

Article

The Structure and Functioning of the Soil Microbial Community as Indicators of Soil Organic Matter Stabilization Under Different Land Use Systems on Gray Forest Soils

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Abstract

Soil organic matter (SOM) stabilization is closely linked to microbial community structure and function, yet reliable biological indicators remain insufficiently defined. This study aimed to identify microbial and biochemical markers of SOM accumulation under different land use systems (cropland, mown with phytomass removal, mown without phytomass removal, and fallow) in gray forest soils. Soil profiles were investigated in four land use types (cropland, mown with phytomass removal, mown without phytomass removal, and fallow) in the Laishevsky District (Russia). Physicochemical properties, SOM fractions, basal respiration, substrate-induced respiration, Biolog EcoPlates, quantitative PCR, and metagenomic data were used to assess microbial diversity and activity. Microbial communities differed substantially among land use systems and soil horizons, with bacterial communities in fallow soils dominated by oligotrophic taxa, such as *RB41*, *Candidatus Udaeobacter*, and *KD4-96*, whereas arable and managed grassland soils showed increased relative abundance of copiotrophic genera, particularly *Pseudomonas* and *Polaromonas*. Fungal communities were primarily represented by *Mortierella*, *Penicillium*, *Trechispora*, and *Metarhizium*, while both bacterial and fungal diversity decreased with soil depth, and metabolic profiling indicated preferential utilization of carbohydrates and carboxylic acids across all land use types. The highest organic matter and total organic carbon (TOC) were in soils under mowing without phytomass removal and fallow land, while arable soils showed the lowest values. Microbial diversity decreased with soil depth across all variants. Hay meadow soils exhibited elevated metabolic activity and higher metabolic quotient (qCO_2), indicating intensified carbon turnover or microbial stress, whereas arable soils were characterized by reduced substrate utilization and simplified community structure. Oligotrophic bacterial taxa were associated with more stable SOM conditions, while copiotrophic dominance reflected rapid carbon turnover. The results demonstrate that microbial community composition, functional activity, and specific taxa (e.g., oligotrophic bacteria, saprotrophic fungi, arbuscular mycorrhizal fungi) can serve as sensitive indicators of SOM stabilization processes. These findings support the development of microbiome-based diagnostic tools for assessing soil carbon dynamics and guiding sustainable land management strategies.

Academic Editors: Roberta Pastorelli and Sara Del Duca

Received: 3 April 2026
Revised: 2 June 2026
Accepted: 5 June 2026
Published: 26 June 2026

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Keywords: SOM; carbon sequestration; microbial community; humification; land use type; gray forest soils; soil microbiome

1. Introduction

The global reserve of organic carbon in the soil (1500 Pg in the 1 m layer) significantly exceeds that of the atmosphere (760 Pg) and living organisms (560 Pg) [1]. Soil organic matter (SOM) therefore represents one of the largest and most dynamic terrestrial carbon pools and plays a central role in climate regulation, nutrient cycling, and maintenance of soil fertility. Most soil organic matter (SOM) is concentrated in the upper 20–30 cm of the soil profile and originates from plant and animal detritus, bacterial and fungal necromass, and root exudates, consisting primarily of organic macromolecules containing carbon, oxygen, nitrogen, phosphorus, and sulfur [2]. The decomposition of organic matter in soils occurs mainly due to bacteria and fungi, which make up more than 95% of the soil biomass, while other soil organisms provide only 10–15% of the destruction of incoming organic matter [3,4]. During the mineralization of organic matter, most carbon is released back into the atmosphere as CO₂ as a result of microbial metabolic activity, whereas a certain proportion is transformed into stable humus compounds that may persist in soil for centuries [5]. Recent studies demonstrate that microbial-derived carbon and microbial transformation processes are major regulators of long-term SOM persistence and carbon sequestration in terrestrial ecosystems [6].

In soils, decomposers are primarily represented by phylogenetically distinct heterotrophic bacteria and fungi that exhibit diverse cellular adaptations and metabolic strategies [7,8]. The relative abundance of bacteria and fungi, as well as their metabolic activity, varies depending on the type of ecosystem and the quality of incoming plant litter, which directly affects the rate of decomposition of organic matter [9,10]. Fungi produce a significantly wider range of extracellular enzymes, which ensures their high capacity to decompose complex biopolymers, including cellulose, hemicellulose, and lignin, which are part of plant litter. Furthermore, fungi are characterized by an adsorption type of nutrition and a mycelial structure, making them key agents in the destruction of organic matter in the soil [11,12]. Bacteria, in contrast to fungi, are characterized by a shorter life cycle and high metabolic activity. They predominantly decompose more easily accessible, nutrient-rich organic compounds. Thus, bacteria and fungi occupy different functional niches in the processes of soil organic matter destruction: fungi act as primary reducers of high-molecular and structurally complex compounds, while bacteria function as the main reducers of accessible low-molecular organic substrates [13,14]. However, in addition to destructive processes, both bacteria and fungi can transform soil organic matter into both humus precursors and humus-like compounds [15]. Microbial necromass formed during these processes can contribute substantially to stable SOM pools and may account for a considerable proportion of mineral-associated organic carbon in soils [16,17].

Conservation agriculture strategies involve the use of agricultural practices that maintain, and preferably increase, soil organic carbon reserves. Long-term changes in the carbon pool are traditionally assessed using direct measurements; however, this approach requires long-term observations. At the same time, the microbial community can serve as a rapid indicator of the direction of carbon processes, since the composition and functional characteristics of microorganisms are closely linked to the mechanisms of carbon accumulation and stabilization in the soil [18–21]. To use the composition and functioning of microbial communities as a diagnostic tool, it is necessary to determine which taxa and/or physiological traits are key to the prevalence of humification processes

over SOM mineralization processes and, accordingly, the formation of stable organic matter reserves [22–24]. Agricultural management practices, particularly tillage intensity, mowing regime, and residue management, strongly influence SOM turnover by altering aeration, substrate availability, and the physicochemical conditions of microbial habitats. Intensive tillage generally accelerates SOM mineralization and reduces carbon storage, whereas reduced disturbance and retention of plant residues promote carbon accumulation and stabilization processes mediated by soil microorganisms [25,26]. Previous studies have shown that land use change can substantially modify microbial diversity, metabolic activity, and the balance between copiotrophic and oligotrophic taxa, thereby affecting carbon cycling pathways and humus formation. However, despite growing evidence linking microbial community structure with SOM dynamics, reliable microbial indicators reflecting the predominance of carbon stabilization over mineralization processes remain insufficiently defined, particularly for gray forest soils under contrasting land use systems.

One method for addressing this issue is the analysis of soils that have been exploited for long periods under various land use practices. Analysis of such soil systems provides the opportunity to identify components of the microbial community, including dominant and stable taxa, specific metabolic traits, and the contribution of microbial biomass, which are functionally linked to the accumulation of stable forms of organic carbon [27,28].

In this regard, the aim of this work was to identify such characteristics of the microbial community that can serve as reliable markers of the direction of SOM accumulation processes and reflect the features of the formation of its stable fractions. We hypothesized that soils characterized by lower anthropogenic disturbance and higher plant residue input would exhibit microbial communities enriched in oligotrophic bacteria, saprotrophic fungi, and metabolically efficient taxa associated with enhanced humification and stable carbon accumulation. To test this hypothesis, soils under arable land, mown systems with and without phytomass removal, and fallow land were compared using an integrated approach combining physicochemical analyses, microbial physiological assays, quantitative PCR, and high-throughput sequencing of bacterial and fungal communities.

2. Materials and Methods

2.1. Soil Sampling

To search for markers of humification processes, four areas with different types of use were selected in the Laishevsky District of the Republic of Tatarstan (Russia): T—arable land (more than 5 years old); G—an area mown with phytomass removal for more than 5 years (simulating a hay meadow); MPh—an area mown without phytomass removal for more than 5 years; FL—fallow land (no agrotechnical measures have been carried out for more than 5 years). Three pits were made at each of the four sites. Soil pits were established on 1 October 2024.

The area (55.564709, 49.137720) where the selected plots are located has a temperate continental climate with warm summers and moderately cold winters. The average annual air temperature is 5.1 °C, and the average annual precipitation is 565.2 mm. The topography of the study region is a gently rolling plain. The sample plots were established on sites with different land use types, which are expected to reduce SOM content (arable land, hay meadow) and accumulate SOM (mown without phytomass removal, fallow land). All four sample plots were previously arable land; they are characterized by similar topography and are located close to each other. This land use pattern has been in place for at least 5 years, which, according to the literature data, is sufficient to establish reliable differences in changes in SOM content. Photographs of the pits are presented in the Appendix A.1.

The soil type of all four studied sections was defined as gray forest in accordance with the “Classification and Diagnostics of Soils of the USSR” (1977) [29]. According to the international soil classification WRB (World Reference Base for soil resources), the soil under study was classified as Haplic Luvisol (Calcaric, Siltic). Geobotanical description of the areas where the sections were laid, and morphological description of the sections are presented in Appendices A.1 and A.2.

Sampling was carried out in accordance with the requirements of GOST 17.4.3.01-2017 and GOST 17.4.4.02-2017 [30,31]. Bulk density was determined using the cutting ring method in accordance with GOST 5180-2015 [32]. Soil samples were collected from each horizon of the soil profile in such a way that each sample was representative of the genetic horizons of a given soil type. One combined sample weighing at least 1 kg was taken from each horizon. The samples delivered to the laboratory were divided into two parts. Soil samples for further DNA extraction were frozen and stored at a temperature of -15°C . Soil samples for further agrochemical analysis were subjected to sample preparation in accordance with GOST 5180-2015, including removal of plant residues and crushing.

2.2. Agrochemical Analysis of Soil Samples

The moisture content of the soil samples was estimated gravimetrically according to GOST 5180–2015. Electrical conductivity and pH were determined according to ISO 10390:2021 [33]. The cation exchange capacity (CEC) was determined according to GOST 17.4.4.01-84 [34]. The organic matter content was determined according to GOST 23740–2016 [35] by burning a sample in a muffle furnace at a temperature of 650°C for 30 min. The total organic carbon (TOC) content was determined by the wet ashing method (ISO 14235:1998) [36] by boiling in a digester at a temperature of 135°C for 30 min in a mixture with a solution of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) and concentrated sulfuric acid (H_2SO_4). To determine DOC, an extract was first prepared in a 0.5 M solution of potassium sulfate K_2SO_4 , after which the content of organic carbon transferred from the soil sample to the extract was determined, similar to the method for determining TOC. The nitrogen content was determined by the Kjeldahl method according to the instructions for the automatic nitrogen analyzer UDK 159 (Velp Scientifica, Usmate, Italy). Nitrates were determined using the ionometric method (GOST 26951-86) [37]. Nitrate ions were extracted from the soil with a 1% solution of potassium alum ($\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) at a soil: solution ratio of 1:2.5 on a mechanical shaker for 3 min. The nitrate concentration in the filtrate was measured using an ion-selective electrode on an Anion 7000 ion meter (AN-ION, Moscow, Russia). Mobile phosphorus and potassium compounds were determined according to GOST 26207-91 [38] (Kirsanov method modified by TsINAO). Extraction was performed with 0.2 M HCl at a soil/solution ratio of 1:5. Phosphorus in the extract was determined colorimetrically (phosphorus–molybdenum complex, $\lambda = 710\text{ nm}$); potassium in the extract was determined on a flame photometer. The results were expressed as mg/kg (P_2O_5 and K_2O) of absolutely dry soil.

