# Preliminary results on the effects of different soil cover methods on the composition of nematode communities

*Picture is for illustration only*

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**Keywords:** biodiversity, microfauna, healthy soil, production technology, sustainable agriculture

## 1. SUMMARY

**In an experiment lasting for five months, changes in the soil nematode community composition were followed at an organic strawberry plantation. The aim of this study was to present the preliminary results of this investigation.**

**Strawberry (*Fragaria x ananassa* 'Asia') 'frigo' seedlings were planted at the end of March 2019, divided into nine plots (2.5 m × 2.2 m), after which they received three types of treatment in three replicates, in a random block arrangement: organic (hay) and inorganic mulch (black geotextile) and uncovered control.**

**Of the soil microfauna, nematode communities were studied. For their characterization, taxonomic and functional diversity indices (taxon richness, effective species number and functional dispersion index) were used, and the trophic composition of the communities and their changes were explored.**

**Soil nematode communities were affected by both the sampling period and the soil cover. The total nematode density decreased in all cases compared to the initial value (t0, pre-planting state). The effective species number was highest in the case of the geotextile cover. Both types of mulch increased the functional diversity of the communities over the five-month period, resulting in significant differences compared to the control plots. Furthermore, significant changes in the community structure were observed by trophic groups between both sampling times and treatments, which were mainly manifested in an increase in the proportion of plant parasitic and fungal-feeding nematodes and a decrease in the proportion of bacterial-feeding and omnivorous nematodes.**

**Although the results were not significant in all cases, they showed that soil cover helps to maintain the taxonomic and functional diversity of soil invertebrates (here, nematodes), which can contribute to the stability of the soil ecosystem and to providing an ecosystem with a positive impact on production.**

### 2. Introduction

Maintaining soil health is essential for agricultural activities and for food safety. Of the many ecosystem services, soil-dwelling invertebrates are of primary importance for soil formation and degradation **[1, 2]**. Of these, the testing of nematodes as indicator organisms, also targeted by us, is recommended by the European Food Safety Authority (EFSA). Due to their species richness, lifestyle characteristics and diversity of feeding, there are various metrics and indices for the multifarious description of their

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communities, which allow the characterization of the processes and changes taking place in the soil food web **[3-5]**.

Soil cover is a common practice in certain agricultural production sectors to prevent moisture loss and crop contamination. In addition, the procedure may increase the organic matter content of the soil (see organic mulch), thus affecting soil biota, including soil-dwelling nematodes, and primary production as well **[6-8]**.

At the same time, the effects of different mulch types on nematode communities, especially their functional aspects, have been sparsely investigated. The aim of the present experiment was to explore the taxonomic and functional diversity of the nematode fauna in a strawberry plantation, the composition of the community, and its changes using organic (hay) and inorganic (geotextile) soil covers, compared to an untreated control in this respect.

## 3. Methods

### *3.1. Location and design of the experiment*

The field experiment took place in Kecskemét, in the experimental garden of John von Neumann University (46° 55' 8.59" É, 19° 41' 8.3" K). The area falls within the temperate climatic zone, where the average annual rainfall is 530 mm and the average annual temperature is 11 °C **[9]**. It is important to note, however, that the year of the study was warmer (12.4 °C) and drier (500 mm) than the 30-year average. The soil of the area can be classified as calcareous sandy soil type ('Calcaric Arenosol') **[10]**, characterized by a slightly basic pH (8.02), and low nitrogen (8 mg/kg $NO_3$+$NO_2$) and organic matter content (0.77 g humus/100 g dry weight).

Before the study, fertilizer was applied twice (on March 18 and May 10; 350 kg/ha 'Phoenix' granulated poultry manure). 'Frigo' strawberry plants (*Fragaria x ananassa* 'Asia') with diameters between 9 and 13 mm were planted in each experimental plot on March 26 in 4-4 twin rows, with 30 × 25 cm row and plant spacing, with a 60 cm cultivation path. The crop was harvested by hand between June 3 and 19.

Soil cover was applied from the time of planting the seedlings. The area was divided into nine plots in a randomized block arrangement (2.5 m × 2.2 m), with three replicates per treatment. The treatments were as follows:

(1) organic mulch (hay), consisting of herbaceous plants, mainly grasses (Poaceae);

(2) inorganic mulch: a water-permeable black geotextile cover made of polypropylene; and

(3) uncovered control.

