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Soil microfauna in municipal solid waste landfills: a case study in the Moscow region, European Russia

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ABSTRACT: The present study focuses on investigating the taxonomic richness and composition of selected groups of soil microfauna, specifically soil ciliates and nematodes, within municipal solid waste landfills located in European Russia. Faunistic surveys were conducted across two landfills, with soil samples collected in the three zones: core sites within the landfill, at the edge of landfills, and control sites located 1 kilometer away from the edges. We revealed distinct faunistic patterns between the landfills core and edge sites vs respective controls. Core sites within the landfills exhibited higher ciliate species richness and nematode abundance. This highlights the landfill effect on soil microfauna community composition. Importantly, certain species of ciliates were exclusively found within the landfills, indicating the presence of a faunistically distinguished community associated with communal waste deposition. Knowing peculiarities of soil microfauna communities in landfills brings ground to further knowledge understanding of soil ecological processes in the landfills. This further opens perspectives for a development of adequate management practices for sustainable communal waste disposal and landfill remediation efforts.
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KEY WORDS: soil fauna, Nematoda, Ciliates, municipal solid waste, open dump.

Почвенная микрофауна полигонов твердых бытовых отходов: на примере Московской области, европейской части России

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РЕЗЮМЕ: Настоящее исследование посвящено изучению таксономического богатства и состава отдельных групп почвенной микрофауны, в частности почвенных инфузорий и нематод, на полигонах твердых бытовых отходов, расположенных в европейской части России. Фаунистические исследования проводились на территории двух полигонах; почвенные образцы отбирались в трех зонах: импактные участки внутри полигонов, буферные участки на краю полигонов и фоновые участки, расположенные в 1 км от границы полигонов. Мы выявили фаунистические закономерности между импактными и буферными участками по сравнению с соответствующими фоновыми участками. Импактные участки полигонов обладали более высоким разнообразием видов инфузорий и высоким обилием нематод. Это подчеркивает влияние полигонов твердых бытовых отходов на состав сообществ почвенной микрофауны. Важно отметить, что определенные виды инфузорий были обнаружены исключительно на территории полигонов, что указывает на наличие фаунистически обособленных сообществ, связанных с депонированием коммунальных отходов. Знание особенностей сообществ почвенной микрофауны на полигонах позволяет продвинуться в понимании почвенно-экологических процессов, протекающих внутри полигонов с твердыми бытовыми отходами. Результаты исследования открывают дополнительные перспективы для разработки соответствующих практик управления устойчивым обращением с коммунальными отходами и усилий по рекультивации свалок.
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КЛЮЧЕВЫЕ СЛОВА: почвенная фауна, нематоды, почвенные инфузории, полигоны твердых бытовых отходов, свалка.

Introduction

Municipal solid waste (MSW) disposal poses a significant and urgent challenge for modern society. With global population growth and the rapid development of consumerism, the production of municipal waste is projected to reach 3.4 million metric tons by 2050 (Sharma *et al*., 2020). Landfills and open dumps remain the prevailing and cost-effective methods of waste disposal (Abdel-Shafy *et al*., 2018; Khalid *et al.*, 2022). However, these methods have a glaring drawback: the contamination of terrestrial ecosystems in the vicinity of the waste disposal areas (Vaverkova *et al*., 2019; Liu *et al*., 2022; Bharath *et al*., 2023). While there have been studies examining the impact of landfill leachate on surface and ground water, the influence of landfills on soil and communities inhabiting it has received insufficient attention (Hamid *et al*., 2018; Montvydienë *et al*., 2020).

Soil microfauna, composed of ciliates and nematodes, represents a vital and highly responsive in time component of terrestrial ecosystems (Acosta-Mercado *et al*., 2004; Bates *et al*., 2013; Bardgett *et al*., 2014; Nielsen *et al*., 2014; Abraham *et al*., 2019). With its abundant presence and trophic reliance on bacterial resources, both ciliates and nematodes may enhance the turnover of soil bacterial biomass (Acosta-Mercado *et al*., 2004). Ciliates also play a key role in activating mineralization processes in soil, thereby improving soil quality and positively influencing plant growth (Díaz *et al*., 2006). Nematodes are apparently the most abundant multicellular organisms in soils (van den Hoogen *et al*., 2019) which play an essential role in soil food webs through microbial communities, grazing, or parasitizing plants and roots, they also affect nutrient cycling and act as prey for larger animals (Yeates *et al*., 1993; Ferris, 2010; Geisen *et al*., 2018). Research on the dynamics and composition of soil ciliate and nematode communities can serve as a valuable approach for assessing and monitoring changes in anthropogenic ecosystems, such as landfills (Bongers, 1990; Stamou *et al*., 2020; Maurya *et al*., 2022). Besides that, certain ciliate and nematode taxa can even be used as indicators of major soil pollutants and environmental stress markers within soil ecosystems (Ferris *et al*., 2001; Díaz *et al*., 2006), further emphasizing the importance of understanding their role in soil ecosystems.

This study investigates the abundance and taxonomic diversity of soil microfauna, including ciliates and nematodes, in municipal solid waste landfills. It sheds light on their faunistic composition and taxonomic richness. This is a prerequisite to study potential functional role of soil microfauna in anthropogenic ecosystems, providing valuable information for sustainable waste management practices.