Extraction of humic and fulvic acids was carried out using the Schnitzer method [39]. To determine the content of HA and FA, a method similar to the determination of TOC was used.

The granulometric composition of the soils was analyzed in accordance with ISO 13220:2020 [40] using a laser diffractometer Microtrac Bluewave (Microtrac Retsch GmbH, Haan, Germany).

2.3. Assessment of the Functioning of the Soil Microbial Community

Respiratory activity (basal respiration) was assessed using a Nexis GC-2030 gas chromatograph and Lab Solution software (Shimadzu, Kyoto, Japan) according to ISO 16072:2002 [41]. A 1 g sample of field-wet soil was placed in a chromatographic vial and incubated for 24 h at 20 °C. Measurements were performed on an Rt-Q-BOND 30 m capillary column with a diameter of 0.53 mm and a layer thickness of the applied phase of 20 µm. The flow rate was 10 mL/min, the injection volume was 10 µL, and the total measurement time was 5 min per sample.

Microbial biomass (C_{mic}) was determined using the substrate-induced respiration method (ISO 14240-1:1997, Part 1) [42]. D-glucose (10 mg per 1 g of soil) was used as an easily accessible substrate, and CO₂ exhalation was recorded on a Shimadzu gas chromatograph Nexis GC-2030 (Japan).

The total metabolic activity of soil microbial communities was determined using the AWCD (average cell color density) index and the commercial Biolog plates, Ecoplates (Biolog Inc., Hayward, CA, USA). Accordingly, 180 µL of the test samples (1:10) were pipetted into each well of a 96-well plate containing the substrate and the redox indicator. The plates were incubated at 25 °C for 120 h. The results were evaluated photometrically on a Multiskan FC (Thermo Fisher Scientific, Waltham, MA, USA) at a wavelength of 595 nm. The biodiversity of the microbial community was assessed using the Shannon index.

2.4. Analysis of Soil Samples Using Molecular Methods

Total DNA extraction from 0.3 g samples was performed using the FastDNA kit. Spin Kit for Soil (MP Bio, Santa Ana, CA, USA). Quantitative assessment of the 16S rRNA gene copies of bacteria and the ITS region of fungal DNA was performed by real-time PCR (ISO 17601:2016) [43] using primers 341f/534r for bacteria and ITS1f/ITS2r for fungi on a thermal cycler. BioRad CFX-96 (Bio-Rad, Munich, Germany). The following program was used for amplification: primary denaturation at 95 °C for 5 min, then 39 three-step cycles at 62–60 °C for 45 s, at 95 °C for 15 s, and at 72 °C for 30 s. Calibration curves were constructed using DNA from *Pseudomonas putida* and *Penicillium notatum*.

Sequencing of the bacterial community was performed on the Illumina platform (Illumina, San Diego, CA, USA). The genomic library was prepared according to the library preparation protocol for 16S metagenomic sequencing (Illumina MiSeq, San Diego, CA, USA). Sequencing data were analyzed using the Quantitative platform Insights Into Microbial Ecology (QIIME) using the DADA2 algorithm. Taxonomic annotation was performed using the SILVA138 and UNITE8 databases. Statistical data processing was performed using the R environment (version 4.4.3). To characterize the diversity of bacterial communities, the Shannon and Simpson indices were calculated using the diversity function of the vegan package. Although total soil DNA was successfully extracted for bacterial community analysis, fungal DNA concentrations in all soil samples from tillage were below the minimum threshold required for library preparation and Illumina sequencing. Consequently, fungal amplicon/metagenomic sequencing could not be performed, and analyses of the fungal community of soils under tillage are not included in this study.

Prediction of the gene sets and metabolic pathways of bacterial communities was performed using the Tax4Fun2 software package. Functional profiles of micromycete communities were created using the FUNGuild database.

To assess the structure of bacterial communities and their relationship with soil physicochemical factors, we performed non-metric multidimensional scaling (NMDS) based on Bray–Curtis distances. NMDS was conducted using the metaMDS function from the vegan package ($k = 2$, $\text{trymax} = 50$). All measured soil variables (pH, organic carbon, total nitrogen, available phosphorus, CEC, etc.) were fitted onto the NMDS ordination using the envfit function (9999 permutations) from the vegan package. To control for false positives due

to multiple testing, we applied Benjamini–Hochberg false discovery rate correction to the p -values of all fitted vectors. Variables with adjusted $p < 0.05$ were considered significant and are highlighted in the biplot. PERMANOVA (permutational multivariate analysis of variance; function `adonis2`, 999 permutations) was used to test the effects of land use type and soil horizon on the Bray–Curtis dissimilarity matrix of bacterial communities. The model included main effects (LandUse + Horizon). Differences in individual soil physico-chemical parameters between land use types and between soil horizons were assessed using the Kruskal–Wallis non-parametric test. Global p -values were adjusted for multiple comparisons across parameters using false discovery rate (FDR) correction. Where the adjusted global test was significant ($p_{\text{adj}} < 0.05$), Dunn’s post hoc test with Holm correction was applied to identify which specific groups differed.

To identify differentially represented taxa, the ANCOM-BC2 (Analysis of Compositions of Microbiomes with Bias Correction 2) from the ANCOMBC package. Taxa were aggregated to the family level. To avoid logarithmization of zero values, a pseudo value (pseudo = 0.1) was used. Differences were considered statistically significant if the adjusted p -value (q -value) was < 0.05 .

2.5. Statistical Data Processing

All data are presented as the mean and standard deviation of triplicate analyses. All histograms show standard deviation values. Means, standard deviations, and Pearson’s correlation coefficients were calculated using Microsoft Excel 2016. Normality testing of the source data was performed using the R package (version 4.4.3) using the Shapiro–Wilk test.

3. Results and Discussion

3.1. Agrochemical Characteristics of Soil Samples

At the first stage, an agrochemical characterization of the soils was carried out (Tables 1 and 2), which revealed that the upper soil horizons were characterized by the lowest moisture content (4–5%), pH (5.9–7.1), and the highest CEC values (21.9–35.9 meq/100 g), regardless of the nature of land use. The established increase in moisture content along the soil profile generally follows the pattern of moisture distribution in gray forest soil in autumn [44] when plant roots do not take moisture from the underlying horizons. However, consistent differences were found in the trends in moisture content increase depending on the type of land use, characteristics of the vegetation cover, and, as a consequence, the presence/absence of roots in the profile at the time of sampling [45]. Thus, in the arable land profile, moisture content in the upper 50 cm remained at the same level (4.7–5.24%).

In general, the soils of all four land use types are characterized by a neutral level of acidity, while the lower pH level in the upper layers of the “fallow”, “mown without phytomass removal” and “hay meadow” areas is associated with the formation of organic acids during the biodegradation of plant residues and leaching [46]. The alkaline reaction of the soil in the lower horizons of the “fallow” and “hay meadow” areas is explained by the presence of carbonates in the parent rock, which leads to an increase in the pH of the environment. The range of CEC values corresponds to those typical for gray forest soils; the decrease in CEC along the soil profile is associated with a higher content of organic matter, low pH values, and optimal soil structure [47,48].

The maximum content of both nitrates and total N was found in the upper soil horizons (2.63–13.67 mg/kg and 1.60–4.76 mg/g, respectively), with the maximum nitrate content being found in soils sampled from arable land and the maximum total N content in fallow soils. The nitrogen content steadily decreases with depth, which is a characteristic feature of gray forest soils and indicates intensive decomposition of organic matter in the

upper part of the profile and weak migration of nitrogen to the lower horizons [49]. The phosphorus content changed similarly, with maximum values characteristic of and for soils sampled from the upper soil horizons, on arable land (242.1 ± 14 mg/kg; very high content) and on fallow land (178.1 ± 24.9 mg/kg; high content). The reason for such a high content of nitrates and phosphates in the upper soil horizons and a decrease in their content along the soil profile is probably the application of organic fertilizers to arable land, in particular based on cattle manure [50] as well as the accumulation of plant residues on fallow land, with subsequent transformation of OM from plant biomass [50]. For potassium, on the contrary, an increase in its content was established in the soils of the lower carbonate horizons, which is probably associated with the accumulation of insoluble forms, as well as the peculiarity of the mineralogical composition of the parent rock [51].

The bulk density of the soil is minimal in the upper horizons ($0.88\text{--}1.08$ g/cm³), increasing towards the lower horizons, reaching $1.54\text{--}1.94$ g/cm³. For arable land, maximum values of density were established for the upper horizon. Soil profiles of different areas had similar particle size distribution with a predominance of sandy and silt fractions. The soil profile horizons from the arable land were consistently characterized by a more uniform particle distribution, whereas differentiation in particle size distribution was observed for other land use types. A lighter particle size distribution was found for the soil under the fallow land compared to the soil under the arable land, which is consistent with the bulk density data. According to the literature, the ratio of fractions of different sizes and, as a consequence, the bulk density of the soil may change with a change in the type of land use due to changes in the vegetation cover in general and root systems in particular, which may contribute to a decrease in density and further affect the particle size distribution [52,53]. In addition, loosening of the soil under the fallow land may occur due to an increase in the number of earthworms [54]. It is important to note that when the fallow land is re-involved in agricultural cultivation, a deterioration in the aggregate state is observed, since the aggregate composition is a dynamic characteristic that is significantly influenced by land use characteristics [45].

Next, we assessed parameters that allowed us to evaluate how the content of various forms of carbon changed due to the change in land use type (Table 3). As expected, the lowest OM content was found in soils sampled from all horizons under arable land (a decrease from 6.8 to 2.2% across the soil profile), while the highest was found in soils sampled in areas where mowing was carried out without removing phytomass (a decrease from 13.2 to 1.4% across the soil profile). It is also worth noting that in the soil profile under arable land, the organic matter content in the upper 50 cm remained constant, and further (from 60 cm) a sharp decrease to 2.1% was observed, whereas in soils of other land use types, the values of this parameter decreased gradually. This effect is likely caused by the periodic application of organic fertilizers and the homogenization of the topsoil due to tillage. It is interesting to note that in the lower part of the soil profile under the fallow land, an accumulation of organic matter of up to 4.8% was observed. The obtained results are consistent with the literature data showing that OM is a sensitive parameter that responds to changes in the type of soil use [45]. Plowing and a sharp decrease in the input of plant residues lead to increased humus mineralization and, as a consequence, to a decrease in the OM content under agricultural soils [45]. In post-agrogenic soils, a tendency for a gradual increase in the organic matter content over time is observed, which is due to the input of a large amount of dead plant residues, the formation of sod, and an increase in underground biomass [52,55,56]. A global meta-analysis (5980 observations) [57] showed that the conversion of arable land to forests and pastures contributes to an increase in carbon stocks by 28.0% and 33.5%, respectively, due to the development of an initially spontaneous phytocenosis, which subsequently becomes zonal. This leads to the accumulation of carbon in the upper horizons of former

arable soils [58]. This phenomenon was recorded for soil samples with the land use types “fallow”, “hay meadow”, and “mown without phytomass removal”; for the sample of the last type, carbon accumulation in the upper layer is most pronounced. This is due to the regular mowing of vegetation and leaving phytomass on the soil surface. In contrast, in the hay meadow area, mown vegetation is removed, and in the fallow area, soil formation processes occur exclusively under natural conditions. The minimum content of HA and FA was found in soils under arable and hayfields; for all types of land use, a decrease in the content of HA and, to a lesser extent, FA was shown; the CHA/CFA ratio changed from 1.02 to 0.12 with the depth of the soil profile, since FA are more mobile and soluble, which is typical for gray forest soils [59–62]. A correlation was established between the contents of OM and TOC ($r = 0.94$), between the contents of OM and FA ($r = 0.79$), and between the contents of OM and HA ($r = 0.86$).

