

Assessment of grey forest soil microbiota under long-term cultivation of energy crops

Serhii Fedorovich Razanov^{1,2*}, Oleksii Alieksieiev¹, Volodymyr Lyknochvor², Oleh Bakhmat³, Ulyana Nedilska³, Volodymyr Balkovskyi², Oksana Mazurak², Olha Hermanovych², Alla Razanova^{1,2}, Halina Ohorodnichuk¹

¹ Vinnytsia National Agrarian University, Soniachna Str. 3, Vinnytsia, 21008, Ukraine

² Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv, Lviv, 79010, Ukraine

³ Higher Educational Institution "Podillia State University", Shevchenko str., 12, Kamianets-Podilskyi, 32316, Ukraine

* Corresponding author's e-mail: razanovsergej65@gmail.com

ABSTRACT

This article presents the results of a study on the impact of long-term cultivation of energy crops (*Silphium perfoliatum* L., *Sida hermaphrodita* (L.) Rusby, *Miscanthus × giganteus*) on the microbiota of grey forest soils in the western region of Ukraine. The relevance of investigating the changes in microbial communities is substantiated by the growing implementation of energy crops as alternative sources of bioenergy and as tools for the ecological stabilization of agro-landscapes. The analysis included the abundance of major physiological groups of microorganisms: ammonifiers, amylolytics, oligonitrotrophs, oligotrophs, pedotrophs, humate-transforming, and cellulolytic organisms. It was established that after more than twenty years of energy crop cultivation, the abundance of most microbial groups increased by 1.3–2.5 times compared to the soils under traditional crop rotation. The most pronounced positive effect was observed under *Sida hermaphrodita*, which contributed to the highest increase in soil biogenicity. At the same time, the soils under *Miscanthus × giganteus* and *Silphium perfoliatum* showed moderate enrichment of microbial complexes along with a reduction in the activity of cellulolytic organisms. The study concluded that long-term cultivation of energy crops does not lead to degradation of the microbiological condition of grey forest soils, but rather enhances their biogenic activity and can be considered a promising strategy for the environmentally sound use of marginal lands.

Keywords: grey forest soil, soil microbiota, energy crops, *Miscanthus × giganteus*, *Silphium perfoliatum*, *Sida hermaphrodita*.

INTRODUCTION

The rational use of soil resources has become increasingly important in the context of global environmental challenges. One of the key factors in preserving soil fertility is the maintenance of an optimal microbiological balance, which determines the pathways of transformation of organic as well as mineral compounds and ensures the stability of agroecosystems. With the rapid expansion of energy crops, such as *Miscanthus × giganteus*, *Silphium perfoliatum*, and *Sida hermaphrodita*, which are capable of providing renewable bioenergy and reducing

the anthropogenic pressure on the environment, particular attention should be given to studying their influence on soil microbiota, especially under long-term cultivation (Kaletnik et al., 2025; Kharytonov et al., 2022). Previous studies have demonstrated that prolonged cultivation of energy crops may either improve soil biogenicity and the structural-functional characteristics of microbial communities, or lead to their depletion, depending on the crop species, soil and climatic conditions, as well as the applied management practices (Razanov et al., 2025). Therefore, the assessment of changes in the microbial complex of grey forest soils under long-term use for the

cultivation of energy crops is both relevant and essential, with significant ecological and practical implications for maintaining soil fertility.

Soil bacteria, fungi, archaea, and actinobacteria play a key role in shaping soil fertility, the decomposition of organic matter, as well as the cycling of essential nutrients such as nitrogen, phosphorus, carbon, and others (Fierer et al., 2007). They contribute to the formation of humus, improve soil structure, enhance water retention, and increase the availability of nutrients (Choi et al., 2021).

Soil microorganisms are a central component of agroecosystem functioning, as they drive the transformation of organic and mineral compounds, participate in nitrogen, carbon, and phosphorus cycling, contribute to humus formation, as well as sustain soil fertility (Singh et al., 2011; Hartmann et al., 2015; Fierer, 2017). For the assessment of soil biogenicity, particular attention is given to specific physiological groups of microorganisms, which differ in their trophic characteristics and functional roles.