Material and methods

STUDY AREA. The study area is located in the European part of Russia within the Moscow oblast. Block design (two blocks in total) with three treatments per block was applied. In each block a landfill core, landfill edge and adjacent 1-km apart located controls were taken. Each treatment was replicated by 3 plots located not less than 20 m apart from each other. Plots within core treatment were located directly on the top of the landfill trapeze and formed a triangle. To prevent the spread of waste, all landfill refuse was mixed with the soil, forming a large mound. Edge plots were located ca. 50 m apart from the landfill hill in the direction of leachate run-on where usually a leachate pond can be formed. Control plots were selected in adjacent forested areas with absence of visible anthropogenic disturbance such as e.g., logging, fires, clear-cutting or trampling.

Block I was located in the vicinity of Balashikha city (N 55.720744°, E 38.062768°). According to the official documentation, the landfill was operated from 2008 to 2014. The approximate mass of accumulated waste by 2015 was about 1200 thousand tons. Total area of the landfill trapeze was about 6 ha and height of 20 m. Sampling was done on 25 September 2021. Landfill was covered by herbaceous ruderal plants (e.g., *Urtica dioica* L., 1753, *Dactylis glomerata* L., 1753, *Solidago virgaurea* L., 1753, *Chelidonium* sp., *Artemisia vulgaris* L., 1753, *Verbascum thapsus* L., 1753, *Hypericum perforatum* L. 1753, *Erysimum barbarea* L., 1753) and partly with shrubs and maple undergrowth (*Rosa canina* L., 1753, *Hippophae rhamnoides* L., 1753, *Acer negundo* L., 1753, *Malus* sp.). Edge plots were represented by wood vegetation (*Acer negundo* L., 1753, *Betula* sp., *Parthenocissus* sp., *Urtica dioica*, *Artemisia vulgaris*, *Carex* sp.). Control plots were located in the nearby located pine forest (*Oxalis* sp., *Vaccinium myrtillus* L., 1753, *Betula* sp., *Pinus sylvestris* L., 1753).

Block II was located in the vicinity of Protvino city (N 54.926797°, E 37.191142°). The landfill was operated from 1971 to 2013. The approximate mass of accumulated waste by 2014 was about 685 thousand tons. Total area of the landfill trapeze was about 3.5 ha and height of ca. 15 m. Sampling was done on 10 October 2021. Landfill was covered by herbaceous ruderal plants (e.g., *Artemisia vulgaris*, *Capsella* sp., *Avena sativa* L., 1753, *Deschampsia cespitosa* (L.) P. Beauv. 1812, *Urtica dioica*, *Dactylis glomerata*) and partly with wood vegetation (*Rubus idaeus* L., 1753, *Malus* sp., *Hippophae rhamnoides*). Edge plots were represented by ruderal plants (*Urtica dioica*, *Artemisia vulgaris*, *Carex* sp., *Tussilago farfara* L., 1753) and invasive maple (*Acer negundo*). Control plots were located in the surrounding pine forest (*Vaccinium myrtillus*, *Sorbus aucuparia* L., 1753, *Sphagnum* sp.).

At the time of the study, there were no signs of active deposition of new waste on the territory of both landfills and, according to visual analysis, both landfills consisted mainly of municipal solid wastes (MSW), such as e.g., products of the petrochemical industry, cellulose processing products, textile, glass, metals, etc.

SAMPLING, EXTRACTION AND ANIMAL IDENTIFICATION. Samples for identification of microfauna (ciliates and nematodes) were collected with a steel corer with diameter of 2.5 cm and 10 cm height. From each plot a mixed sample consisting of 3 subsamples was collected and sealed into a single plastic bag and transported to the laboratory.

Collected soil samples were air-dried before the analysis of ciliates. Quantitative studies of soil ciliates were carried out using a method based on direct counting of ciliate cells in 1 ml of soil suspension, followed by recalculation of the number per 1 g of air-dry soil (Nikitina, 2011). A 1 g of air-dry soil was moistened with a small amount of settled water and thoroughly pounded to obtain a homogeneous suspension. The resulting mushy soil mass was transferred into a test tube, several times washing off the remaining soil from the walls of the cup. The total volume of used water was 9 ml (1:10 dilution). All subsequent 3 replications were prepared in the same way. The test tube rack was kept at room temperature before microscopic examination after 3-4 days. Then 1 ml of soil suspension was collected with a sterile pipette and applied to a glass slide in 2-3 drops, the entire volume of liquid was gradually inspected at 1000x magnification using Motic BA310E microscope and the total number of ciliates was recorded in each drop. Thus, the number of ciliates in 1 ml was calculated from each of all three replications. The number of ciliates in 1 g of air-dry soil was calculated using the following formula:

A = X * B, where A is the number of cells in 1 g of air-dry soil, X — arithmetic mean number of ciliates in 1 ml of suspension, B — the number of ml used to dilute I g of soil.