DOC accounts for less than 2% of the total carbon pool in the soil, but it plays a key role in maintaining microbial activity, contributing to increased soil fertility and carbon sequestration [63]. In connection with the above, it is advisable to assess not only the total carbon reserves but also their distribution among fractions. No relationship has been established between the DOC content and the soil horizon for soils with the land use types “fallow”, “hay meadow”, and “mown without phytomass removal”. In the soil under arable land, a decrease in the DOC content was found with an increase in the depth of the soil profile. A correlation was not established between the OM content and DOC ($r = 0.27$). According to the literature, a decrease in the DOC content with depth is possible due to decomposition and sorption processes; cases of its different distribution among soil horizons have also been described [64–66]. Thus, it has been shown that the downward migration of dissolved organic compounds can enrich the lower soil horizons with organic carbon, facilitating its accumulation at depth [66]. Migration losses of humus can also be caused by physicochemical processes, such as the dissolution of organic matter in water under the influence of acidification or alkalization, which leads to their movement along the profile [64]. This confirms the possibility of enriching the lower soil horizons with organic carbon in the form of soluble fractions, which is observed in the profile of soils with the land use types “fallow land”, “hay meadow”, and “mown without phytomass removal”.

Table 1. Agrochemical characteristics of the soil.

Plot	Soil Horizons	Sample Code	Humidity		pH		CEC		N (Total)		Nitrates (NO ₃ ⁻)		Phosphorus (P ₂ O ₅)		Potassium (K ₂ O)	
			%	SD		SD	meq/100 g	SD	mg/g	SD	mg/kg	SD	mg/kg	SD	mg/kg	SD
FL	Ad (0–7 cm)	FL7	4.51	0.61	6.30	0.77	28.50	6.10	4.76	0.60	4.04	0.38	178.10	24.93	160.74	18.81
	Ast pah (7–30 cm)	FL30	6.33	0.85	6.71	0.73	27.50	6.90	1.96	0.10	1.41	0.12	127.96	14.33	139.73	9.64
	A1 (30–55 cm)	FL55	5.86	0.32	6.90	0.75	29.20	5.20	1.52	0.22	1.13	0.00	158.90	14.14	130.39	14.08
	A1A2 (55–80 cm)	FL80	3.34	0.49	7.10	0.99	19.00	2.60	0.69	0.08	0.95	0.04	89.55	6.54	111.72	6.37
	A2B (80–100 cm)	FL100	7.88	1.07	7.48	0.99	15.90	3.10	0.22	0.02	0.96	0.08	45.30	5.07	753.28	45.95
	Bk (100–140 cm)	FL140	9.93	1.03	8.06	1.04	11.80	0.80	1.12	0.07	1.32	0.05	50.77	5.74	671.09	99.99
MPH	Ad (0–6 cm)	MPh6	3.78	0.43	5.91	0.71	32.60	6.80	3.48	0.17	3.93	0.18	60.74	4.56	212.10	12.73
	Ast pah (6–20 cm)	MPh20	8.22	1.18	6.62	0.91	28.70	3.70	1.96	0.20	1.72	0.08	137.56	10.18	177.08	8.85
	A1A2 (20–35 cm)	MPh35	8.82	1.12	6.65	0.85	22.20	1.20	1.48	0.18	1.21	0.06	75.68	10.29	151.40	13.47
	A2B (35–100 cm)	MPh100	11.20	0.80	6.90	0.99	25.50	5.30	0.61	0.08	1.19	0.00	106.62	8.00	65.03	3.90
	Bk (100 cm and below)	MPh > 100	10.46	1.14	7.00	0.59	19.70	0.30	0.12	0.01	1.11	0.05	53.28	2.98	83.70	10.80
T	Apah (0–15 cm)	T15	4.70	0.59	7.10	0.60	29.30	0.30	1.60	0.21	13.67	1.26	151.43	21.96	174.75	19.40
	A1 (15–50 cm)	T50	5.24	0.28	7.25	0.92	30.00	7.00	0.44	0.04	3.93	0.18	242.11	14.04	170.08	10.03
	A1A2 (50–60 cm)	T60	3.90	0.20	7.27	0.71	23.20	1.60	0.23	0.03	1.56	0.00	85.28	12.71	72.03	9.80
	A2B (60–86 cm)	T86	8.07	0.95	7.06	0.55	19.80	2.20	0.44	0.03	1.33	0.06	91.68	11.19	186.42	22.93
	Bk (86–160 cm)	T160	14.13	0.85	6.85	0.62	16.70	1.50	0.54	0.05	4.89	0.06	103.42	8.79	230.77	24.23
G	Ad (0–2 cm)	G2	5.19	0.50	6.54	0.63	21.90	0.50	2.30	0.20	2.63	0.09	138.63	15.80	170.08	20.41
	Ast pah (2–24 cm)	G24	13.07	1.53	6.56	0.40	35.90	1.10	1.95	0.22	3.05	0.07	195.17	16.00	121.05	16.58
	A1 (24–51 cm)	G51	17.51	1.30	6.80	0.48	44.80	2.60	1.84	0.26	1.99	0.02	127.96	15.10	67.36	5.39
	A1A2 (51–70 cm)	G70	12.81	0.85	6.97	0.36	25.10	5.10	0.67	0.09	1.35	0.05	82.08	10.67	67.36	7.54
	A2B (70–104 cm)	G104	15.76	2.08	7.10	0.43	29.20	7.00	0.66	0.07	1.29	0.05	122.62	10.79	142.06	15.34
	Bk (104–164 cm)	G164	12.32	1.39	7.85	0.68	13.00	1.60	0.19	0.02	1.48	0.09	36.55	5.48	694.58	96.55
	BSk (164 cm and below)	G > 164	15.51	2.25	8.10	1.00	11.60	2.20	0.18	0.02	1.80	0.12	33.64	2.83	694.58	70.15

Table 2. Physical characteristics of soil.

Plot	Soil Horizons	Sample Code	Bulk Density of Soil		Sand Fraction, 2–0.05 mm		Silt Fraction, 0.05–0.002 mm		Clay Fraction, <0.001 mm	
			g/cm ³	SD	%ob.		%ob.		%ob.	
FL	Ad (0–7 cm)	FL7	0.89	0.57	57.76		0.00		42.24	
	Ast pah (7–30 cm)	FL30	1.55	0.11	44.62		0.42		54.96	
	A1 (30–55 cm)	FL55	1.85	0.13	44.90		1.33		53.77	
	A1A2 (55–80 cm)	FL80	1.79	0.09	45.36		1.01		53.63	
	A2B (80–100 cm)	FL100	1.68	0.02	43.83		1.43		54.74	
	Bk (100–140 cm)	FL140	1.94	0.13	41.67		0.12		58.21	
MPh	Ad (0–6 cm)	MPh6	0.90	0.25	61.90		0.00		38.10	
	Ast pah (6–20 cm)	MPh20	1.29	0.15	44.07		0.67		55.26	
	A1A2 (20–35 cm)	MPh35	1.20	0.12	36.96		0.97		62.07	
	A2B (35–100 cm)	MPh100	1.54	0.06	38.20		1.00		60.80	
	Bk (100 cm and below)	MPh > 100	1.39	0.02	50.82		0.79		48.39	
T	Apah (0–15 cm)	T15	1.08	0.18	51.72		0.69		47.59	
	A1 (15–50 cm)	T50	1.12	0.06	51.45		1.00		47.55	
	A1A2 (50–60 cm)	T60	1.09	0.02	50.16		3.36		46.48	
	A2B (60–86 cm)	T86	1.26	0.06	50.73		1.16		48.11	
	Bk (86–160 cm)	T160	1.93	0.03	47.21		0.25		52.54	
G	Ad (0–2 cm)	G2	0.88	0.18	51.56		0.35		48.09	
	Ast pah (2–24 cm)	G24	1.74	0.13	51.86		0.23		47.91	
	A1 (24–51 cm)	G51	1.72	0.17	45.18		0.37		54.45	
	A1A2 (51–70 cm)	G70	1.30	0.08	49.96		1.64		48.40	
	A2B (70–104 cm)	G104	1.90	0.04	31.62		1.57		66.81	
	Bk (104–164 cm)	G164	1.83	0.03	53.10		0.38		46.52	
	BSk (164 cm and below)	G > 164	1.74	0.02	38.52		0.71		60.77	

Table 3. Content of OM, TOC, DOC, FA, and HA in soil samples.

Plot	Soil Horizons	Sample Code	OM		TOC		DOC		FA		HA	
			%	SD	mg/g	SD	mg/g	SD	g/kg	SD	g/kg	SD
FL	Ad (0–7 cm)	FL7	7.60	0.93	32.00	1.36	0.18	0.01	35.70	1.17	33.23	0.26
	Ast pah (7–30 cm)	FL30	5.00	0.48	21.58	1.46	0.42	0.02	31.74	1.75	20.38	0.39
	A1 (30–55 cm)	FL55	4.80	0.57	20.31	0.26	0.32	0.00	30.57	1.36	17.46	0.32
	A1A2 (55–80 cm)	FL80	2.60	0.25	9.02	0.19	0.09	0.01	22.52	1.36	10.77	0.39
	A2B (80–100 cm)	FL100	4.00	0.40	7.17	0.29	0.44	0.02	23.23	0.78	2.79	0.19
	Bk (100–140 cm)	FL140	4.80	0.30	3.70	0.06	0.40	0.04	24.99	0.32	NA	
MPh	Ad (0–6 cm)	MPh6	13.20	1.02	62.24	0.13	0.80	0.04	40.11	1.82	41.02	0.26
	Ast pah (6–20 cm)	MPh20	6.00	0.52	26.77	0.94	0.34	0.02	32.00	0.19	24.08	0.45
	A1A2 (20–35 cm)	MPh35	5.80	0.44	22.33	1.23	0.50	0.04	28.30	0.39	21.87	0.45
	A2B (35–100 cm)	MPh100	5.00	0.52	20.05	0.45	0.70	0.06	25.77	0.06	13.17	0.06
	Bk (100 cm and below)	MPh > 100	1.40	0.16	4.19	1.07	0.61	0.05	23.43	0.06	5.91	0.45
T	Apah (0–15 cm)	T15	6.80	0.64	27.68	0.36	0.83	0.02	36.08	1.95	27.78	0.26
	A1 (15–50 cm)	T50	6.20	0.36	26.09	0.71	0.76	0.07	30.63	0.00	19.08	0.13
	A1A2 (50–60 cm)	T60	2.20	0.11	8.57	0.71	0.50	0.04	25.90	0.06	9.09	0.26
	A2B (60–86 cm)	T86	2.20	0.29	5.09	0.29	0.47	0.02	28.82	1.95	7.01	0.26
	Bk (86–160 cm)	T160	3.40	0.19	6.17	0.00	0.28	0.01	27.58	0.06	3.44	0.06
G	Ad (0–2 cm)	G2	6.80	0.50	30.47	1.27	0.26	0.01	34.33	0.71	23.43	0.32
	Ast pah (2–24 cm)	G24	5.80	0.31	23.98	1.07	0.23	0.01	32.06	1.30	20.18	0.45
	A1 (24–51 cm)	G51	6.20	0.90	24.99	1.88	0.29	0.02	32.51	0.97	14.80	0.78
	A1A2 (51–70 cm)	G70	5.00	0.53	11.84	0.29	0.16	0.01	32.00	2.40	6.17	0.19
	A2B (70–104 cm)	G104	4.20	0.55	7.82	0.10	0.11	0.01	22.65	0.84	3.12	0.13
	Bk (104–164 cm)	G164	3.00	0.32	3.12	0.19	0.54	0.02	21.48	0.06	NA	
	BCk (164 cm and below)	G > 164	4.00	0.42	3.50	0.84	0.38	0.04	19.99	1.04	NA	

Thus, the most humus-saturated are the upper horizons of soils under mown soil without removal of phytomass (Ad, Ast pah) and deposits (Ad, Ast pah).