In particular, heterotrophic ammonifiers are involved in the ammonification process, converting organic nitrogen into ammonium forms available to plants (Wang et al., 2018). Additionally, within anaerobic microsites of the soil, dissimilatory nitrate and nitrite reduction to ammonium (DNRA) takes place. The key enzyme of this pathway is cytochrome c nitrite reductase, encoded by the *nrfA* gene. Metagenomic studies indicate that the microorganisms carrying the *nrfA* gene are widespread in soils of various types, including arable lands. They contribute to nitrogen retention in agroecosystems by transforming nitrate and nitrite into NH_4^+ , which is less prone to leaching and more readily available for plant uptake (Heo et al., 2020; Saghäi and Hallin, 2024; Hird et al., 2025). Amylolytic microorganisms (various representatives of the genera *Bacillus*, *Aspergillus*, *Penicillium*) degrade starch into simpler compounds, thereby providing a source of easily available carbon that sustains microbial consortium activity and contributes to improved soil fertility. Such organisms are found both in the rhizosphere and in natural biotopes (Ekka and Namdeo, 2018).

Oligotrophs in soils constitute the «background» microbial community that underpins ecosystem stability, particularly on low-fertility substrates and within deeper soil horizons. Comparative genomic studies demonstrate the reduction of energy-intensive

metabolic pathways and the presence of multiple adaptive mechanisms to resource limitations in these microorganisms (Dragone et al., 2024).

Pedotrophs and microorganisms involved in the transformation of humate compounds participate in the decomposition of lignin and humic acids, thereby contributing to the formation of stable humic substances. As a result, soil structure, water-holding capacity, and organic carbon stability are improved. These processes are driven by enzymatic complexes, including laccases and peroxidases (Demyanyuk et al., 2018).

Cellulolytic microorganisms (*Trichoderma*, *Penicillium*, *Aspergillus*, *Cellvibrio*) are responsible for the breakdown of cellulose, the main component of plant residues. Their activity determines the rate of organic matter mineralization and the replenishment of the pool of available carbon, which directly influences nutrient cycling and soil fertility (Zhang et al., 2023; Ma et al., 2024).

Thus, the studied groups of microorganisms (ammonifiers, amylolytic, oligonitrotrophs, oligotrophs, pedotrophs, humate-transforming, and cellulolytic) perform key functions in soil ecosystems: they ensure the mineralization of organic compounds and nitrogen availability, the decomposition of carbohydrates and maintenance of the carbon balance, humification, as well as soil resilience to stress factors. The activity of these physiological groups determines the course of biogeochemical cycles and correlates with crop productivity; therefore, they are considered indicators for assessing the biogenic activity of soils (Bardgett and van der Putten, 2014; Adomako et al., 2022).

Scientific evidence indicates that soil microbiota is highly responsive to long-term cultivation of agricultural crops, primarily through changes in the abundance of ammonifiers, pedotrophs, humate-transforming, and cellulolytic microorganisms. These shifts determine both the rate of mineralization processes and the degree of organic matter preservation (Hartman et al., 2018; Suman et al., 2022; Ahammed et al., 2025). At the same time, for several crops that are not traditional for Ukraine, including *Miscanthus × giganteus*, *Sida hermaphrodita*, and *Silphium perfoliatum*, the volume of research remains limited, highlighting the need for in-depth studies of their effects on soil biota.

It is important to note that the significance of perennial energy crops extends beyond soil and

ecological aspects alone (Okhota et al., 2024). Their cultivation represents a crucial component of sustainable development strategies in the national economy (Pryshliak et al., 2022). These crops provide renewable sources of bioenergy, thereby reducing the dependence on fossil fuels (Lohosha et al., 2025). Moreover, they are characterized by high biomass productivity even on marginal lands (unsuitable for food crop production), while simultaneously enhancing the ecological stability of agro-landscapes (Scarlat et al., 2015; Ramirez et al., 2024; Kharytonov et al., 2025).