Geotextile was placed on the soil prior to planting, while hay was replaced roughly once a month so that a soil cover of at least 2 to 3 cm could be ensured at all times. During blooming and ripening, a hay cover was applied in the control plots to prevent contamination, and it was removed after the harvest. The area was not cultivated in the year prior to the experiment.

All cultivation activities (fertilization, irrigation, weeding) were carried out in accordance with the national and EU rules of organic farming. Irrigation was performed using a micro-nozzle system, always adjusted to the weekly rainfall. Weeding was carried out once a month by hand.

### *3.2. Nematode sampling and analysis*

During the experiment, soil samples were taken twice for analyses of the nematode communities: before planting the strawberry seedlings (date $t_0$, March 20, 2019), and more than two months after the harvest (date $t_1$, August 28, 2019). Composite samples were mixed from ten random subsamples with diameters of 2 cm taken between the seedling rows from a depth of 0-15 cm, and these were stored in separate plastic bags at 4 °C until the nematode extraction.

Nematodes were extracted from 50 g subsamples by the standard Baermann funnel method over 48 hours **[11]**, they were counted under a stereomicroscope (×50 magnification), and the number of individuals per g was given for soil dry weight. The animals were killed by heat treatment and preserved in 4% formalin. To determine the community structure, at least 100 individuals per sample were identified at the genus or family level (except for samples with lower individual numbers where all individuals were identified) based on the identification key of Bongers **[12]**. The relative frequency of nematode taxa per 100 individuals was used to determine the population density of a given taxon.

Nematodes were classified into feeding/trophic groups (bacterial-feeders, fungal-feeders, herbivores and plant parasites, omnivores and predators), and into c-p ('colonizer-persister') 1-5 categories similar to the r-K lifestyle classification, making c-p 1 the extreme r strategist species and c-p 5 the extreme K strategist species **[3,13]**.

### *3.3. Statistical methods*

For statistical analyses, version 3.6.2 of the R program (R Development Core Team, 2019) and its software packages 'FD', 'ggplot2', 'lsmeans','nlme' and 'vegan' were used **[14-18]**. During the statistical analyses, the assumptions of normality, homogeneity of variances, and independence of the samples were examined in each case. The significance level was determined at $p < 0.05$.

*Table 1. Contributions (%) of nematode taxa to total soil nematodes averaged by trophic groups and their functional traits under different mulch treatments at two sampling times. Genera and family marked in bold were the most dominant nematode taxa during the survey.*