3.2. Physiological Activity of Microbial Communities

It has been proven that soil microorganisms play a central role in the formation, transformation, and stabilization of SOM. Microbial necromass can account for more than 50% of TOC in both natural and agrarian ecosystems, with fungi often making a greater contribution than bacteria due to their slower decomposition rate and greater stability in the soil [16,67–70]. Currently, a known parameter is the carbon use efficiency of microorganisms (CUE), which shows the proportion of carbon assimilated by the microbial community that is used for microbial biomass growth compared to the proportion of carbon released into the atmosphere through respiration. This indicator is a key integrative indicator predicting the global accumulation of organic carbon in the soil. Isotopic methods are used to determine this indicator; their widespread use is impossible due to the cost and complexity of their organization for agricultural enterprises [71–74]. In order to identify simple and widely applicable markers of the prevalence of SOM accumulation processes over its mineralization, the composition and functioning of the soil microbial community were assessed.

One of the parameters that can be used to assess the prevalence of microbial biomass accumulation over organic matter mineralization is qCO_2 (Table 4). The highest qCO_2 values were found in soil microbial communities under mown crops, both with and without phytomass removal (0.19–0.72) and without (0.15–0.91), while the lowest values were found in soil microbial communities under arable land (0.10–0.43). High qCO_2 values in mown soils and in mown crops with phytomass removal may indicate either active decomposition with rapid turnover of substances (due to the abundant supply of fresh plant residues) [75] or increased microbial stress caused by management practices such as frequent mowing without fertilization, in which case more carbon is lost through respiration per unit of biomass, resulting in less biomass accumulation and subsequently less necromass, i.e., lower effective carbon use efficiency [76–78] qCO_2 increases when microorganisms decompose nutrient-poor substrates with high C:N or C:P ratios, at the expense of additional respiration expended on providing nitrogen or phosphorus [79–83]. Low qCO_2 in arable soils may reflect adaptation to chronic resource scarcity [84], consistent with the low carbon and nitrogen contents of arable soils.

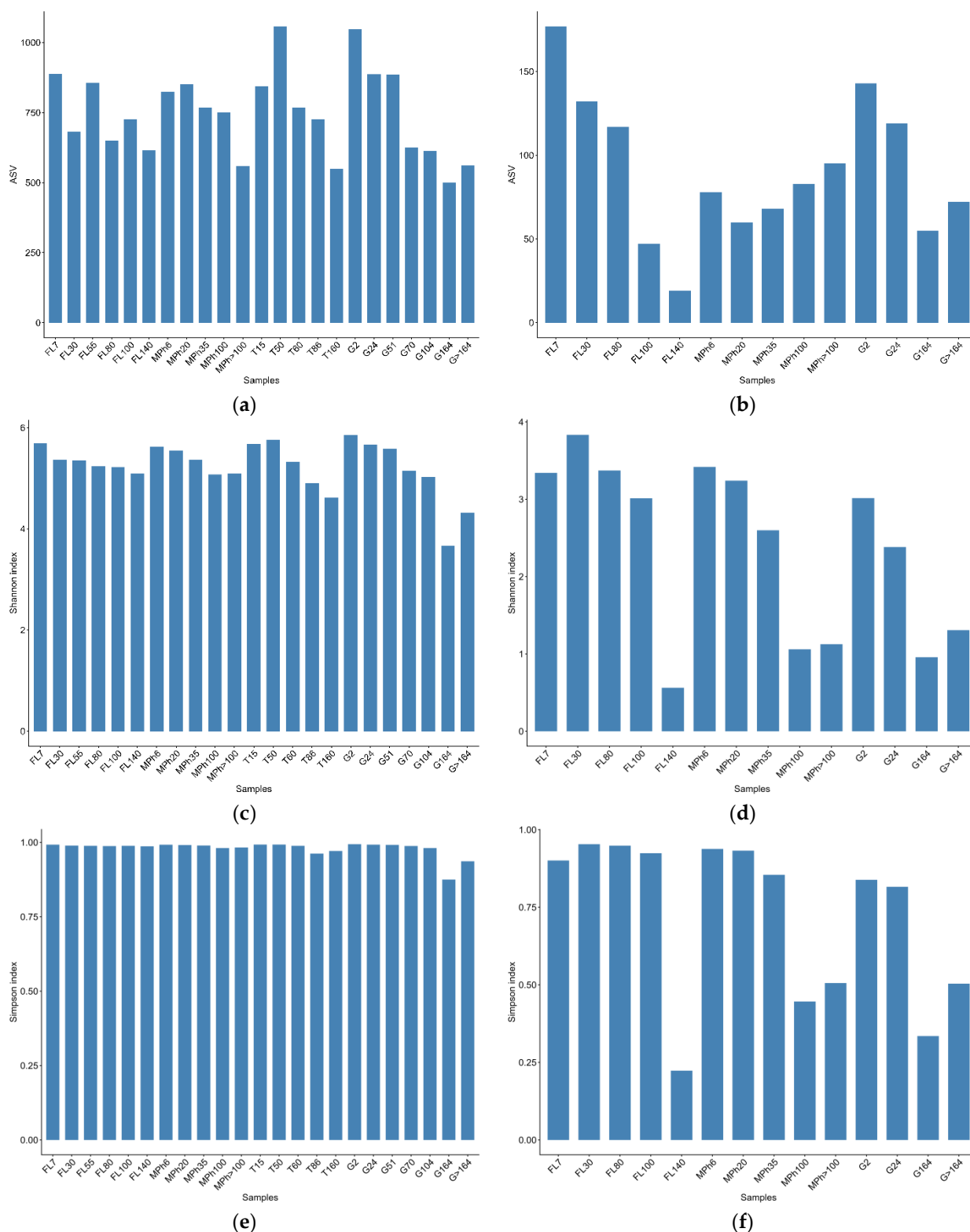
Next, the consumption rate of carbon-containing substrates was assessed. It was found that carbohydrates and carboxylic acids are used most intensively in all horizons, which is associated with their high bioavailability for most microorganisms [21,85–87]. The microbial community of the upper soil horizons is characterized by high metabolic activity in relation to all substrates; with increasing depth of the soil profile, the consumption rate of carboxylic acids increases and decreases for all remaining substrate groups, with the maximum decrease established for amino groups and phenolic compounds. It is worth noting that in the soil under arable land, in general, lower values of the consumption rate for all substrates were established. The combination of low qCO_2 and weak substrate consumption suggests reduced microbial turnover and slower carbon cycling under resource-limited conditions. Thus, the use of qCO_2 alone cannot be a marker of OM accumulation processes in the soil.

Table 4. Analysis of the functional activity of soil microbial communities.

Plot	Soil Horizons	Sample Code	qCO ₂	Substrate Consumption Rate Based on Data Biolog Ecoplate					
			mgCO ₂ —C g ^{−1} Cmic h ^{−1}	Amine	Phenol	Polymer	Amino Acid	Carboxylic Acid	Carbohydrate
FL	Ad (0–7 cm)	FL7	0.33	17.96	10.03	5.93	25.13	17.20	23.76
	Ast pah (7–30 cm)	FL30	0.53	11.99	0.00	43.95	12.91	19.07	12.08
	A1 (30–55 cm)	FL55	0.36	0.00	2.92	14.37	2.49	72.49	7.73
	A1A2 (55–80 cm)	FL80	0.05	15.73	6.01	10.77	1.95	56.51	9.02
	A2B (80–100 cm)	FL100	0.38	0.00	1.53	9.86	10.96	64.49	13.16
	Bk (100–140 cm)	FL140	0.37	0.00	0.39	10.65	16.74	42.88	29.34
MPh	Ad (0–6 cm)	MPh6	0.15	12.57	12.26	16.50	21.83	19.86	16.99
	Ast pah (6–20 cm)	MPh20	0.91	13.53	9.36	13.03	19.66	21.56	22.86
	A1A2 (20–35 cm)	MPh35	0.42	0.78	0.00	16.15	14.78	26.67	41.62
	A2B (35–100 cm)	MPh100	0.42	4.58	9.72	6.90	17.45	35.63	25.72
	Bk (100 cm and below)	MPh > 100	0.52	0.00	0.18	9.14	7.08	74.52	9.08
T	Apah (0–15 cm)	T15	0.19	14.10	13.39	14.15	21.98	17.61	18.78
	A1 (15–50 cm)	T50	0.43	14.11	14.19	12.78	26.81	19.49	12.61
	A1A2 (50–60 cm)	T60	0.10	0.47	1.02	43.58	25.66	26.50	2.77
	A2B (60–86 cm)	T86	0.18	7.77	0.00	8.84	42.34	26.22	14.82
	Bk (86–160 cm)	T160	0.13	0.00	0.00	18.35	9.43	60.33	11.89
G	Ad (0–2 cm)	G2	0.72	19.14	13.01	13.84	23.53	16.19	14.30
	Ast pah (2–24 cm)	G24	0.61	19.71	15.26	16.77	17.63	17.65	12.98
	A1 (24–51 cm)	G51	0.51	16.97	19.99	16.75	28.27	10.94	7.08
	A1A2 (51–70 cm)	G70	0.42	23.40	30.48	10.59	13.89	14.55	7.10
	A2B (70–104 cm)	G104	0.39	15.01	10.27	20.53	27.78	18.05	8.36
	Bk (104–164 cm)	G164	0.26	2.15	7.03	14.86	9.12	48.16	18.67
	BCK (164 cm and below)	G > 164	0.19	33.83	0.00	23.06	20.21	14.68	8.22

3.3. Composition and Functioning of Microbial Communities in Soil Samples

After filtering out unidentified ASVs at the phylum and organelle levels, a total of 2429 phylotypes were identified for the 16S rRNA gene and 445 for the ITS2 fragment libraries. To assess the influence of land use on the formation of microbial communities in different soil horizons, α -diversity was estimated. Figure 1 shows the abundance of bacterial and fungal ASVs, as well as the Shannon, Simpson, and Chao1 indices.