In this context, increasing attention has been directed toward the effects of perennial energy crops on the physico-chemical and biological properties of soils. For *Miscanthus × giganteus*, it has been demonstrated that long-term cultivation promotes the accumulation of organic carbon and reduces the intensity of mineralization processes (Fu et al., 2022; Vodiak et al., 2022). It has also been revealed that the genetic characteristics of *Miscanthus* species play a decisive role in shaping and functioning of root and rhizosphere microbiomes. Niuniu Ji and co-authors reported that plant genotype has a stronger influence on the composition of major prokaryotic communities, as well as on the stability of their interactions in the root zone than environmental conditions. These findings suggest that when cultivating energy crops, it is essential to consider genetic diversity to optimize the utilization of beneficial microorganisms (Ji et al., 2023). For *Silphium perfoliatum*, its perennial root system and annual leaf litter contribute to soil enrichment with organic matter, improvement of water permeability, and favorable conditions for soil fauna. Field studies have recorded a higher mean aggregate mass and an increased carbon content under this crop (Peni et al., 2020; Grunwald et al., 2021). The extensive root system and considerable annual input of plant residues into the soil under *Sida hermaphrodita* create a stable rhizosphere environment that promotes organic carbon accumulation, improves soil structure and water regime, as well as exerts a generally positive influence on soil quality (Kitczak et al., 2022). A review of scientific publications indicates that, although the interaction between energy crops and soil microbiota has been actively studied, there is still a significant lack of experimental data for the grey forest soils in the western region of Ukraine. This gap highlights the necessity of conducting targeted research in this area.

The aim of this study was to determine the specific features of microbiota formation in grey forest soils under long-term cultivation of energy crops (*Silphium perfoliatum*, *Sida hermaphrodita* and *Miscanthus × giganteus*), to assess changes in the abundance of key physiological groups of microorganisms, as well as to evaluate their contribution to the biogenicity and stability of soil ecosystems.

MATERIALS AND METHODS

The study was conducted at permanent experimental plots in the western region of Ukraine (geographic coordinates: 49°54'14" N, 24°05'10" E; 258 m a.s.l.). The climate is moderately continental, with a mean annual air temperature of approximately 8.5–8.8 °C. The mean annual precipitation ranges between 700 and 850 mm, with a maximum in summer (June–July) and a minimum during winter. The soils at the experimental sites are classified as grey forest soils, light loam texture, and low in humus content.

The objects of investigation were the microbial communities of grey forest soils under long-term cultivation (>21 years) of energy crops: *Miscanthus × giganteus*, *Silphium perfoliatum* L., *Sida hermaphrodita* (L.) Rusby, and arable land under a crop rotation (winter wheat, spring barley, pea, soybean). Four experimental variants were studied: (1) crop rotation soils, (2) soils under *Silphium perfoliatum*, (3) soils under *Sida hermaphrodita*, and (4) soils under *Miscanthus × giganteus*. Throughout the 21-year cultivation period of energy crops (*Silphium perfoliatum*, *Sida hermaphrodita*, *Miscanthus × giganteus*), no mineral fertilizers were applied. In contrast, the control crop rotation was managed under conventional technology, including the application of NPK fertilizers in accordance with regional recommendations.

Soil samples were collected from the 0–20 cm layer in three replicates according to the national standard DSTU 4287:2004 “Soil quality. Sampling”(2005). Each composite sample consisted of at least five subsamples per plot. Laboratory analyses were conducted at the Institute of Applied Biotechnology (Kyiv, Ukraine). The abundance of the main physiological groups of microorganisms (ammonifiers, amylolytics, oligonitrotrophs, oligotrophs, pedotrophs, humate-transforming, and cellulolytic microorganisms) was determined

by plating soil suspensions on selective agar media in accordance with DSTU 7847:2015 “Determination of microbial abundance in soil by plating on solid media” (2016). Soil suspensions were prepared in serial dilutions and incubated at 28 ± 1 °C for 5–7 days. Results were recorded as the number of colony-forming units (CFU) per 1 g of oven-dry soil. Sterile instruments and media were used to avoid contamination.

The experimental data were processed using methods of variation statistics in the Statistica software package. The differences between treatments were evaluated by analysis of variance (ANOVA), with significance levels tested using Tukey's HSD and Fisher's LSD tests at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of the study, conducted under the conditions of the Western region of Ukraine, demonstrated that long-term cultivation of energy crops (more than 20 years) has a significant impact on the microbiological status of grey forest soil. Notable changes were observed in the abundance of the main physiological groups of microorganisms – ammonifiers, amylolytics, oligonitrophiles, oligotrophs, pedotrophs, humate-transforming, and cellulose-degrading organisms (Table 1).