| **Group and taxon** | **Body mass** | **c-p†** | **Control** | | **Hay** | | **Geotextile** | |
|---|---|---|---|---|---|---|---|---|
| | | | **$t_0$** | **$t_1$** | **$t_0$** | **$t_1$** | **$t_0$** | **$t_1$** |
| | **µg** | | **%** | | | | | |
| Bacterivores | | | 60.55 aA‡ | 61.62 aA | 62.21 aA | 40.76 aB | 72.20 aA | 46.20 aB |
| ***Acrobeles sp.*** | 0.6 | 2 | 32.76 | 26.14 | 38.12 | 16.82 | 46.24 | 8.42 |
| ***Acrobeloides sp.*** | 0.227 | 2 | 10.04 | 8.64 | 5.42 | 11.86 | 7.64 | 11.64 |
| *Bastiania sp.* | 0.161 | 3 | 0.00 | 0.28 | 0.00 | 0.00 | 0.00 | 0.40 |
| *Cephalobus sp.* | 0.257 | 2 | 4.30 | 12.98 | 5.27 | 5.34 | 5.46 | 13.26 |
| *Cervidellus sp.* | 0.152 | 2 | 6.80 | 4.33 | 3.31 | 1.90 | 6.11 | 4.70 |
| *Chiloplacus sp.* | 0.508 | 2 | 1.14 | 1.55 | 6.00 | 0.65 | 3.11 | 3.88 |
| *Eucephalobus sp.* | 0.243 | 2 | 0.00 | 0.38 | 0.00 | 0.63 | 0.56 | 1.61 |
| *Heterocephalobus sp.* | 0.356 | 2 | 0.29 | 4.60 | 1.76 | 0.00 | 0.33 | 0.40 |
| *Microlaimus sp.* | 0.146 | 3 | 0.00 | 0.28 | 0.00 | 0.00 | 0.00 | 0.00 |
| Monhysteridae sp. | 0.358 | 2 | 0.00 | 0.38 | 0.00 | 0.00 | 0.00 | 1.89 |
| Panagrolaimidae sp. | 0.655 | 1 | 1.17 | 0.38 | 0.00 | 1.66 | 0.89 | 0.00 |
| *Plectus sp.* | 0.902 | 2 | 0.00 | 0.69 | 0.87 | 0.00 | 0.31 | 0.00 |
| *Prismatolaimus sp.* | 0.413 | 3 | 2.34 | 0.99 | 0.29 | 1.90 | 1.27 | 0.00 |
| *Tylocephalus sp.* | 0.214 | 2 | 0.87 | 0.00 | 0.29 | 0.00 | 0.00 | 0.00 |
| Rhabditidae sp. | 5.037 | 1 | 0.84 | 0.00 | 0.88 | 0.00 | 0.28 | 0.00 |
| | | | | | | | | |
| Fungivores | | | 6.91 aB | 17.09 aA | 5.03 aB | 15.93 aA | 5.13 aB | 16.24 aA |
| *Aphelenchoides sp.* | 0.145 | 2 | 1.42 | 0.56 | 0.90 | 5.45 | 0.00 | 0.00 |
| *Aphelenchus sp.* | 0.231 | 2 | 3.49 | 4.64 | 2.68 | 2.58 | 1.43 | 5.27 |
| *Ditylenchus sp.* | 0.494 | 2 | 1.42 | 10.93 | 1.17 | 3.07 | 0.28 | 5.30 |
| *Filenchus sp.* | 0.098 | 2 | 0.58 | 0.66 | 0.00 | 4.83 | 0.32 | 4.24 |
| *Tylencholaimellus sp.* | 0.564 | 4 | 0.00 | 0.30 | 0.28 | 0.00 | 3.10 | 1.43 |
| | | | | | | | | |
| Herbivores | | | 11.3 aA | 8.40 bA | 8.06 aB | 32.43 aA | 6.93 aB | 26.21 abA |
| *Meloidogyne sp.* | 24.219 | 3 | 0.00 | 0.00 | 0.28 | 0.00 | 0.00 | 0.80 |
| *Pratylenchus sp.* | 0.126 | 3 | 2.52 | 3.85 | 0.61 | 3.40 | 1.64 | 10.71 |
| **Telotylenchidae sp.** | 0.46 | 3 | 8.78 | 4.25 | 7.17 | 29.03 | 4.98 | 14.70 |
| Tylenchidae sp. | 0.16 | 2 | 0.00 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Xiphinema sp.* | 5.668 | 5 | 0.00 | 0.00 | 0.00 | 0.00 | 0.31 | 0.00 |
| | | | | | | | | |
| Mindenevők / Omnivores | | | 19.03 aA | 12.89 aA | 23.56 aA | 10.88 aB | 15.74 aA | 10.72 aA |
| *Aporcelaimellus sp.* | 9.079 | 5 | 4.21 | 1.21 | 5.52 | 0.95 | 2.04 | 4.95 |
| *Carcharolaimus sp.* | 3.727 | 4 | 0.58 | 0.38 | 0.00 | 0.00 | 0.00 | 0.61 |
| *Ecumenicus sp.* | 0.705 | 4 | 5.49 | 0.97 | 7.22 | 3.33 | 6.92 | 1.26 |
| *Eudorylaimus sp.* | 3.09 | 4 | 3.44 | 8.47 | 4.39 | 6.28 | 2.59 | 2.24 |
| *Kochinema sp.* | 0.646 | 4 | 0.00 | 0.61 | 0.57 | 0.00 | 0.56 | 0.63 |
| *Microdorylaimus sp.* | 0.201 | 4 | 3.09 | 0.00 | 1.13 | 0.00 | 1.52 | 0.00 |
| *Paraxonchium sp.* | 5.7 | 5 | 2.22 | 1.25 | 4.73 | 0.32 | 2.11 | 1.03 |
| | | | | | | | | |
| Ragadozók / Predators | | | 2.21 aA | 0.00 aA | 1.14 aA | 0.00 aA | 0.00 aA | 0.63 aA |
| *Discolaimus sp.* | 2.928 | 5 | 1.64 | 0.00 | 1.14 | 0.00 | 0.00 | 0.63 |
| *Mylonchulus sp.* | 1.745 | 4 | 0.57 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Teljes taxon gazdagság / Total taxon richness** | | | **24** | **27** | **24** | **18** | **24** | **23** |