For qualitative studies of soil ciliates, the non-flooded petri dish method described by Foissner (1987) was used. About 10 ml of water from the flooded petri dishes were withdrawn at intervals of 24 hours for up to 14 days. Live specimens were observed under a powerful brightfield, phase contrast, and darkfield oil immersion objective (magnifications 100–1000; Motic BA310E). For intravital coloring of ciliates, a solution of methyl-green stain was used. The cilia movement and digestive vacuoles were observed in a drop of water with the addition of sodium bicarbonate solution. Species identification was carried out using the following keys: Bick, 1972; Lepinis, Geltser *et al*., 1973; Corliss, 1979; Foissner, 1987, 1995; Lynn *et al*., 2002; Kuppers, 2020. The Corliss's monograph (Corliss, 1979), has been used as a complementary source of information for the identification of soil ciliate families. The actual taxonomic information for the species is given according to the CilCat: The World Ciliate Catalog (Aescht, 2019).

Nematodes were extracted using the Baermann method (Baermann, 1917) modified by Butenko *et al*. (2017). Briefly, soil samples of known weight (up to 23 g) were placed in a bag made of unwoven Agrospan-30© textile (6 × 8 cm), which was placed into a funnel with the diameter of 12 cm with the tube (2 ml) through a silicon hose and filled with water to top level. The nematode extraction lasted 48 h at 20 °C. The identification and counting of nematodes at the genus level was carried out on live specimens using a light microscope (Olympus BX-43) and a stereomicroscope (MBI-3) using the nematode morphology as suggested in the literature (Goodey, 1963; Paramonov, 1964; Andrassy, 1984; Siddiqui, 2000). Nematode genera abundance was then calculated into the number of individuals per gram dry soil (ind. g^{-1}). All nematode genera were allocated into feeding groups (Bongers, Bongers, 1998; Yeates, 2003) as follows: bacterial-feeding, hyphal-feeding, omnivorous, plant-associated and plant-feeding nematodes (predatory nematodes were not found in the samples). To assess the maturity of nematode communities, we set the C-P value for each nematode genus as proposed by Bongers and Bongers (1998) with following recalculation to Maturity index (C-P) and Maturity index for free-living taxa (MI) for each site as a measure of disturbance (Neher, Darby, 2016).

DATA ANALYSES. All data presented in the text are given as average values ± SE for the treatment (n = 6). The significance of differences in abundance and taxonomic groups of microfauna between treatments (Core, Edge, and Control) was checked using the Kruskal-Wallis (KW) test, utilizing the stats package ver. 4.3.0. If the KW test returned significant results, further analysis was conducted to determine the significance of differences between means using the Dunn test from the FSA package ver. 0.9.4 (Ogle *et al*., 2023), with Holm correction applied for multiple comparisons. The abundance and taxonomic groups of microfauna in the studied landfills were visualized utilizing ggplot2 ver. 3.4.2 (Wickham, 2016) for better understanding and interpretation of the differences.

The faunistic similarity of microfauna communities in the studied landfills was evaluated using hierarchical cluster analysis. Prior to analysis, the data were organized into a species x communities matrix with presence-absence data for ciliates and a species x communities matrix with abundance data for nematodes. The dissimilarities between nematode communities were measured using the Euclidean distance, based on their abundance profiles, while a binary method, utilizing presence-absence data, was employed for ciliates. The resulting dendrograms were constructed using the Ward2 algorithm, facilitating the identification of distinct clusters representing similar nematode and ciliate assemblages. Statistical support for the resulting clusters was provided using multiscale bootstrap with 10,000 resamplings, conducted using the pvclust package 2.2-0 (Suzuki, Shimodaira, 2006). Also, for each cluster AU (Approximate Unbiased) probability values which provide statistical support and reliability and BP (bootstrap probability) values that indicate the level of support for the branches within the dendrogram, were calculated. The dendrograms obtained from the hierarchical cluster analysis were enhanced and visually improved using Microsoft PowerPoint 2019.

All analyses were performed in R 4.3.0 (R Core Team 2023) with RStudio interface (R studio Inc.).

Results

CILIATES. Fourty species of ciliates from 20 genera were found. Complete list of species found in different plot types from studied landfills is given in Table 1. The highest total species richness was recorded in the core plots — 30 species. Fewer were identified in control plots — 26 species, and the lowest species richness was observed in the edge plots — 19 (Table 1). Only one species, *Colpoda aspera* Kahl, 1926, was recorded in all studied plots. Additionally, several other species were frequently observed

Table 1. Species list of soil microfauna (ciliates and nematodes) found on the territory of studied municipal solid waste landfills in the vicinity of Balashikha and Protvino cities. Total abundance is given as individuals in 1 g^{-1} of dry soil ± SE.
Таблица 1. Список видов почвенной микрофауны (инфузории и нематоды) найденных на территории исследованных полигонов бытовых отходов в окрестностях г. Балашиха и Протвино. Общая численность представлена в виде экземпляров в 1 $г^{-1}$ сухой почвы ± станд. ошибка средней.