The analysis of the obtained results revealed substantial differences in the effects of various energy crops on specific groups of microorganisms (Figure 1). The abundance of ammonifiers in the soils under *Silphium perfoliatum* was 1.5 times higher, under *Sida hermaphrodita* – 2.5 times higher, and under *Miscanthus × giganteus* – 1.6 times higher compared to the soils of the conventional crop rotation. The abundance of amylolytic microorganisms increased by 1.3, 2.1, and 1.3 times, while oligonitrophs increased

by 1.4, 2.2, and 1.1 times, respectively. A comparable pattern was observed for oligotrophs, pedotrophs, and humate-transforming organisms. Under *Silphium perfoliatum*, their numbers increased 1.7, 1.7, and 2.7 times; under *Sida hermaphrodita* – 2.0, 1.7, and 5.1 times; and under *Miscanthus × giganteus* – 1.7, 1.7, and 1.8 times, respectively. In contrast, the impact on cellulolytic microorganisms diverged from this general trend. Their abundance in the soils under *Silphium perfoliatum* and *Miscanthus × giganteus* was lower by 1.05 and 1.6 times, respectively, whereas under *Sida hermaphrodita* it was higher by 1.09 times, compared to the control.

Overall, the highest levels of all studied groups of microorganisms were observed under *Sida hermaphrodita*. The abundance of ammonifiers exceeded the values in the soils under crop rotation by 2.5 times, under *Silphium perfoliatum* by 1.7 times, and under *Miscanthus × giganteus* by 1.5 times; amylolytics – by 2.1, 1.6, and 1.6 times; and oligotrophs – by 2.0, 1.2, and 1.2 times, respectively.

The number of pedotrophs under *Sida hermaphrodita* was 1.7 times higher compared to the soils of crop rotation and 1.03 times higher than under *Miscanthus × giganteus*, whereas under *Silphium perfoliatum* no significant difference was recorded (24.8 ± 3.1 vs. 24.7 ± 3.0 million CFU/g of soil).

The most pronounced differences were observed for humate-transforming microorganisms: their abundance under *Sida hermaphrodita* exceeded the control by 5.1 times, under *Silphium perfoliatum* by 1.9 times, and under *Miscanthus × giganteus* by 2.8 times. The summarized data revealed a clear trend toward increasing soil biogenicity under energy crops (Figure 2). Specifically, it was 1.42 times higher under *Miscanthus × giganteus*, 1.45 times higher under *Silphium perfoliatum*, and 2.0 times higher

Table 1. Content of microorganisms in grey forest soil, million CFU/g of soil (n=3, M ± m)

Physiological group of microorganisms	Crop rotation (control)	<i>Silphium perfoliatum</i>	<i>Sida hermaphrodita</i>	<i>Miscanthus × giganteus</i>
Ammonifiers	8.5 ± 1.0	12.7 ± 1.5	21.2 ± 2.7	13.9 ± 1.8
Amylolytics	14.0 ± 1.7	18.7 ± 2.3	29.8 ± 3.7	17.9 ± 2.2
Oligonitrotrophs	12.8 ± 1.5	18.1 ± 2.2	28.9 ± 3.6	14.4 ± 1.8
Oligotrophs	20.2 ± 2.5	35.9 ± 4.4	41.9 ± 5.2	34.0 ± 4.1
Pedotrophs	14.1 ± 1.7	24.7 ± 3.0	24.8 ± 3.1	23.9 ± 3.0
Humate-transforming	1.9 ± 0.3	5.1 ± 0.7	9.8 ± 1.2	3.5 ± 0.4
Cellulolytic	91.5 ± 11.2	87.0 ± 10.7	99.8 ± 12.2	58.2 ± 7.1