† c-p (colonizer-persister) 1-5 values: classification similar to r-K life history categories, 1=extreme r-strategists, 5=extreme K-strategists **[3]**.
‡ Lower case and upper case letters indicate differences among treatments within each sampling period and between sampling dates, respectively (Tukey-Kramer adjusted $p < 0.05$).

For each plot, the taxonomic and functional diversity of the nematode communities were estimated. The former was expressed by taxon richness and the effective species number or Hill index **[19]**, while the latter was expressed by a multivariate indicator based on multiple functional traits (body weight, c-p group, feeding strategy), the so-called functional dispersion (FDis) **[14]**. The values of the functional traits were obtained from the automatic calculation system of the NINJA (Nematode INdicator Joint Analysis) database **[20].**

Linear mixed models were used to test the effects of different mulch treatments (uncovered control, hay, geotextile), sampling times ($t_0$ and $t_1$) and their interactions on the nematode communities (diversity indices, total density, distribution of trophic groups). The lack of spatial and temporal independence between the individual samples resulting from the sampling design was taken into account by including the variables 'Plot' and 'Sampling time' as random factors. For multiple comparisons, the least square means method was used with Tukey's adjusted p values **[16]**.

To understand the effects of treatments and the sampling period on the taxonomic composition of the communities, nested permutational multivariate analysis of variance (PERMANOVA, Bray-Curtis index, number of permutations: 999) was performed, the results of which were plotted using non-metric multidimensional scaling (NMDS).

## 4. Results and discussion

### *4.1. Diversity and abundance*

The study confirmed the presence of 34 nematode taxa (29 genera, 5 family-level categories), representing a total of 22 taxonomic families (***Table 1***). Taxon richness varied between 7 and 21 genera per plot. After the harvest, the highest taxon number (27) was found in the control, while the lowest (18) was shown by the plot treated with organic mulch (***Table 1***). After the five months, taxon richness was significantly reduced in the case of hay cover (***Table 2***).

According to the effective species number which, in addition to species richness, also takes into account the abundance of the different species, the soils covered with geotextile exhibited the most diverse nematode community (***Table 2***). This type of mulch had a positive effect on taxonomic $\alpha$ diversity by the end of August, showing a significant difference compared to the plots treated with hay.

Functional diversity (FDis) showed significantly higher values for both soil cover treatments compared to the initial state, as well as to the control (***Table 2***).

Regarding the total density of nematodes, as opposed to the soil cover treatments, the sampling period proved to be the determining factor, as the numbers of individuals decreased from 5.58-14.58 for the plots in the spring to 1.56-4.88 per g of dry soil by the end of the summer (***Table 2***).

The results supported our first hypothesis that the presence of cover is a dominant factor for the soil nematode community. In other words, the nematode taxon has been shown to be suitable for indicating changes in soil biota caused by agricultural practices **[21, 22]**. The diversity values of nematodes were in agreement with the results of a previous study, also involving strawberry plantations **[23]**.