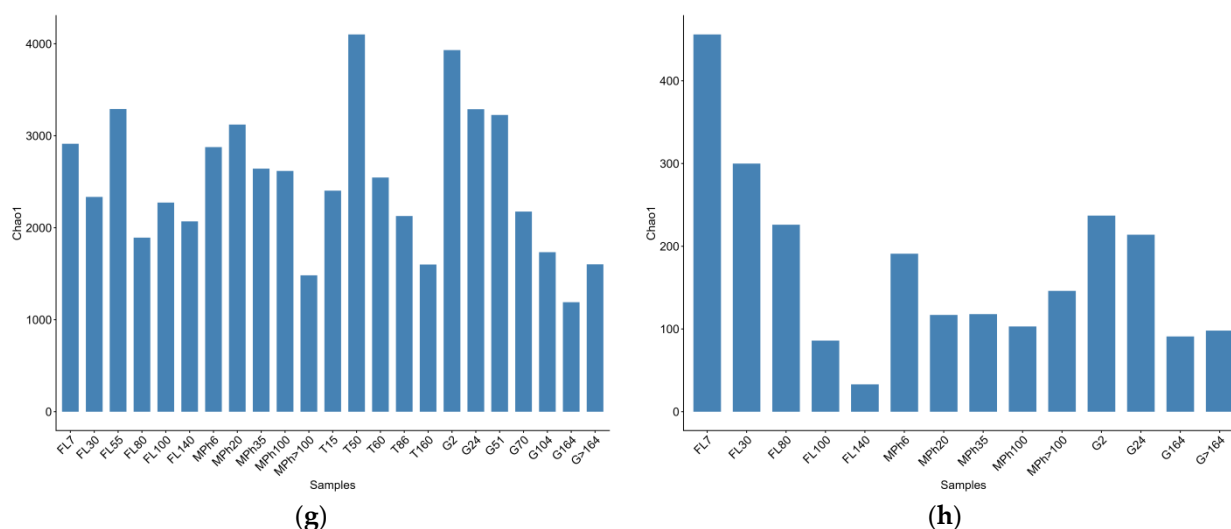


Figure 1. Change in the abundance of bacterial (a) and fungal (b) ASV, the Shannon index of bacterial (c) and fungal (d) communities, the Simpson index of bacterial (e) and fungal (f) communities, and the Chao1 index of bacterial (g) and fungal (h) communities in soil samples collected from areas where different types of land use are implemented. T—arable land (implemented for more than 5 years); G—an area where mowing with the removal of phytomass has been carried out for more than 5 years (imitation of a hay meadow); MPh—an area where mowing without the removal of phytomass has been carried out for more than 5 years; FL—fallow land (implemented for more than 5 years).

A decrease in bacterial taxon richness with soil depth was observed across all land use types, with variation in the magnitude and vertical distribution among variants. In fallow soils (FL), ASV richness decreased from 889 in the Ad horizon to 616 in the Bk horizon. Grassland soils (G) showed a decrease from 824 to 559 ASVs, while the highest richness was recorded in the upper Ad horizon (1048 ASVs). In arable soils (T), the maximum bacterial richness occurred in the A1 horizon (1058 ASVs), followed by a decrease to 550 ASVs in the deeper horizons. Grassland soils (G) maintained high richness in the upper horizons (1048–886 ASVs), with lower values in deeper layers.

Fungal ASV richness varied with depth depending on land use. In FL soils, richness decreased from 177 in the Ad horizon to 19 in the Bk horizon. In G soils, richness decreased from 237 to 98 ASVs with depth. In MPh soils, richness increased from 78 ASVs in the surface Ad horizon to 95 ASVs in the Bk horizon. Fungal data from T soils were excluded due to low sequencing quality resulting from insufficient DNA concentrations.

Bacterial alpha diversity indices (Shannon, Simpson, and Chao1) showed consistently high values across all land use types, with vertical variation among horizons. In FL soils, Shannon values ranged from 5.10 to 5.69, Simpson values were approximately 0.99, and Chao1 ranged from 1892 to 2913 in upper horizons, with lower values in deeper horizons. In MPh soils, Shannon values ranged from 5.36 to 5.62, Simpson from 0.989 to 0.992, and Chao1 from 2642 to 3122 in the upper and middle horizons, with reduced values in deeper layers. In T soils, Shannon values ranged from 5.68 to 5.76 in the upper horizons and 4.62 to 4.90 in deeper horizons; Chao1 decreased from 2403–4101 to 1600–2127 with depth. In G soils, Shannon reached 5.86 in upper horizons, Simpson was 0.994, and Chao1 exceeded 3900; in the Bk horizon, Shannon was 3.66, Simpson was 0.87, and Chao1 was 1190.

Fungal alpha diversity indices varied across land use types and soil horizons. In FL soils, Shannon values ranged from 3.34 to 3.83 in upper horizons and decreased to 0.56 in the Bk horizon; Simpson values ranged from 0.90 to 0.95 in upper horizons and decreased to 0.22 in deeper horizons; Chao1 ranged from 300 to 456 in upper horizons and de-

creased to 33 in the Bk horizon. In MPh soils, Shannon decreased from 3.42 to 1.06, Simpson from 0.85 to 0.45, and Chao1 from 200 to 103 with depth. In G soils, Shannon values ranged from 2.38 to 3.01 in upper horizons and 0.96 to 1.31 in lower horizons; Simpson ranged from 0.82 to 0.84 in upper horizons and 0.33 to 0.50 in deeper horizons; Chao1 ranged from 214–237 in upper horizons and 91–98 in lower horizons.

When comparing sections in areas with different types of land use, a clear tendency towards unification of the composition of soil bacteria and fungi is revealed as we move deeper into the soil profile for each group of sections.

The structure of bacterial communities in the studied soil profiles shows complex distribution patterns, which are determined by both the type of economic land use and the depth of the soil profile (Figure 2). The soils of the fallow area (FL) are characterized by a relatively uniform bacterial community structure without a pronounced dominance of one genus. In all soil horizons, the genera *MB-A2-108*, *KD4-96*, *Rokubacteriales*, *RB41*, and *Candidatus Udaeobacter* were characterized by high relative abundance.

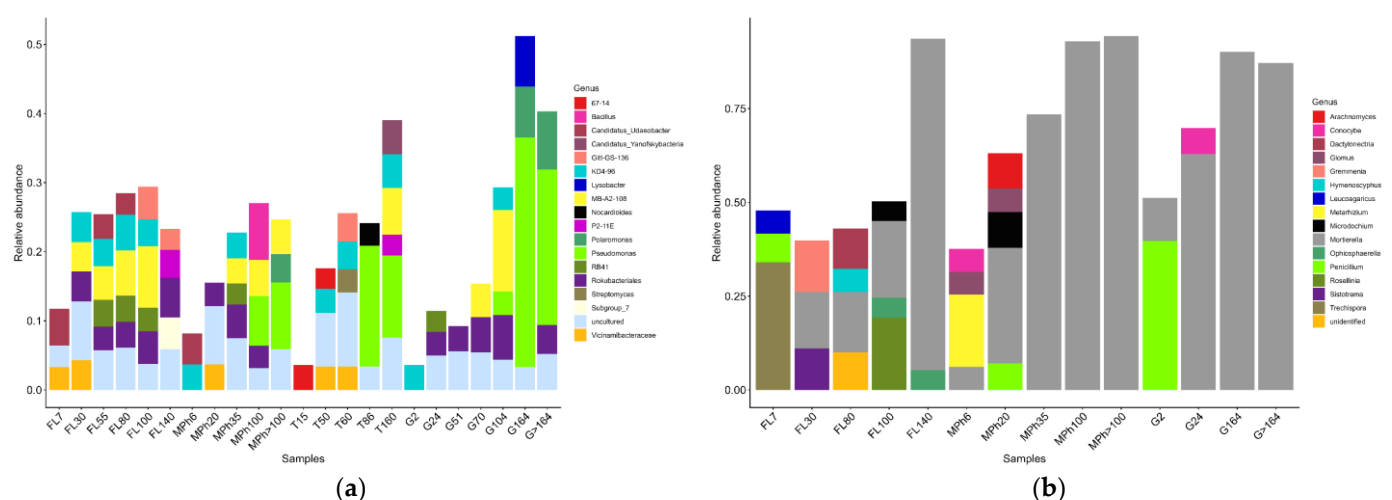


Figure 2. Change in the relative abundance of dominant (>3%) bacterial genera (a) and dominant (>5%) fungal genera (b) in soil samples collected from areas where different types of land use are implemented. T—arable land; G—an area where mowing is carried out with the removal of phytomass; MPh—an area where mowing is carried out without the removal of phytomass; FL—fallow land.

Significant representation of oligotrophic taxa (genera *RB41*, *Candidatus Udaeobacter*) indicates stable, undisturbed conditions and gradual decomposition of organic matter. The relative abundance of bacteria of the genera *Pseudomonas* and *Polaromonas* is not high, which distinguishes FL samples from other samples (MPh, T, and G). In soils under hayfield without phytomass removal, an increase in the relative abundance of bacteria of the genera *Pseudomonas* and *Polaromonas* is observed, especially in the A2B and Bk horizons. At the same time, a high relative abundance of *MB-A2-108*, *KD4-96* is maintained, which reflects the intermediate position of the ecosystem between natural and agrocenoses, combining oligotrophic and copiotrophic groups. In the soil communities of the hayfield meadow, bacteria of the genus *Pseudomonas* dominate, the proportion of which in the lower soil layers exceeds 30–40% of the total abundance. The relative abundance of the genera *Polaromonas* and *Lysobacter* also increases, indicating high microbiological activity and intensive organic matter transformation processes. Microbial communities in arable soils differ from those in other land use types; they are dominated by rapidly growing copiotrophic bacteria: the relative abundance of bacteria of the genus *Pseudomonas* increases, as do *MB-A2-108* and *KD4-96*. In the A2B and Bk horizons, the contribution of bacteria of the genus *Pseudomonas* becomes one of the determining factors, in-

dicating high trophic status, while the representation of oligotrophic groups characteristic of fallow soils decreases.

Analysis of the fungal community composition reveals a similar trend: the most pronounced taxonomic heterogeneity is observed in the fallow area, particularly in the upper soil layers. Representatives of the genera *Trechispora* and *Penicillium*, *Dactylonectria*, and *Hymenoscyphus* make significant contributions to the fungal community structure of the soils beneath the fallow land. The relative abundance of the genus *Mortierella* increases with increasing soil horizon depth; it is worth noting that this trend is characteristic of all analyzed variants. In the upper horizons (Ad and Ast pah) of soils mown without phytomass removal, fungi of the genera *Metarhizium*, *Arachnomycetes*, *Conocybe*, and *Glomus* were characterized by high relative abundance; in soil samples collected below 20 cm, fungi of the genus *Mortierella* predominate. Fungal communities in hay meadow soils are also characterized by a predominance of the genus *Mortierella* in most of the studied samples, with the exception of the upper soil horizon (Ad), which was dominated by fungi of the genus *Penicillium*. The authors interpret with great caution the insufficient amount of fungal DNA and the impossibility of obtaining data on the composition of the fungal community in soils under arable land as a poor and sparse fungal community in the soil under this type of land use.

