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Assessment of the soil microbial community under energy crops (*Panicum virgatum L.* and *Miscanthus giganteus*): a case study from Ukraine

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Abstract

Bioenergy crops are a promising alternative for energy production. They can be grown on inaccessible, degraded, marginal land that is not economically viable for traditional agriculture. At the same time, a broader evaluation of the environmental and ecological impact of energy crop cultivation on land is needed. There are still knowledge gaps regarding the mechanisms underlying soil carbon accumulation, especially concerning the involvement of the soil microbiome in facilitating these processes during energy crop cultivation. The present study determined the main ecotrophic and taxonomic groups of soil microbial communities and the direction of soil microbiological processes under *Panicum virgatum L.* and *Miscanthus giganteus* growth. Research results showed an initial impact of energy crop growth on soil microbial communities in the soil-climatic conditions of Ukrainian forest-steppe zones. Soils under energy crops are characterized by the highest abundances of the investigated ecological and trophic microbial groups (by 1.2–3 times) compared to control (soil under grassland). The coefficients of microbiological processes determine the high potential for increasing soil fertility under energy crop cultivation. Statistical analysis of the results confirmed a medium and strong correlation between soil microbiological parameters and soil organic carbon content during energy crop growth. Therefore, energy crops can act as ecosystem engineers, improving soil biological and chemical properties and supporting soil ecosystem sustainability.

1. Introduction

In recent years, achieving energy autonomy from non-renewable sources has become a paramount human endeavour. As a result, nations are actively pursuing strategies to increase the use of biomass for energy production within existing frameworks. Bioenergy crops are a promising substitute for fossil fuels in energy production endeavours. Increasing the cultivation of bioenergy crops on agricultural land may pose sustainability challenges due to competition with food production. However, energy crops can be grown on inaccessible, degraded, contaminated and marginal land that is not economically viable for conventional agriculture (Brami et al., 2020). Nevertheless, a comprehensive assessment of the environmental and ecological impacts of growing energy crops on land is essential to improve the sustainability quotient of the sector.

The cultivation of *Miscanthus giganteus* (*M. giganteus*) as a bioenergy crop is attractive due to the plants' vigorous growth of the plants and their ability to produce high yields under various environmental conditions. The cultivation of *M. giganteus* represents a favourable avenue for land rejuvenation and climate change mitigation due to its ability to increase soil organic matter (Kane et al., 2023). The cultivation of *M. giganteus* offers a plethora of environmental benefits due to its minimal requirements for fertiliser and weed control, extended growth periods of 10 to 25 years, and the adoption of no-till farming practices. In addition, *M. giganteus* shows promise in providing ecosystem services, including the potential to increase soil organic carbon reserves and reduce metal bioavailability by accumulating in the rhizosphere at contaminated sites. Consequently, *M. giganteus* cultivation is suitable for revitalising degraded marginal lands (Brami et al., 2020). However, there are gaps in our knowledge of the mechanisms that facilitate soil carbon accumulation under *M. giganteus* cultivation, in particular the role of the soil microbiome in facilitating these processes.

Switchgrass (*Panicum virgatum L.*) is emerging as another promising candidate for biofuel production. This perennial grass has high photosynthetic efficiency and significant potential for biomass production (Taranenko et al., 2019; Taranenko et al., 2021). Widespread integration of this perennial biofuel crop holds promise for redirecting land use towards more sustainable, biomass-centric energy systems, with consequent impacts on soil ecosystems. Switchgrass can release up to 20% of its fixed carbon into the rhizosphere through exudation. Despite the growing agronomic understanding of switchgrass, its influence on the soil microbial community remains a relatively underexplored topic (He et al., 2017).

The cultivation of bioenergy significantly impacts on the composition of soil microbial communities and their associated organisms, thus shaping the complex soil food web in which they live. This influence has a direct impact on the dynamics of nutrient cycling and overall crop productivity. As ecosystem engineers, plants play a crucial role in shaping their immediate environment. In addition to the inputs inherent in the cultivation of bioenergy crops, a number of site-specific variables, including soil texture and climatic conditions, further modulate the composition of soil bacterial and fungal communities (Leichty et al., 2021).