### *4.2. Composition of the nematode communities*

*4.2.1. Trophic classification of nematodes and its taxonomic aspects*

The most dominant taxa were the following: *Acrobeles* (29.9%), Telotylenchidae (9.79%), *Acrobeloides* (9.45%). Although the majority of nematodes were present in the case of each treatment and period (***Table 1***), the taxonomic composition of the communities was significantly affected by these factors (PERMANOVA: $R^2$=0.13, p=0.016 and $R^2$=0.28, p=0.004; ***Figure 1***). Representatives of the genera *Microdorylaimus*, *Mylonchulus*, *Tylocephalus*, *Xiphinema* and the family Rhabditidae were present only in spring, before the planting of the seedlings (t0), while *Bastiana*, *Microlaimus*, Monhysteridae

*Table 2. The results of linear mixed models testing the effects of mulch treatments, sampling periods ($t_0$ and $t_1$), and their interaction on soil nematode communities (diversity indices and total density).*

| | Kontroll / Control | | Széna / Hay | | Agroszövet / Geotextile | |
|---|---|---|---|---|---|---|
| | $t_0$ | $t_1$ | $t_0$ | $t_1$ | $t_0$ | $t_1$ |
| Taxon richness | 17.67 aA† | 18.00 aA | 17.67 aA | 12.00 aB | 16.67 aA | 15.67 aA |
| Hill index | 9.94 aA | 9.62 abA | 9.13 aA | 7.68 bA | 7.39 aB | 11.94 aA |
| FDis‡ | 0.28 aA | 0.23 bA | 0.27 aB | 0.35 aA | 0.22 aB | 0.35 aA |
| Total density ($g^{-1}$ dry soil) | 7.69 bA | 3.25 aB | 7.30 bA | 2.37 aB | 11.33 aA | 1.71 aB |

† Lower case and upper case letters indicate differences among treatments within each sampling period and between sampling dates, respectively (Tukey-Kramer adjusted p < 0.05).
‡ FDis: Functional dispersion

and Tylenchidae could only be detected in the post-harvest period (t1). While *Microlaimus*, *Mylonchulus* and Tylenchidae were present only in the control plots, the genus *Xiphinema* was present only in the case of the geotextile cover.

The trophic group of bacterial-feeding nematodes was the most diverse with 15 taxa, followed in number by omnivores (7), herbivores (5) and fungal-feeders (5). The predator group was represented by only 2 genera (***Table 1***).

Although bacterial-feeders dominated in almost all soil samples (25% - 80.4%), their proportion decreased significantly as a result of the treatments (***Table 1***). This negative trend was mainly due to a significant decline in the populations of the genus *Acrobeles* populous bacterial-feeding taxon (50.3%) (***Table 1***). An opposite change was observed for the genus *Acrobeloides*. The genus *Cephalobus* was present in higher proportions after the harvest period, with the exception of the hay cover. While *Heterocephalobus* appeared to prefer the control plots, the populations of the genus *Chiloplacus* were negatively affected by the hay cover (***Table 1***).

The proportion of fungal-feeding nematodes in the communities increased significantly over time (between 2.5% and 27.7%) (***Table 1***). The dominant genera were *Ditylenchus* and *Aphelenchus*, with 35.3% and 29.4% of the group, respectively. The former became more abundant in each summer end sample, regardless of the treatment. The percentage of the genus *Filenchus* increased within the communities for both mulch types (***Table 1***). Similar situations were observed for the genus *Aphelenchoides* in the plots with organic mulch and the genus *Aphelenchus* in the plots covered with geotextile.

Herbivores (or plant parasites) accounted for 1.9 to 40% of the nematode communities. Both cover types had a significant positive effect on the trophic group, which was more significant compared to the control primarily in the case of hay (***Table 1***). The most abundant taxon proved to be the family Telotylenchidae (72.9%). The genus *Pratylenchus* became more significant in post-harvest samples for each treatment, especially in the case of the geotextile cover. *Meloidogyne*, known as a strawberry pest, occurred only sporadically and only with low densities, irrespective of the treatment (***Table 1***).

Of the omnivorous nematodes, the genera *Eudorylaimus*, *Ecumenicus* and *Aporcelaimellus* were the most common (74.7%). They accounted for 1.8% to 29.2% of the total nematode community, which decreased significantly during the growing season, mainly in the case of organic mulch (***Table 1***). At the genus level, the presence of *Ecumenicus* decreased consistently for all treatments (***Table 1***). The presence of the genus *Paraxonchium* was adversely affected by the hay cover, while *Aporcelaimellus* responded differently to the different cover methods: positively to geotextile cover, negatively to hay mulch (***Table 1***). The lack of treatment had a favorable effect on the members of the genus *Eudorylaimus*.