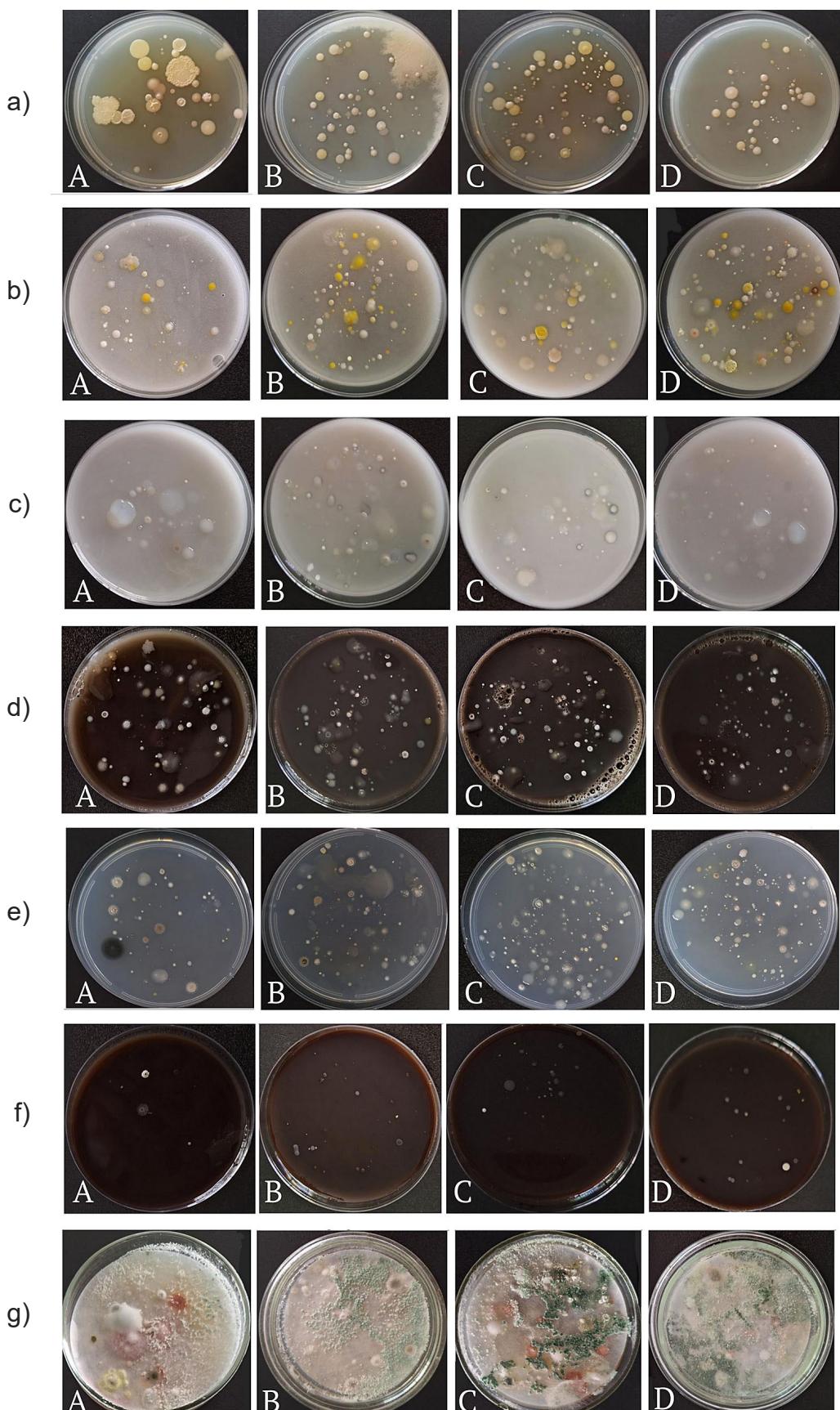


Figure 1. Morphological characteristics of colonies of main physiological groups of microorganisms in grey forest soil: (a) ammonifiers; (b) amylolytics; (c) oligonitrotrophs; (d) oligotrophs; (e) pedotrophs; (f) humate-transforming; (g) cellulolytic (A – soil from field crop rotation; B – soil from *Silphium perfoliatum* cultivation; C – soil from *Sida hermaphrodita* cultivation; D – soil from *Miscanthus × giganteus* cultivation)