Species	Balashikha			Protvino		
	Core	Edge	Control	Core	Edge	Control
CILIATA						
Acineria uncinata Tucolesco, 1962			+	+		+
Anteholosticha monilata (Kahl, 1928) Berger, 2003			+			+
Aspidisca cicada (Müller, 1786) Claparède et Lachmann, 1858	+		+		+	
Blepharisma steini Kahl, 1932	+		+			
Chilodonella uncinata (Ehrenberg, 1838) Strand, 1928		+	+	+	+	+
Colpidium colpoda (Losana, 1829) Ganner et Foissner, 1989	+	+				
Colpoda aspera Kahl, 1926	+	+	+	+	+	+
Colpoda cucullus Muller, 1773	+		+			+
Colpoda inflata (Stokes, 1884) Kahl, 1931	+			+	+	+
Colpoda sp.	+		+	+		
Colpoda steinii Maupas, 1883			+			
Cyclidium sp.	+		+		+	
Drepanomonas revoluta Penard, 1922						+
Drepanomonas sphagni Kahl, 1931	+	+				+
Euplotes aediculatus Pierson, 1943			+			+
Euplotes moebiusi Kahl 1932						+

Table 1 (contonued).
Таблица 1 (продолжение).

Species	Balashikha			Protvino		
	Core	Edge	Control	Core	Edge	Control
Euplotes mutabilis Tuffrau, 1960	+					+
Euplotes sp.			+		+	
Frontonia sp.			+		+	
Gonostomum affine (Stein, 1859) Sterki, 1878				+	+	
Leptopharynx costatus Mermod, 1914			+			+
Litonotus sp.			+			+
Oxytricha chlorelligera Kahl, 1932	+	+	+		+	+
Oxytricha longa Gelei et Szabados, 1950	+	+	+	+		
Oxytricha sp.	+	+		+		
Plesiocaryon elongatum (Shewiakoff, 1892) Foissner, Agatha et Berger, 2002	+		+	+	+	+
Prorodon sp.	+	+		+		
Protocyclidium citrullus (Cohn, 1866) Foissner, Agatha et Berger, 2002	+			+		+
Pseudokeronopsis flava (Cohn, 1866) Wirnsberger, Larsen et Uhlig, 1987				+		
Spathidium sp.	+	+	+			+
Spathidium spathula (Müller, 1773) Dujardin, 1841			+			+
Strobilidium sp.	+			+		
Stylonychia pustulata (Müller, 1786) Ehrenberg, 1838	+					
Stylonychia putrina Stokes, 1885		+		+		
Tachysoma chilensis (Bürger, 1905) Berger, 1999	+	+		+	+	
Tachysoma pellionellum (Müller, 1773) Borror, 1972	+	+				

Table 1 (contonued).
Таблица 1 (продолжение).

Species	Balashikha			Protvino		
	Core	Edge	Control	Core	Edge	Control
Trithigmostoma cucullulus (Müller, 1786) Jankowski, 1967	+					
Trithigmostoma sp.	+					
Uroleptopsis citrina Kahl, 1932	+			+		
Uronema nigricans (Müller, 1786) Florentin, 1901	+			+		
Total species richness	25	12	20	17	11	18
Total abundance	7182 ± 3854	2926 ± 1746	4139 ± 474	1603 ± 482	1150 ± 109	6244 ± 218
NEMATODA						
Eucephalobus				+	+	+
Panagrolaimus	+	+	+	+	+	+
Rhabditis		+		+		
Aphelenchoides	+		+			
Eudorylaimus		+		+		
Ditylenchus	+		+			
Tylenchus	+	+	+	+		
Helicotylenchus		+		+		
Pratylenchus		+		+		
Total genera richness	4	6	4	7	2	2
C-P index	1.8 ±0	2.4 ± 0	1,8 ±0	1.8 ± 0.3	1.5 ± 0,3	1.8 ± 0.2
MI index	1.6 ± 0	2.5 ± 0.3	1.7 ± 0.0	1.9 ± 0.2	1.4 ± 0.3	1.9 ± 0.1
Total abundance	96 ± 5	57 ± 3	35 ± 4	53 ± 13	13 ± 4	21 ± 15

and found in 5 out of the 6 studied plot types: *Plesiocaryon elongatum* (Schewiakoff, 1892) Foissner, Agatha et Berger, 2002, *Chilodonella uncinata* (Ehrenberg, 1838) Strand, 1928, and *Oxytricha chlorelligera* Kahl, 1932. Certain species such as *Euplotes aediculatus* Pierson, 1943, *Anteholosticha monilata* (Kahl, 1928) Berger, 2003, *Leptopharynx costatus* Mermod, 1914, and *Spathidium spathula* (Müller, 1773) Dujardin, 1841 were exclusively found in the control plots, whereas species like *Strobilidium* sp., *Stylonychia pustulata* (Müller, 1786) Ehrenberg, 1835, *Trithigmostoma cucullulus* (Müller, 1786) Jankowski, 1967, *Trithigmostoma* sp., *Uroleptopsis citrina* Kahl, 1932, and *Uronema nigricans* (Müller, 1786) Florentin, 1901 were exclusively found within the landfills (Table 1).