The potential function of bacterial communities at different stages of composting of chicken manure and chicken manure with biochar was predicted using the Tax4Fun2 software package [88] the results are given in the Supplementary Materials. Microbial communities of fallow soils (FL) were found to exhibit higher activity of energy metabolism (e.g., ko00020 Citrate cycle) and amino acid pathways compared to those of arable land (T). The treatments with mown vegetation (MPh and G) showed intermediate values, with MPh samples exhibiting increased carbohydrate metabolism at shallow depths. Arable land (T) was characterized by lower levels of xenobiotic degradation but higher stress response indices, such as ABC transporters (ko02010). For most metabolic pathways, the abundance of bacteria capable of potentially implementing them decreased with depth. In shallow horizons (0–15 cm), carbon/nutrient cycling (ko01200) predominates; in deeper horizons (>50 cm), information transfer pathways (ko02020) prevail. Fallow soils (FL) exhibit the least depth variability, indicating community stability. Microbial communities in FL and G support diverse metabolism, for example, higher values for the secondary metabolite synthesis pathway (ko01110). Biosynthesis of secondary metabolites, while the microbial community of arable lands (T) switches to metabolic pathways, is associated with degradation and resistance development. According to the data obtained, the microbial community of the fallow soil best preserves key soil processes. From the point of view of soil carbon transformation, the most interesting are ko01200 (carbon metabolism), ko01230 (degradation of lignins of plant biomass), ko00680 (methane metabolism), and ko00720 (oxidation–reduction reactions that play a central role in carbon transformations). They reflect the role of microorganisms in the decomposition of organic matter [89] Glycolysis (ko00010) and the tricarboxylic acid cycle (ko00020) stimulate catabolism, and their intensity was found to be higher in disturbed soils. The FL microbial community is characterized by the accumulation of biomass with stable/high values of ko01200 (0.0259–0.0263) and ko00680 (0.0064–0.0069, increasing with depth), which minimizes SOC losses. Microbial communities MPh and G are characterized by increased biomass decomposition (ko01230), but retain the fixation potential due to high accumulation (ko01200, ko00680). On arable lands (T), SOC losses increase due to rapid biomass turnover (ko01230, ko01200). In the surface horizons (<20 cm), depending on the type of use, SOC accumulation occurs predominantly (ko01200, ko00680), while in deeper horizons (>50 cm), SOC losses prevail. Thus, in sample T60, ko01200 was 0.0263, which indicates a slight increase in SOC at low inputs; in sample FL55, ko00680

was 0.0069, which indicates SOC conservation. In fallow land and hayfields (FL, G), carbon is better retained in the deep soil layers due to the oxidation–reduction balance (ko00720). Based on metabolic pathway data, it was shown that the microbial communities of soils under fallow land and hayfield demonstrated higher SOC fixation/oxidation and less excessive degradation, promoting humus formation; the microbial community of arable land (T) is characterized by intense catabolism, which can lead to SOC losses.

Differences between land use systems reflect variations in the balance between carbon accumulation and loss processes. Grassland soils (G) and soils mown without phytomass removal (MPh) are characterized by a continuous input of plant residues and root exudates, which supports microbial metabolic pathways associated with the biosynthesis of amino acids, fatty acids, and secondary metabolites. The biosynthesis of secondary metabolites, including phenolics and terpenoids, promotes the formation of chemically complex organic matter characterized by relatively high persistence in the soil. In contrast, arable soils (T) are typically characterized by an increased relative abundance of pathways associated with oxidative phosphorylation, pyruvate metabolism, and aromatic compound degradation, reflecting the increased intensity of mineralization associated with soil disturbance. Intense microbial respiration leads to greater CO₂ release and a decrease in stable soil carbon stocks. Fallow soils (FL) exhibit intermediate functional characteristics, with a relatively balanced representation of biosynthetic and decomposition pathways. However, reduced plant productivity may limit the supply of fresh organic substrates, potentially leading to the gradual depletion of labile carbon fractions.

Differences in metabolic pathways with depth indicate a shift from rapid decomposition of plant-derived carbohydrates in the surface horizons to increased importance of carbon fixation, amino acid metabolism, and lipid metabolism in deeper layers. These processes reflect the adaptation of microbial communities to reduced substrate availability and increased reliance on the recycling of microbial residues.

For the micromycete communities using the FUNGuild bioinformatics tool (Table 5), over 86.4% of the high-throughput sequences were classified into seven functional groups based on their trophic modes: symbiotroph, saprotroph–symbiotroph, saprotroph, pathotroph–symbiotroph, pathotroph–saprotroph–symbiotroph, pathotroph–saprotroph, and pathotroph. Only high-confidence FUNGuild assignments (“likely” and “highly likely”) were included in the present analysis, so 13.4% of the high-throughput reads remained unclassified. When interpreting the results, it is worth considering that the trophic modes assigned to fungal ecological groups using FUNGuild are predictions and not direct measurements. Furthermore, the use of the ITS1F-ITS2 primer favored the dominance of the Ascomycota and Basidiomycota phyla and led to an underestimation of the relative abundance of Zygomycota, Chytridiomycota, and thermophilic fungi in the compost samples, which may partially influence the obtained results.

Fungi, through the metabolic processes they carry out, contribute to both the accumulation of SOM and its mineralization [90]. Saprotrophs decompose plant residues and SOM with the release of CO₂ [91]. Fungi belonging to the group of pathotroph–saprotroph are opportunistic degraders of necromasses [92]. Symbiotrophs (mainly mycorrhizal fungi) transfer plant-derived carbon into the soil, promoting the formation of stable organomineral complexes, stabilization of soil aggregates, and accumulation of necromass (chitin and melanin) [90]. According to the data obtained (Table 5), fungal communities of fallow soils (FL) were characterized by a high relative abundance of saprotrophs, a moderate presence of symbiotrophs, and a significant proportion of mixed pathotroph–saprotroph type. This composition of the fungal community indicates active decomposition of accumulated plant litter, moderate formation of stable carbon pools, and a relatively balanced, but decomposition-oriented, carbon cycle. A strong dominance of saprotrophs was established in the upper horizon, which is associated with the active

mineralization of fresh plant residues. Deeper layers (80–140 cm) are characterized by an increase in mixed trophic strategies, indicating the use of stabilized fractions of organic matter. The fungal community of soils mown without phytomass removal was characterized by the maximum cumulative abundance of saprotrophic fungi, an increased proportion of symbiotrophs (saprotrophs), and a relatively low abundance of pathotrophs. The periodic addition of mown phytomass to the soil leads to a continuous supply of substrate for decomposers, stimulation of fungi that process plant residues, and intensive formation of microbial carbon (microbial necromass). Saprotrophs predominate in the upper soil horizons, while symbiotrophs predominate in the deeper horizons. Despite high decomposition activity, the continuous supply of leaf litter promotes the formation of stable microbial carbon, a relatively high carbon accumulation potential, and an active yet productive carbon cycle. The fungal community of soils under a hay meadow (G) was characterized by a strong dominance of the saprotroph–symbiotroph group, with a relatively low number of symbiotrophs and a low abundance of pathotrophs. A marked increase in symbiotrophic groups was observed in deeper horizons (>100 cm), indicating carbon transfer via root-associated fungi. These soils are characterized by efficient microbial transformation of carbon from phytomass, a significant contribution of fungal biomass to stable soil organic matter, and a high potential for long-term carbon sequestration.

Table 5. Differences in the relative abundance of fungi of different trophic groups in soil samples collected from areas where different types of land use are implemented. G—an area where mowing is carried out with the removal of phytomass; MPh—an area where mowing is carried out without the removal of phytomass; FL—fallow land. The color scale shows the increase in parameter values from red (minimum values) to green (maximum values).

Trophic Mode	FL7	FL30	FL80	FL100	FL140	MPh6	MPh20	MPh35	MPh100	MPh > 100	G2	G24	G164	G > 164
Symbiotroph	0.026	0.053	0.078	0.057	0.001	0.192	0.089	0.014	0.000	0.000	0.020	0.000	0.000	0.000
Saprotroph–Symbiotroph	0.051	0.231	0.175	0.208	0.884	0.065	0.340	0.737	0.929	0.945	0.122	0.630	0.916	0.878
Saprotroph	0.508	0.133	0.091	0.086	0.051	0.148	0.129	0.076	0.022	0.025	0.115	0.099	0.015	0.014
Pathotroph–Symbiotroph	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.000
Pathotroph–Saprotroph–Symbiotroph	0.096	0.021	0.020	0.016	0.000	0.041	0.073	0.001	0.002	0.002	0.407	0.019	0.002	0.001
Pathotroph–Saprotroph	0.098	0.098	0.086	0.312	0.060	0.223	0.135	0.053	0.008	0.015	0.104	0.029	0.014	0.019
Pathotroph	0.029	0.154	0.108	0.032	0.000	0.025	0.020	0.030	0.004	0.001	0.024	0.038	0.000	0.004

Regardless of land use type, all soils in the surface horizons demonstrated a predominance of saprotrophic metabolism, active decomposition of plant litter, and rapid carbon turnover. With increasing soil horizon depth, the proportion of mixed and symbiotrophic groups increases, root exudates are utilized, and stable organic matter is formed. Deep soil horizons were characterized by a greater contribution of slow-metabolizing fungi to stabilized carbon reserves.

It was found that the relative abundance of fungal genera capable of forming arbovascular mycorrhiza (*Glomus*, *Rhizophagus*, *Claroideoglomus*, *Microdominikia*, *Paraglomus*, *Diversisporaceae*, *Septoglomus*) was maximum in MPh samples (0.7–16.5%), minimum in G samples (0.002–1.279%). This indirectly confirms the obtained results on the most effective humification processes in soils where mowing is implemented without the removal

of phytomass. The highest abundance of AMF is in the upper horizons, the genus with the maximum abundance is *Glomus*) Please note that due to a technical issue, soil samples from arable land were excluded from the analysis. The remaining three land use options were considered when interpreting the results.

The NMDS ordination of bacterial communities based on Bray–Curtis distances demonstrated that both soil physicochemical properties and land use practices contributed to the structuring of microbial communities across the studied soil profiles (Figure 3). The relatively low stress value of the ordination (Stress = 0.11) indicates a reliable representation of multidimensional community dissimilarities in two-dimensional space. The ordination pattern revealed partial separation of bacterial communities according to both soil horizon and land use type. Surface and subsurface horizons generally clustered closer together, whereas deeper horizons tended to shift along the NMDS1 axis, indicating progressive changes in community composition with soil depth. This pattern is consistent with the vertical stratification of resource availability and physicochemical conditions commonly observed in forest and agricultural soils. Among all analyzed environmental variables, only pH showed a statistically significant relationship with bacterial community structure after multiple-testing correction ($\text{envfit}()$, $p_{\text{adj}} < 0.05$). The pH vector exhibited the greatest directional fit along the positive NMDS1 axis, indicating that shifts in bacterial community composition were primarily associated with changes in soil acidity. The dominant role of pH in structuring bacterial communities agrees with numerous studies demonstrating that soil pH is one of the strongest predictors of bacterial diversity and taxonomic composition across terrestrial ecosystems, because it directly influences nutrient solubility, membrane transport processes, enzyme activity, and competitive interactions among microbial taxa.