Predatory nematodes were rare in our samples, they were mostly absent (their proportion was 0% to 5.7% for the whole community). They were represented by the following two genera: *Discolaimus* (84.6%) and *Mylonchus* (15.4%).

Various results have been reported in the literature regarding the application of similar treatments, ranging from an increase in nematode diversity **[24]**, through treatment resulting in no change **[25]**, to a decrease in the taxonomic diversity of nematodes

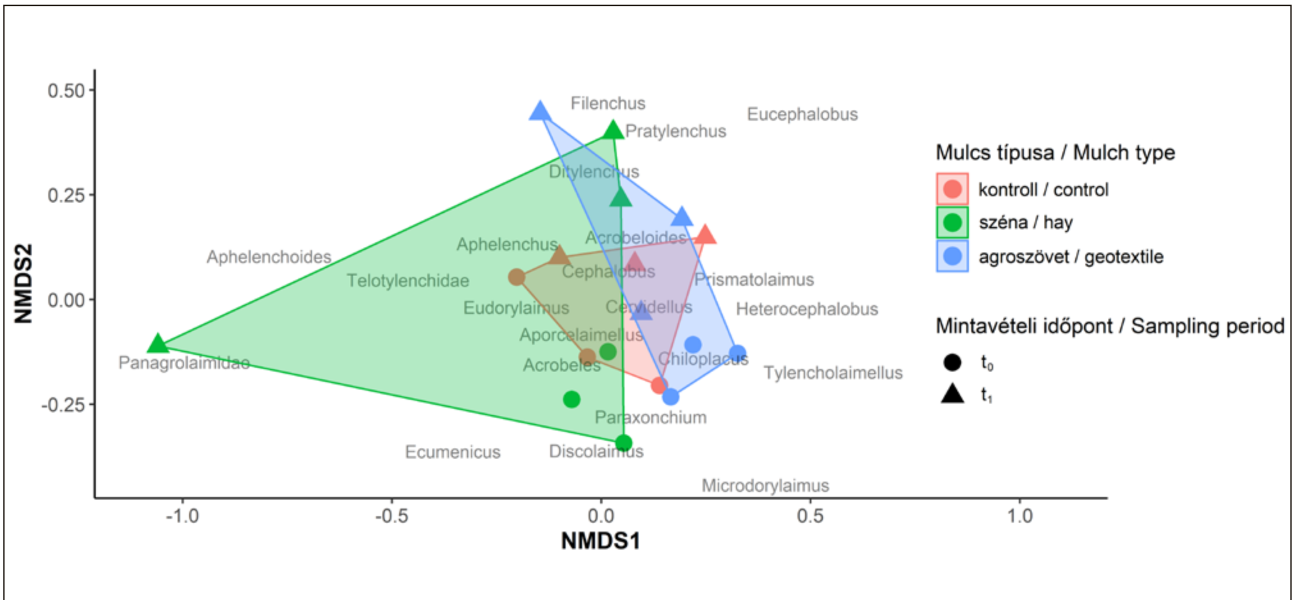


*Figure 1. Non-metric multidimensional scaling (NMDS) ordination plots of nematode communities by mulch treatments (no: non-mulching, organic: mulching with grass hay, inorganic: mulching with black geotextile) at pre-plant (t0) and post-harvest (t1) time periods.*

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[e.g., **26**]. The effect of the organic mulch applied depends significantly on the quality and type of the organic matter that makes it up (C:N ratio, contaminants, nematotoxic components, etc.), the presence of antagonists, as well as the duration and frequency of the application **[24]**. In our case, the drought, the low number of rainy days and the low total precipitation during the critical period (45.8 mm during July and August) may have had a significant effect on the population dynamics of nematodes **[27].**

## 5. Conclusions

The results support that soil cover has a beneficial effect on soil fauna diversity. Contrary to our expectations, mulch could not offset the effects of the summer drought and thus increase the abundance of nematodes, but it did have a positive effect on the functional diversity of nematodes. This can lead to a more stable soil ecosystem, which increases the functional resilience and adaptive capacity of the soil biota, which can lay the groundwork for a more viable and sustainable agricultural production.

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