Average ciliate species richness significantly differed between treatments (Fig. 1B, Table 2) and followed the same trend with total species number (Table 1). Highest average species number was found in core plots, then it was

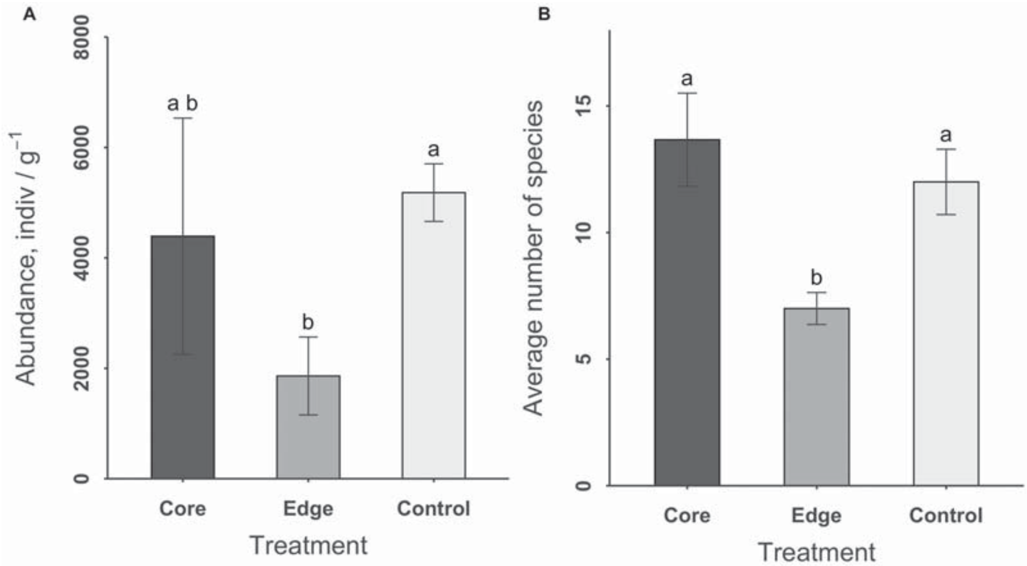

Fig. 1. Abundance, individuals per g^{-1} ± SE (A), and average number of species ± SE (B) of ciliates (n = 6) in the different plots of the studied municipal solid waste landfills in European Russia. Similar letters above bars indicate the absence of significant differences between mean values for the studied treatment. Comparisons were recognized as significant at the < 0.05 *p*-level (Dunn's test with Holm correction).
Рис. 1. Численность, экз. на $г^{-1}$ ± станд. ошибка средней (A) и среднее количество видов ± станд. ошибка средней (B) инфузорий (n = 6) на разных участках исследованных полигонов твердых бытовых отходов европейской части России. Одинаковые буквы над столбцами указывают на отсутствие статистически значимых различий между средними значениями. Сравнения признаны значимыми при *p*-уровне < 0,05 (критерий Данна с поправкой Хольма).

Table 2. Results of Kruskal-Wallis (KW) test showing the significance of differences in abundance and taxonomic groups of microfauna among different treatments (Core, Edge, and Control) in the studied municipal solid waste landfills. The table presents *p*-values obtained from the KW test, indicating the level of significance for each comparison.
Таблица 2. Результаты теста Краскела-Уоллиса (KW), иллюстрирующие статистическую значимость различий в численности и таксономических группах микрофауны между исследованными типами участков (ядро, край и контроль) на исследованных полигонах твердых бытовых отходов. В таблице представлены *p*-значения, полученные из теста KW, с указанием уровня значимости для каждого сравнения.

Variable	Kruskal-Wallis test statistic	Degrees of freedom	*p*-value
Ciliate abundance	6.7595	2	0.03406
Ciliate species richness	9.3993	2	0.009098
Nematoda abundance	6.3935	2	0.0409
Nematoda genera richness	1.1058	2	0.5753
C-P index	0.3883	2	0.8235
MI index	1,286	2	0.5257

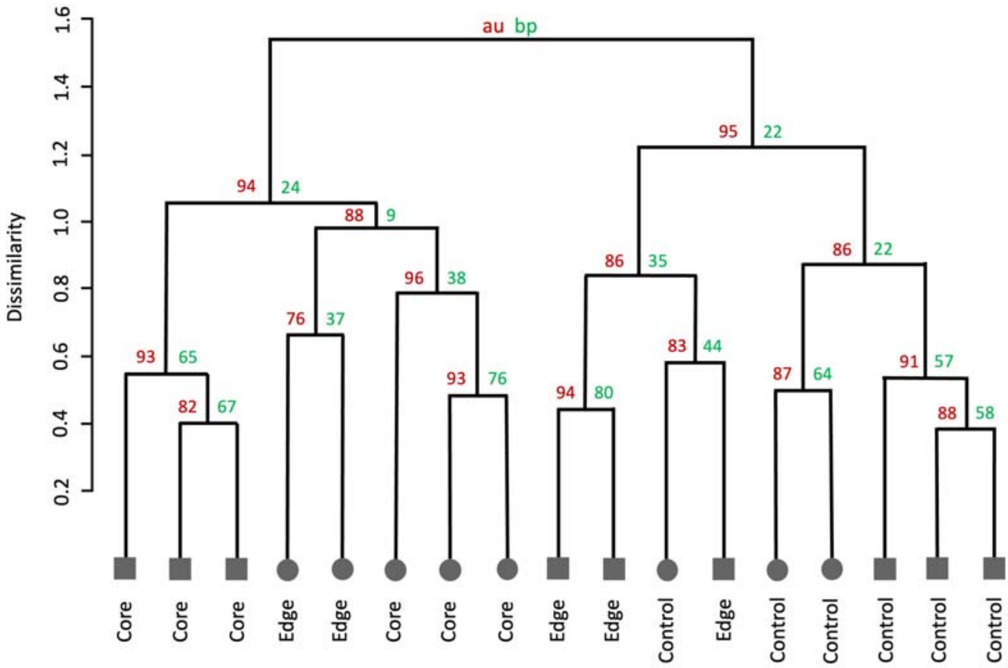

Fig. 2. Dendrogram showing the hierarchical clustering of ciliate faunal communities in different landfills (Balashikha — circles, Protvino — squares) and plots (Core, Edge and Control) using the binary method (presence-absence data) and Ward2 algorithm. One plot was excluded from the analysis due to the absence of a variance. The vertical axis represents the dissimilarity between clusters, with lower branches indicating greater similarity.