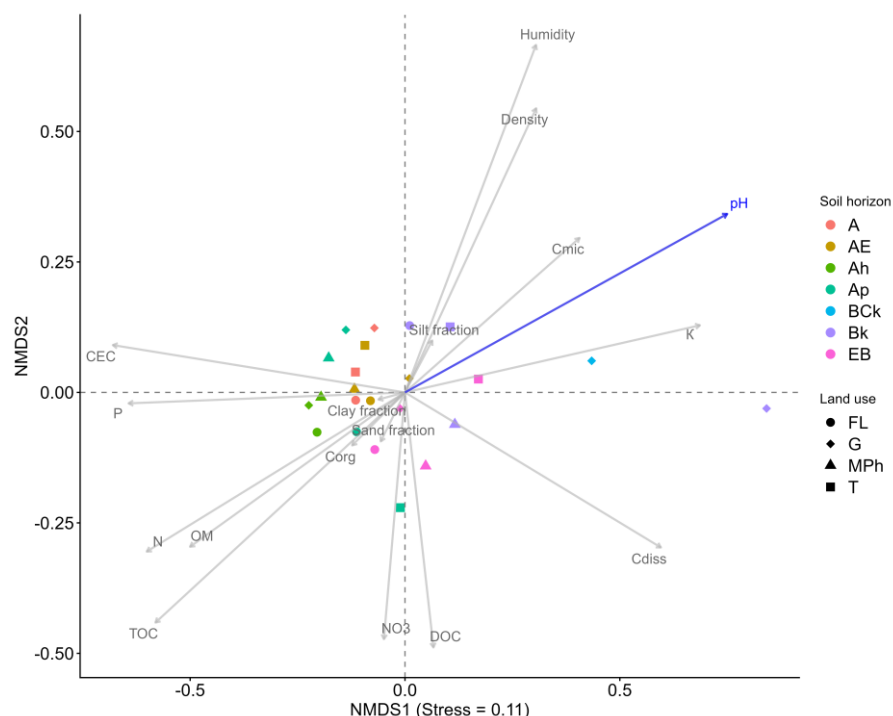


Figure 3. NMDS ordination of bacterial communities (Bray–Curtis) with fitted soil vectors. Significant factors ($\text{envfit}()$, $p_{\text{adj}} < 0.05$) are highlighted in blue (pH), non-significant in gray.

In contrast, other soil properties, including total organic carbon (TOC), organic matter (OM), total nitrogen (N), dissolved organic carbon (DOC), cation exchange capacity (CEC), soil texture fractions, and microbial biomass carbon (Cmic), did not show statistically significant independent effects on bacterial community composition. Never-

theless, their vector orientations provide insight into secondary ecological gradients influencing microbial distribution. For example, TOC, OM, and nitrogen vectors were oriented toward the negative NMDS1 region, where several upper-horizon samples from fallow and low-disturbance systems were grouped, suggesting that carbon-rich environments support distinct bacterial assemblages associated with greater SOM accumulation. Conversely, vectors related to pH, potassium, and microbial biomass carbon were directed toward the positive NMDS1 region, corresponding mainly to deeper or more disturbed soil horizons. The relatively weak contribution of most agrochemical parameters compared with pH may indicate substantial functional redundancy within the bacterial community, where different taxa can occupy similar ecological niches under varying nutrient conditions. At the same time, the clustering pattern suggests that land use intensity indirectly affects bacterial community composition through modifications of soil chemical properties and carbon availability. Fallow soils and soils subjected to reduced disturbance tended to occupy ordination regions associated with higher SOM-related variables, whereas arable soils showed displacement toward conditions associated with increased mineralization and altered nutrient dynamics. Importantly, the overlap among some land use variants indicates that bacterial community composition is not determined solely by management practice but also by vertical heterogeneity within the soil profile. Soil depth likely acts as an integrated ecological filter through changes in oxygen availability, substrate quality, moisture regime, and root-derived carbon inputs. This interpretation is supported by the gradual separation of lower horizons from upper horizons across all land use systems. Overall, the NMDS analysis suggests that pH is the principal environmental factor controlling bacterial community structure in the studied gray forest soils, whereas land use practices influence microbial assemblages indirectly through their effects on SOM dynamics, nutrient availability, and physicochemical stratification of the soil profile.

To assess the effects of land use and soil depth on physicochemical soil properties, non-parametric Kruskal–Wallis tests were applied because the data did not satisfy normality assumptions according to the Shapiro–Wilk test and the sample size was limited (Table 6). For significant effects, pairwise comparisons were further evaluated using Dunn’s post hoc test with Holm correction, while FDR correction was additionally applied to account for multiple testing.

Table 6. Results of Kruskal–Wallis and Dunn’s post hoc tests for differences in soil physicochemical properties among land use types and soil horizons.

Parameter	LandUse_p	Horizon_p	LandUse_p_adj	Horizon_p_adj
OM	0.832	0.022	0.884	0.052
TOC	0.805	0.003	0.884	0.050
Density	0.235	0.064	0.842	0.100
CEC	0.813	0.012	0.884	0.052
pH	0.276	0.041	0.842	0.084
N	0.509	0.009	0.884	0.052
Sand fraction	0.557	0.113	0.884	0.148
Silt fraction	0.297	0.210	0.842	0.223
Clay fraction	0.823	0.011	0.884	0.052
P	0.676	0.019	0.884	0.052
K	0.827	0.210	0.884	0.223
NO3	0.095	0.101	0.537	0.144
Corg	0.996	0.056	0.996	0.096
Cdiss	0.573	0.018	0.884	0.052
Cmic	0.715	0.045	0.884	0.084
DOC	0.020	0.973	0.273	0.973
Humidity	0.032	0.155	0.273	0.188

The statistical analysis demonstrated that soil horizon exerted a stronger influence on soil physicochemical properties than land use type. Before FDR correction, several parameters, including TOC, total nitrogen, CEC, clay fraction, phosphorus, pH, C_{mic}, and plant-derived carbon (C_{plant}), differed significantly among soil horizons ($p < 0.05$). However, after FDR adjustment, these effects became marginal and remained close to the significance threshold ($p_{\text{adj}} = 0.05\text{--}0.10$). In particular, TOC showed the strongest depth-related trend ($p = 0.003$; $p_{\text{adj}} = 0.050$), indicating pronounced vertical stratification of organic carbon within the soil profile. Similar tendencies were observed for OM, CEC, total nitrogen, clay fraction, phosphorus, and C_{plant} ($p_{\text{adj}} = 0.052$), suggesting coordinated changes in nutrient availability, organic matter accumulation, and mineral composition with increasing soil depth.

These results are consistent with the ecological differentiation of soil horizons, where upper layers receive greater inputs of plant residues and root exudates and therefore contain higher concentrations of organic substrates and nutrients supporting microbial activity. In contrast, deeper horizons are characterized by reduced carbon availability and altered physicochemical conditions, which likely contribute to the observed shifts in microbial community composition and metabolic functioning. The near-significant decrease in microbial biomass carbon with depth (C_{mic}, $p = 0.045$; $p_{\text{adj}} = 0.084$) further supports the interpretation that microbial abundance and activity are closely linked to vertical gradients of SOM and nutrient availability.

In comparison, land use type had a weaker direct effect on most measured soil parameters. No significant differences among land use systems were detected after FDR correction for OM, TOC, nutrient concentrations, soil texture fractions, CEC, or pH. Nevertheless, dissolved organic carbon (DOC) and soil humidity showed significant differences prior to multiple-testing correction (DOC: $p = 0.020$; humidity: $p = 0.032$), suggesting that management practices primarily influence the more labile and dynamic components of the soil environment rather than stable bulk soil characteristics. Although these effects did not remain significant after FDR correction ($p_{\text{adj}} = 0.273$), the observed trends indicate that mowing regime and tillage may modify soil moisture conditions and the availability of soluble organic substrates, which in turn can affect microbial metabolism and SOM turnover.

The relatively limited effect of land use compared with soil horizon may reflect the inherent stability of the physicochemical properties of gray forest soils over the time scale considered in this study (>5 years). At the same time, the stronger depth-related differentiation emphasizes the importance of vertical heterogeneity in regulating microbial habitats, substrate accessibility, and carbon cycling processes. This interpretation is supported by the NMDS analysis, where bacterial community structure was associated more strongly with edaphic gradients, particularly pH and SOM-related variables, than with categorical land use separation alone.

Overall, the statistical analyses suggest that soil depth represents the principal driver of physicochemical differentiation in the studied soils, whereas land use practices exert subtler effects that are primarily expressed through changes in labile carbon pools and moisture conditions rather than through major alterations of bulk soil chemistry.

To identify taxa that could be indicators of SOM accumulation, the ANCOM-BC2 method was used (Figure 4). For this analysis, all samples were divided into two groups—1) with a predominance of SOM accumulation processes; 2) with a predominance of SOM mineralization processes—as determined by NMDS and data on potential metabolic activity and trophic composition.

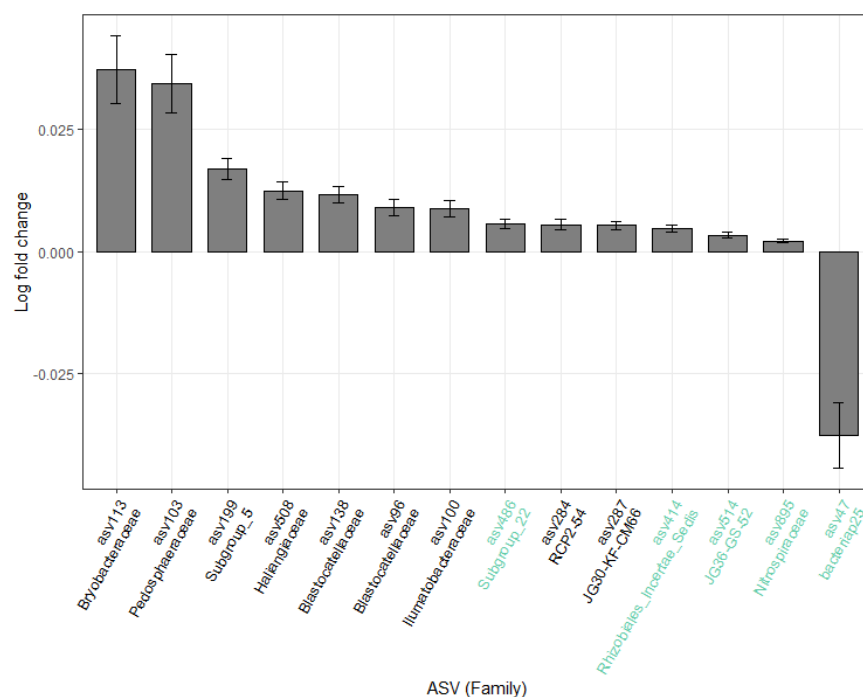


Figure 4. Identification of taxa that may be indicators of intensive humification using the AN-COM-BC2 method. Taxa that are statistically significant with an adjusted p -value (q -value) < 0.05 are highlighted in green.

In the group of samples associated with SOM accumulation processes, enrichment of representatives of the Bradyrhizobiaceae family was observed, followed by representatives of Paedospirillaceae and representatives of the Acidobacteriota_9 subgroup. These microorganisms are typically associated with the rhizosphere environment, the use of plant-derived substrates and the efficient incorporation of organic carbon into microbial biomass [93–95]. Representatives of Haliangiaceae and Blastocatellaceae also showed enrichment in soils characterized by elevated carbon content, indicating their possible involvement in the decomposition of structurally complex organic compounds and subsequent humification processes.

Moderate positive enrichment was found for Ilumatobacteraceae and Acidobacteriota Subgroup_2, microorganisms often associated with oligotrophic environments and the cycling of relatively stable carbon substrates. Minor but consistent positive responses were noted for RCP2-54, JG30-KF-CM45, and Rhizobiales. Incertae Sedis, JG36-GS-52 and Nitrospiraceae, indicating their involvement in coupled carbon and nitrogen transformations that support the gradual accumulation of soil organic matter.

