found in great abundance everywhere, also classifies as a beneficial addition. The presence of *Acacia crassicapa* Cunn. ex Benth leaf litter altered trophic composition of nematodes and increased the ratio of predators by 123.9% when compared to uncovered plots [41].

Based on our results, *Prionchulus punctatus* benefited from the conditions of mulching. This is probably due to the higher sensitivity of this species to the extremities of soil moisture or soil temperature: the *Prionchulus* was only found in mulched plots, where abiotic conditions are more evenly balanced [40].

Our experiment sheds light on a potential benefit of in-situ composting of municipal leaf litter in crop protection. The fact that predatory nematodes benefit from the presence of leaf litter will most likely add a notion towards changing our perception: leaf litter is often considered waste; however, we showed that when used as mulch, it enhanced predatory nematodes and may lead to greater suppression of plant parasitic nematodes as well as improve the soil properties and crop health [59].

5. Conclusions

As a conclusion, our first hypothesis can be accepted because a mulch of MGW leaf litter had a significant positive effect on predatory mononchid nematodes. However, both their individual numbers and taxonomic richness remained quite low, especially in unmulched plots, so it was impossible to make a reliable judgement on our second hypothesis regarding species composition. For a deeper understanding of the impact of organic mulching on mononchids, the following essential study milestones are envisaged: (i) investigating the microhabitat preference of predatory nematodes, (ii) monitoring the long-term effect of different mulch materials (e.g., straw, walnut leaf litter, green yard waste compost) on mononchid populations, (iii) examining soil parameters (e.g., soil temperature, soil moisture content), and (iv) monitoring the predatory-prey ratios throughout the whole year in different depths that may be responsible for the change in the number of mononchids.

Author Contributions: Conceptualization R.P. and F.T.; methodology: F.T. and P.I.N.; investigation: R.P. and P.I.N.; software and data curation: M.Z.; visualization: M.Z. and R.P.; writing—original draft: R.P., F.T.B. and M.Z.; writing—review and editing: F.T.B., P.I.N. and F.T.; funding acquisition: R.P., P.I.N. and F.T.; supervision: F.T. and P.I.N. All authors have read and agreed to the published version of the manuscript.

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Appendix A

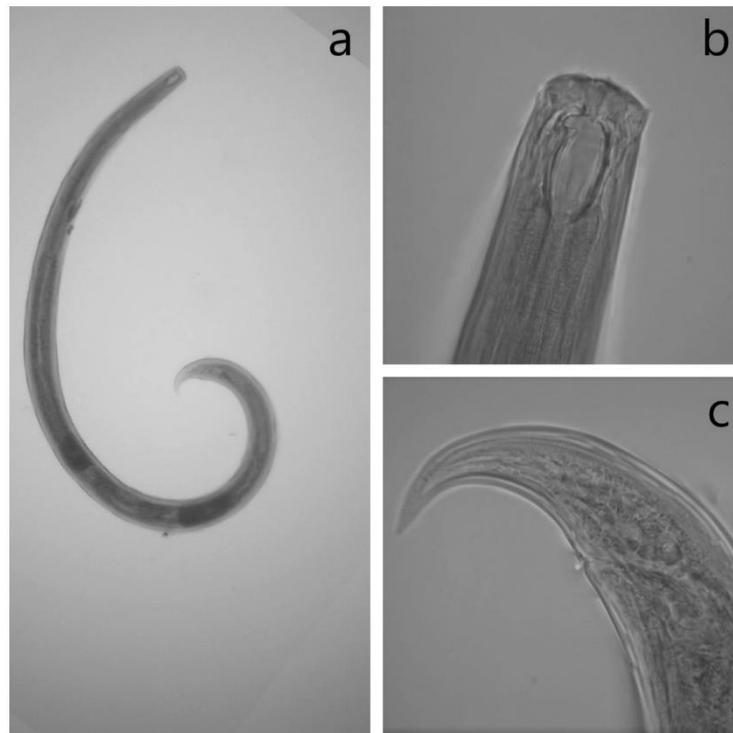


Figure A1. *Clarkus papillatus* (Bastian, 1865): (a) habitus, (b) lip region, (c) tail; at 200× magnification: (a), at 400× magnification: (b,c) Gödöllő, Hungary.

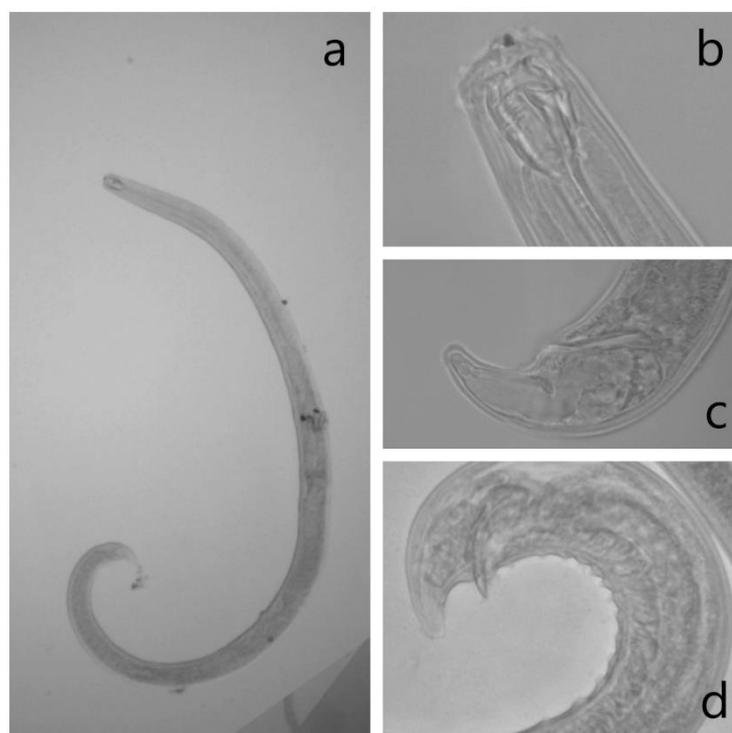


Figure A2. *Mylonchulus brachyuris* (Bütschli, 1873): (a) habitus of female, (b) lip region of female, (c) tail of female, (d) posterior part of male; at 200× magnification: a, at 400× magnification: (b–d) Gödöllő, Hungary.



Figure A3. *Prionchulus punctatus* (Cobb, 1917): (a) habitus, (b) lip region, (c) tail; at 200× magnification: (a), at 400× magnification: (b,c) Gödöllő, Hungary.

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