Рис. 2. Дендрограмма, иллюстрирующая иерархическую кластеризацию сообществ инфузорий на разных полигонах (Балашиха — круги, Протвино — квадраты) и участках (Импактный, Буферный и Фоновый) с использованием бинарного метода (данные наличия-отсутствия) и алгоритма Ward2. Один участок был исключен из анализа из-за отсутствия дисперсии. Вертикальная ось представляет несходство между кластерами, нижние ветви указывают на большее сходство.

slightly lower in controls and the lowest average number of species was observed in edge plots. Average abundance also significantly differed between studied treatments (Table 2). The highest ciliate abundance was recorded in control plots (Fig. 1A), which was 2.7 times higher than in edge plots. The abundance of ciliates in core plots was only slightly lower than in control plots (Fig. 1A).

According to the cluster analysis, the ciliate faunal communities in the studied zones were found to be zone-specific, with little or no mixing or overlap observed between them (Fig. 2). Meanwhile, the faunal communities of different landfills and their adjacent territories showed moderate mixing. There was a relatively high faunistic similarity observed between the core plots of the landfill in the vicinity of Balashikha. A similar trend was observed for the core plots of the landfill in Protvino. The control and edge plots within different landfills exhibited slightly lower similarity within their respective groups, indicating a tendency for mixing both within and between different landfills (Fig. 2).

NEMATODES. In total we found soil nematodes from nine genera belonging to nine families (Table 1). In Protvino sites the total number of genera was highest (seven) in the ñore plots. In Balashikha the number of genera was slightly higher in edge (six), than in core and control plots (four on each). Nematode genus richness (Fig. 3B), the C-P values and MI indices didn’t significantly differ between studied treatments

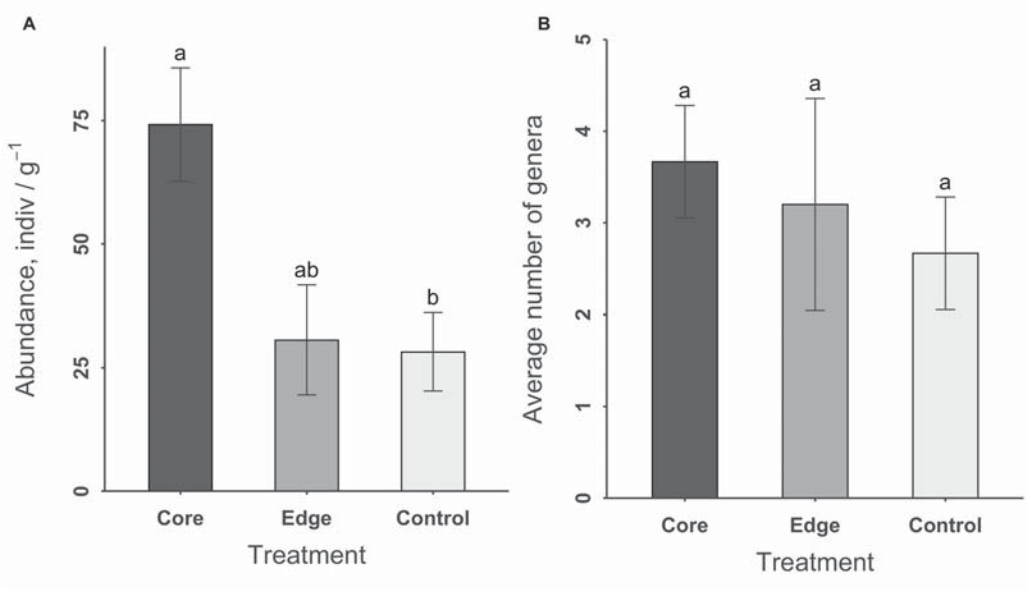

Fig. 3. Abundance, individuals per g^{-1} ± SE (A), and average number of genera ± SE (B) of nematodes (n = 6) in the different plots of the studied municipal solid waste landfills in European Russia. Similar letters above bars indicate the absence of significant differences between mean values for the studied treatment. Comparisons were recognized as significant at the < 0.06 p-level (Dunn's test with Holm correction).
Рис. 3. Численность, экз. на г$^{-1}$ ± станд. ошибка средней (А) и среднее количество родов ± станд. ошибка средней (В) нематод (n = 6) на разных участках исследованных полигонов твердых бытовых отходов европейской части России. Одинаковые буквы над столбцами указывают на отсутствие статистически значимых различий между средними значениями. Сравнения признаны значимыми при р-уровне < 0,06 (критерий Данна с поправкой Хольма).

according to KW test (Table 2). However, the MI index in Balashikha was slightly higher in the edge than in the other plots. The total abundance (Fig. 3A) significantly differed between studied treatments according to KW test (Table 2). The highest abundance was observed in the core plots (Fig. 3A) and was more than twice higher than abundance at the edge and control plots.