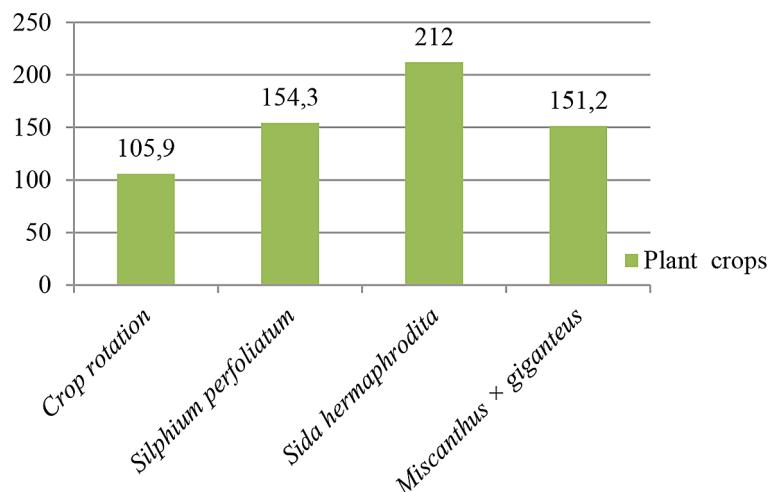


Figure 2. Soil biogenicity of grey forest soil under different plant species

under *Sida hermaphrodita*, compared to the control. The highest soil biogenicity was recorded under *Sida hermaphrodita*.

The evaluation of microbiological indicators showed that the soils under different crops differed in the level of microbiota enrichment. Field crop rotation soil was characterized by rich content of ammonifiers, amylolytics, oligonitrotrophs, oligotrophs, and pedotrophs; very poor content of humate-transforming microorganisms; predominance of mineral nitrogen compound transformation processes ($K_{M\text{-}I} = 1.64$); very low intensity of organic matter transformation ($K_{OMT} = 13.7$). The soil under *Silphium perfoliatum* was distinguished by rich content of ammonifiers and oligotrophs; abundant content of amylolytics, oligonitrotrophs, and pedotrophs; poor content of humate-transforming microorganisms and low intensity of organic matter transformation ($K_{OMT} = 21.4$). The soil under *Sida hermaphrodita* was characterized by very rich content of ammonifiers, amylolytics, oligonitrotrophs, and oligotrophs; rich content of pedotrophs; moderate content of humate-transforming microorganisms; the highest intensity of organic matter transformation ($K_{OMT} = 36.3$). The soil under *Miscanthus × giganteus* was distinguished by very rich content of ammonifiers and oligotrophs; rich content of amylolytics, oligonitrotrophs, and pedotrophs; very poor content of humate-transforming microorganisms; low intensity of organic matter transformation ($K_{OMT} = 24.7$). The obtained results indicate a positive effect of energy crops on the microbiological activity of Grey Forest soil. The established hierarchy of effectiveness (*Sida hermaphrodita* > *Silphium*

perfoliatum > *Miscanthus × giganteus*) may be attributed to differences in root exudates, the composition of plant residues, and the intensity of biological nutrient cycling under different crops. Of particular importance is the increase in the abundance of humus-transforming microorganisms, which reflects the activation of humification processes and the improvement of the structural-aggregate state of soil. The highest values recorded under *Sida hermaphrodita* (a 5.1-fold increase) point to especially favorable conditions for soil formation processes.

The revealed changes in soil microbiological activity are of considerable agroecological significance, as they indicate enhanced biological fertility and greater stability of agroecosystems under the cultivation of energy crops.

DISCUSSION

The obtained results confirm the substantial impact of long-term energy crop cultivation on the formation of the microbial complex of grey forest soil. An increase in the abundance of major physiological groups of microorganisms (ammonifiers, amylolytic microorganisms, oligonitrotrophs, pedotrophs, and oligotrophs) was recorded, indicating intensified mineralization and transformation processes in the soil, as well as the activation of biological nutrient cycling.

In the soils under *Silphium perfoliatum*, *Sida hermaphrodita*, and *Miscanthus × giganteus*, the abundance of the main groups of microorganisms was higher than in the soils under crop rotation. This confirms the capacity of energy crops to

stimulate soil biological activity and sustain soil fertility. Similar findings have been reported by other researchers, who noted the positive effects of perennial crops on microbial biomass and soil enzymatic activity (Gintaras et al., 2015; Ruf & Emmerling, 2020; Pantigoso et al., 2022).