In contrast, the p25 bacterium showed a marked negative log fold change, indicating a connection with soils characterized by lower organic carbon content or with conditions favorable for more intense mineralization processes.

Overall, the results suggest that soil organic matter accumulation may be associated with microbial consortia comprising taxa capable of transforming complex organic substrates, microorganisms adapted to low nutrient availability, bacteria involved in atmospheric nitrogen fixation that may support plant and microbial growth, and fungal communities dominated by saprotrophic nutritional strategies. Such microbial community structure appears to reflect conditions favoring the gradual transformation and stabilization of carbon rather than its rapid mineralization, potentially contributing to longer-term carbon retention in soil.

4. Conclusions

Land use type strongly influenced SOM accumulation and microbial community structure in gray forest soils. The highest SOM and TOC contents were observed in fallow soils and soils mown without phytomass removal, whereas arable soils showed the lowest carbon reserves and reduced microbial functional diversity. Across all land use systems, microbial diversity declined with soil depth.

The most important finding of this study is the identification of microbial indicators associated with SOM stabilization. Fallow soils were enriched in oligotrophic bacterial taxa, such as *RB41*, *Candidatus Udaeobacter*, and *KD4-96*, which were linked to stable carbon accumulation and reduced SOM mineralization. In contrast, arable and intensively managed soils were dominated by copiotrophic genera, particularly *Pseudomonas* and *Polaromonas*, reflecting accelerated carbon turnover. Functional analyses further demonstrated that less disturbed systems maintained more stable and metabolically diverse microbial communities supporting carbon retention.

These results demonstrate that microbial taxonomic composition and functional activity can serve as sensitive indicators of SOM stabilization processes in gray forest soils. The study provides a basis for the development of microbiome-based approaches for monitoring soil carbon dynamics and evaluating the sustainability of land use practices.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/soilsystems10070071/s1>. Table S1: Prediction functional profiles from metagenomic 16S rRNA data; Table S2: Fungal community datasets by ecological guild.

Author Contributions: Conceptualization, S.S. and P.K.; methodology, N.D.; software, D.T.; validation, V.B., D.T., and P.K.; formal analysis, D.T.; investigation, D.T.; resources, V.B.; data curation, D.T.; writing—original draft preparation, V.B.; writing—review and editing, P.K.; visualization, D.T.; supervision, N.D.; project administration, P.K.; funding acquisition, N.D. All authors have read and agreed to the published version of the manuscript.

Funding: This work has been supported by the grants the Russian Science Foundation, RSF 24-76-00066.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author due to landowner privacy.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

SOM	Soil organic matter
SOC	Soil organic carbon
TOC	Total organic carbon
Cmic	Microbial biomass carbon
DOC	Dissolved organic carbon
NMDS	Non-metric multidimensional scaling
OM	Organic matter
CEC	Cation exchange capacity
FDR	False discovery rate
GOST	State standard of Russia
AMF	Arbuscular mycorrhizal fungi

Appendix A

Appendix A.1

Table A1. Soil profiles laid out on sites with different types of land use.

Type of Land Use	Fallow Land	Meadow Where Biomass is Mown and Left on the Soil	Arable Land	Hay Meadow
Photographic image of soil cut				
Vegetation cover composition and projective cover area (%)	Bush grass (<i>Calamagrostis epigejos</i>)—60%; field thistle (<i>Cirsium arvense</i>)—40%	Couch grass (<i>Elytrigia repens</i>)—70%; white clover (<i>Trifolium repens</i>)—18%; common yarrow (<i>Achillea millefolium</i>)—10%; dandelion (<i>Taraxacum officinale</i>)—2%	Absent	Couch grass (<i>Elytrigia repens</i>)—70%; clover white (<i>Trifolium repens</i>)—20%; plantain medium (<i>Plantago media</i>)—10%

Appendix A.2

Table A2. Morphological description of soil sections.

Section 1 (Arable land)			Section 2 (Hay meadow)		
Horizon	Depth, cm	Morphological signs	Horizon	Depth, cm	Morphological signs
Apah	0–15	Gray, dry, light loamy, coarsely lumpy, structureless, with crop residues and silica dusting, loose, sharp, smooth transition	Ad	0–2	-
A1	15–50	Gray, dry, medium loamy, lumpy, with siliceous powder and small roots, very hard, clear transition, wavy	Ast pah	2–24	Gray, fresh, medium loamy, lumpy, structureless, with siliceous powder and small roots and isolated inclusions of gravel, very hard, the transition is clear, even
A1A2	50–60	Light gray, dry, medium loamy, finely lumpy, finely nutty, structureless, with abundant silica powder and small roots, very hard, sharp transition, wavy	A1	24–51	Gray, moist, medium loamy, loose-granular-lumpy, with siliceous powder and roots, hard, gradual transition, even
A2B	60–86	Light gray-brown, dry, light loamy, prismatic, structureless, with abundant silica powder, the transition is sharp, wavy	A1A2	51–70	Light brown-gray, fresh, medium loamy, finely lumpy, nutty, with abundant silica powder and humus streaks, hard, clear, smooth transition
Bk	86–160	Yellowish-grayish-brown, fresh, heavy loamy, finely lumpy, prismatic, very hard, effervesces below 100 cm	A2B	70–104	Grayish-brown, fresh, heavy loamy, prismatic, with humus streaks, hard, abrupt transition, smooth
			Bk	104–164	Light brown, fresh, medium loamy, finely lumpy, prismatic, effervescing in the lower part, solid, gradual smooth transition, humus streaks present, carbonate spots from 140 cm
			Bck	164 (and below)	Yellowish-light brown, fresh, light loamy, powdery, with carbonate spots
Section 3 (Fallow land)			Section 4 (Meadow where biomass is mown and left on the soil)		
Horizon	Depth, cm	Morphological signs	Horizon	Depth, cm	Morphological signs
Ad	0–7	-	Ad	0–6	-
Ast pah	7:30	Gray, dry, medium loamy, granular, with plant roots and silica dusting, hard, abrupt transition, smooth	Ast pah	6–20	Gray, dry, medium loamy, lumpy, structureless, with silica dusting, plant roots and crop residues, solid, clear transition, smooth
A1	30–55	Gray, dry, light loamy, lumpy and structureless, with plant roots, coals, the presence of cockchafer larvae, very hard, smooth, gradual transition	A1A2	20–35	Gray, fresh, light loamy, lumpy, nutty, with plant roots and silica dusting, very hard, with a sharp, even transition
A1A2	55–80	Light brown-gray, dry, light loamy, finely nutty, prismatic, with abundant silica powder and small roots, the transition is sharp, wavy	A2B	35–100	Whitish-grayish-brown, fresh, medium loamy, lumpy-powdery, with silica dusting and plant roots, very hard, smooth, gradual transition
A2B	80–100	Grayish-brown, dry, medium loamy, prismatic, lumpy, with silica powder, plant roots present, very hard, sharp transition, wavy	Bk	100 (and below)	Grayish-brown, fresh, medium loamy, prismatic, lumpy, with individual plant roots and humus streaks, effervesces below 100 cm, very hard
Bk	100–140	Yellowish-light-brown, fresh, medium loamy, prismatic, finely lumpy, very hard, with isolated plant roots, boils below 100 cm			

Appendix B

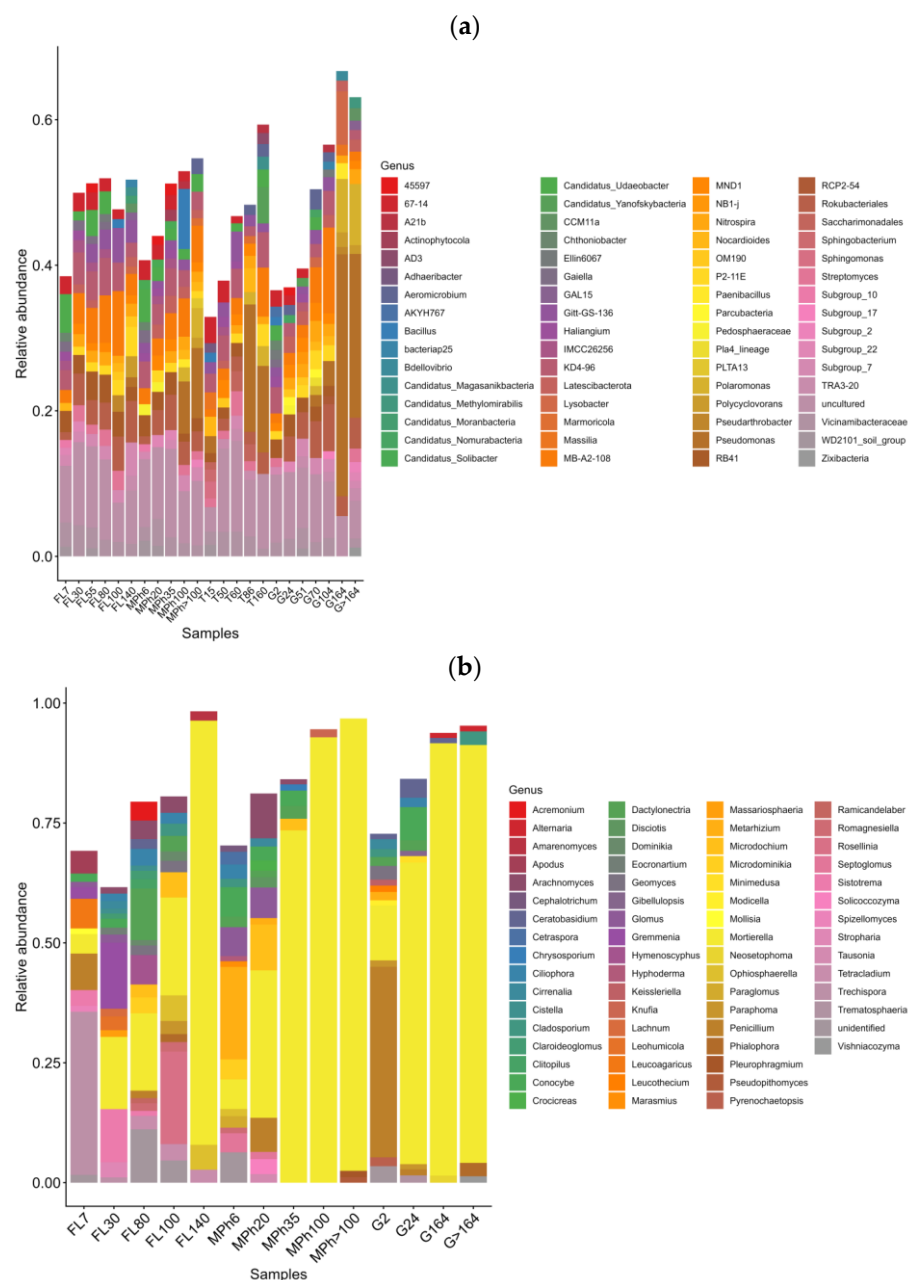


Figure A1. Change in the relative abundance of dominant (>1%) bacterial genera (a) and dominant (15%) fungal genera (b) in soil samples collected from areas where different types of land use are implemented. T—arable land; G—an area where mowing is carried out with the removal of phytomass; MPPh—an area where mowing is carried out without the removal of phytomass; FL—fallow land.

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