Cluster analysis revealed a high modulating effect of the landfill on the taxonomic composition of nematode communities (Fig. 4). The core and control plots from the landfill in the vicinity of Balashikha formed distinct clusters, which were clearly separated from the studied plots in Protvino. However, the nematode communities from the edge plots of the landfill in Balashikha were observed to be mixed with those from the studied plots in Protvino. Within Balashikha landfill clusters highly overlapped, and the most distinct were the edge and control plots.

Discussion

To our knowledge our study is the first attempt to assess the diversity of ciliates within solid waste landfill sites. This assessment was particularly challenging due to the limited availability of published data on the abundance and species composition of soil ciliates specifically in the European Russia region (Tribun, 2021; Tribun *et al.*, 2022). Soil samples (e.g., 100–500 ml) can potentially contain a wide range of 10 to over 400 species of free-living ciliates (Foissner, 1998; Foissner *et al.*, 2005; Chao *et al.*, 2006) although this number may represent only a modest share of the actual species pool present due to the limitations of reactivating resting cysts using current methods (Foissner *et al.*, 2002). In turn, although there have been several surveys of soil nematode and ciliate communities in the landfills (see, e.g., Khan, Chandra, 2017; Stamou *et al.*, 2020; Maurya *et al.*, 2022), they have mainly concerned operat-

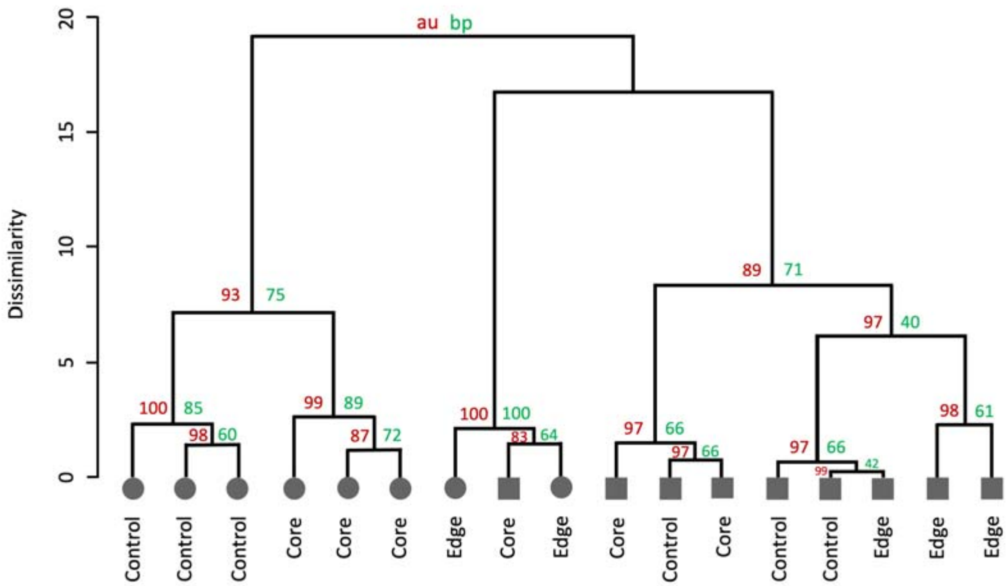

Fig. 4. Dendrogram illustrating the hierarchical clustering of nematode faunal communities in different landfills (Balashikha — circles, Protvino — squares) and plots (Core, Edge, and Control) using the Euclidean distance and Ward2 algorithm. One plot was excluded from the analysis due to the absence of variance. The vertical axis represents the dissimilarity between clusters, with lower branches indicating greater similarity.
Рис. 4. Дендрограмма, иллюстрирующая иерархическую кластеризацию сообществ нематод на разных полигонах (Балашиха — круги, Протвино — квадраты) и участках (Импактный, Буферный и Фоновый) основанную на Евклидовом расстоянии и алгоритме Ward2. Один участок был исключен из анализа из-за отсутствия дисперсии. Вертикальная ось представляет несходство между кластерами, нижние ветви указывают на большее сходство.

ed and not abandoned ones and have been conducted in tropical and Mediterranean regions. Unfortunately, nematodes were identified only to the genus level due to similar to ciliates' difficulties, which, nevertheless, allows using all the approved metrics for assessing the environment conditions (Bongers, Bongers, 1998) and verify patterns obtained for ciliates. These limitations along with a small number of replicates do not allow to call our study fully comprehensive, but it provides new insights on the regional microfauna taxonomic pools. This is particularly true for ciliates.