Particularly noteworthy is *Sida hermaphrodita*, under which the highest increases were recorded for ammonifiers (2.5-fold), amylolytic microorganisms (2.1-fold), pedotrophs (1.7-fold), and humate-transforming microorganisms (5.1-fold). Such a pronounced effect may be associated with the specific properties of root exudates of *Sida hermaphrodita*, which create a favorable environment for microbial development, as well as with the high content of readily available organic compounds in its plant residues.

The increase in the abundance of humate-transforming microorganisms, particularly under *Sida hermaphrodita*, is of special significance for soil formation processes, as these microorganisms are responsible for the synthesis of humic substances and the development of soil aggregate structure. This is consistent with the findings of Taranenko et al. (2025), who reported enhanced soil biogenicity under non-traditional energy crops in Ukraine.

The differences in the effects on cellulolytic microorganisms deserve particular attention. Their abundance under *Sida hermaphrodita* exceeded the control, whereas under *Silphium perfoliatum* and *Miscanthus × giganteus* it was lower. This indicates a qualitative difference in plant residues: *Sida hermaphrodita* apparently produces less lignified cellulose components that are more readily subject to microbial decomposition. Such distinctions confirm that different energy crops exert differential effects on microbial communities depending on the biochemical composition of plant residues and root exudates (Berg et al., 2020; Peni et al., 2020).

The established hierarchy of effectiveness (*Sida hermaphrodita* > *Silphium perfoliatum* > *Miscanthus × giganteus*) may also be associated with differences in root exudate intensity and rooting depth. *Sida hermaphrodita*, with its powerful taproot system, is capable of creating more intensive rhizosphere interactions and ensuring a continuous supply of organic exudates to the soil.

The obtained results have important practical implications for the development of sustainable land-use systems. Energy crops can serve not only

as a source of renewable bioenergy, but also as an effective tool for the biological reclamation of degraded and marginal lands (Honcharuk et al., 2024; Razanov et al., 2024). The particularly strong positive effect of *Sida hermaphrodita* highlights its potential to combine high energy productivity with ecological functions of soil restoration.

CONCLUSIONS

Long-term cultivation of energy crops (*Silphium perfoliatum* L., *Sida hermaphrodita* (L.) Rusby, *Miscanthus × giganteus*) for more than 20 years leads to significant positive changes in the microbiological state of grey forest soil compared to conventional crop rotation. A reliable increase was established in the abundance of key functional groups of microorganisms: ammonifiers (1.5–2.5 times), amylolytic microorganisms (1.3–2.1 times), oligonitrotrophs (1.1–2.2 times), oligotrophs (1.7–2.0 times), pedotrophs (1.7 times), and humate-transforming microorganisms (1.8–5.1 times).

The highest microbiological activity was recorded in the soils under *Sida hermaphrodita*, where the abundance of ammonifiers exceeded the control by 2.5 times, amylolytic microorganisms by 2.1 times, and humate-transforming microorganisms by 5.1 times, indicating particularly intensive processes of biological transformation of organic matter.

An efficiency hierarchy of energy crops in their influence on soil microbiological activity was established: *Sida hermaphrodita* > *Silphium perfoliatum* > *Miscanthus × giganteus*, which correlates with soil biogenicity levels and the intensity of biogeochemical processes. The impact on cellulolytic microorganisms was crop-specific: their abundance under *Sida hermaphrodita* exceeded the control by 9%, whereas under *Silphium perfoliatum* and *Miscanthus × giganteus* it was lower by 5% and 37%, respectively, reflecting differences in the biochemical composition of plant residues.

The overall biogenicity of the grey forest soil under energy crops exceeded that of crop rotation: 1.42 times higher under *Miscanthus × giganteus*, 1.45 times higher under *Silphium perfoliatum*, and 2.0 times higher under *Sida hermaphrodita*. Long-term cultivation of energy crops, particularly *Sida hermaphrodita*, enhances the biological fertility of grey forest soil, promotes

the formation of stable microbial communities, and can be considered an environmentally sound strategy for the agricultural use of marginal and low-productive lands while simultaneously producing renewable biomass. The results of this study justify the introduction of energy crops into sustainable farming systems as an effective tool for biological reclamation of soils and the preservation of their ecosystem functions.

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