The landfill core sites exhibit a notably higher abundance of ciliates, while the peripheral areas show a significant decrease of this parameter, with again increased levels observed in control areas. According to Zalyaletdinova (2016), the abundance of protozoans in soil can indirectly indicate bacterial population density and reflect the processes occurring in a given area. This suggests that bacterial biomass is higher in the core and control sites, while the edge sites of landfills experience a decline and slower soil mineralization processes. In leachate-affected areas, the abundance of ciliates was significantly lower than in control areas. Similar pattern was discovered in 2022 (see Maurya *et al*., 2022), in a comparative study of the diversity of ciliates under the influence of soil pollution with landfill leachate which demonstrated that landfill leachate significantly alters the physical and chemical parameters of contaminated soil, leading to a notable reduction in ciliate community diversity.

Although the ciliate abundance in the landfill core and control areas was comparable, the core areas displayed a higher species richness compared to the slightly lower number observed in the control sites. When species composition was compared across all sampled areas, significant similarity was observed among ciliate species in the landfill core, while no significant similarity was found in the control areas. This difference can be attributed to the homogeneous soil type that was investigated (Zalyaletdinova, 2016). Based on the findings of Mazei (2007) at a homogeneous biotope scale, the number of protozoan species is influenced by the available niche space within the environment.

Despite the presence of diverse soil ciliate communities, the studied locations predominantly exhibited eurybiotic species. *C. aspera*, a species of ciliate found in all soil samples, is widely distributed in soil and freshwater environments. It usually thrives in mesosaprobic water bodies, which have moderate levels of organic pollution (Nikitina 2011). Moreover, this species is frequently found in humus-peaty gley soils (Shatilovich, 2010). Species such as *P. elongatum*, *C. uncinata*, and *O. chlorelligera*, which are commonly found in the study areas, are also eurybiotic representatives of soil ciliate fauna (Dixon, 1939). These species are capable for cyst formation and can survive in resource-limited environments for a certain period of time. Additionally, recent research has revealed that *C. uncinata* can act as a facultative endoparasite of mosquito larvae from the *Culex*, *Aedes*, and *Anopheles* genera, causing chronic and fatal infections in the natural mosquito population in Delhi and its surrounding areas in Northern India (Bina Pani Das, 2003).

Species found exclusively in the control sites, such as *A. monilata*, *L. costatus*, and *E. aediculatus* are widely distributed in marine and freshwater environments, as well as in soil and moss. Notably, the species *S. spathula* primarily prey on smaller ciliates and other protist species (Foissner *et al*., 1996). In the future, species found exclusively in control areas around landfills could serve as indicator species, but this aspect certainly requires further investigation.

Ciliate species detected exclusively in the core areas of landfills like *Strobilidium* sp., are commonly found in freshwater detritus (Maeda, 1986). Overall, species from this genus prefer shallow freshwater bodies and primarily feed on green algae and diatoms (Foissner, 1992). Another core-zone attribured species *U. nigricans* is widely distributed in both marine and freshwater environments, especially in heavily polluted waters. It is known to be a parasite of marine fish and is found in active sediments with high organic content. Limited research has been conducted on the ecology of free-living ciliates. However, based on the available information, ciliate species forming distinct assemblages within landfill sites are often compost-associated and frequently found in highly polluted and stagnant waters.

Similar to ciliates, the abundance and taxonomic richness of nematodes demonstrate peaks in the core zone due to the dominance of bacterial-feeding genera (*Eucephalobus*, *Panagrolaimus* and *Rhabditis*). This confirms the anticipated high bacterial activity and biomass within the core plots (Bongers and Bongers, 1998).

The reclamation process after the end of landfill operation is carried out using transported substrates with an associated seed and microbial bank (Kim, Lee, 2005; Stamou *et al*., 2020) and is significantly different from the restoration and growth of natural plant communities. This difference apparently significantly affects the nematode communities within the Balashikha landfill, where all sites are highly clustered. The highest nematode diversity and even MI index value of this block was noted within the edge site, which is quite typical for undisturbed ecotone ecosystems. Surprisingly, the highest abundance was noted for omnivorous nematodes (*Eudorylaimus* spp.), which are considered a sensitive group to pollutants and disturbances (Bongers, Bongers, 1998). This can indicate the absence of a significant amount of toxicant and pollutant runoff already 50 m from the landfill seven years after the end of landfill operation. However, this assumption requires additional studies of soil properties, as evidenced by the growth of ruderal species in the edge plot forest, indicating the high content of nitrogen and phosphorus in the soil (for example, *Urtica dioica*).

In contrast to Balashikha, within the Protvino landfill, nematode communities slightly differed between the three site types, which is associated with a small diversity within the edge and control plots and the dominance of bacterial-feeding nematodes. The low diversity and abundance within the edge plot is in line with the data obtained for ciliates and is likely caused by landfill leachate input and continued contamination of the area close to landfill, which is also supported by the dominance of exclusively ruderal and invasive vegetation. The low abundance and diversity within the control pine forest is most likely caused by the extremely poor organic matter content in its xerophilic soil.

Our data suggests that soil microfauna can thrive shortly after abandonment of severely disturbed substrates during communal solid waste deposition. At the absence of physical disturbance ciliate and nematode communities rearrange themselves quickly to utilize excessive resources from the waste mobilized by bacteria. Knowing peculiarities of soil microfauna communities in landfills provides new insights for understanding soil ecological restoration processes in the abandoned landfills. This further opens perspectives for a development of adequate management practices for sustainable communal waste disposal and landfill rehabilitation.

Compliance with ethical standards

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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