Microbial abundance is a fundamental determinant of soil quality and crop productivity within the soil ecosystem. Declines in the abundance of microbial communities can lead to declines in essential soil functions critical for maintaining soil health and agricultural yields. The population dynamics of soil fungi and bacteria are subject to temporal variation influenced by crop management practices. In particular, persistent cropping practices tend to reduce the abundance of essential bacterial taxa that central to providing essential soil ecosystem services such as nitrogen fixation and disease suppression. Similarly, long-term cropping can reduce the abundance of key soil fungal taxa that act as biological control agents against soil-borne pathogens (Zahida et al., 2020). In addition, long-term cropping has been associated with a reduction in phosphorus (P) and carbon (C) content of microbial biomass (Zhou et al., 2014; Katsalirou et al., 2016). Studies have also documented relatively lower soil microbial biomass under continuous cropping systems due to fluctuations in resource availability or crop residues within monocultures (Brumme et al., 2009; McDaniel et al., 2014). Consequently, declines in microbial biomass may foreshadow reduced inputs of organic matter and crop residues to the soil, highlighting the need to increase crop diversity to support soil organic matter and microbial biomass levels. Unfortunately, there are gaps in research on microbiological parameters of soils under energy crop production.

Soil microorganisms comprise different physiological groups involved in different biological processes such as ammonification, nitrogen fixation, nitrate reduction and the breakdown of cellulose and other essential plant components. The microbiota controls many soil processes, ranging from the mobilisation to the accumulation of chemical elements. However, it's necessary to recognize that micro biocenosis - an indicator of abundance and taxonomic structure - is highly variable, showing spatial and temporal variations depending on plant species, climatic conditions, soil composition, and agricultural practices. Microorganisms are an important and driving force in the restoration of soil ecosystems (Pysarenko et.al., 2022a), including disturbed soils that have undergone human influence and have a negative impact on the environment (Pysarenko et.al., 2022b). Microbiomes are highly sensitive to environmental cues due to their large surface area in contact with the environment. At the same time, their rapid reproductive rate allows them to adapt rapidly to environmental changes induced by various factors. Despite progress, there are still gaps in our knowledge of the mechanisms governing soil carbon sequestration, particularly the contribution of the soil microbiome in facilitating these processes in the context of energy crop cultivation. Therefore, studies that elucidate soil microbiology are crucial for understanding the dynamics of geochemical, biochemical and biophysical interactions at the Earth's surface, especially in the context of global climate change. The study of soil microbiocenosis under energy crop cultivation can become an indicator of soil quality and a tool for determining the direction of microbiological processes, enabling the formation and accumulation processes of soil matter.

The study aimed to analyze the main ecotrophic and taxonomic groups of soil microbial communities and to determine the direction of soil microbiological processes under switchgrass (*Panicum virgatum L.*) and *M. giganteus* cultivation.

2. Material and methods

The study of soil chemical and microbiological indicators was conducted at the experimental field of the Poltava State Agrarian University (forest-steppe zone of Ukraine). Energy crops have been grown in the experimental field for seven years. Determination of studied parameters was conducted in three variants: 1) soil under switchgrass (*Panicum virgatum L.*) growth, 2) soil under *M. giganteus* growth, 3) soil under grassland (control). Twelve top-soil samples (0-30) cm were collected using diagonal schemes during 3rd decade of May 2023. A pedological probe took the soil samples by ISO 11464:2006 (soil samples for determining chemical parameters) and ISO 18400-206:2018 (soil samples for determining biological parameters). In the laboratory, soil samples were cleaned of plant remains, stone roots and insect remains, air dried (for determining chemical parameters according to ISO 11464:2006), ground and sieved through a 2 mm mesh sieve. The experiment included the determination of the following microbiological parameters: the number of microorganisms of the main ecotrophic and taxonomic groups (Ammonifying bacteria, Streptomyces, Amylolytic microorganisms, Pedotrophic microorganisms, Oligotrophic microorganisms and microscopic Fungi) and the direction of microbiological processes in the soil.

Ecological and trophic groups of soil microorganisms were identified by inoculation of dilutions of soil suspensions onto selective nutrient media (DSTU 7847:2015): Ammonifying bacteria were cultured on meat peptone agar (MPA); Streptomyces and mineral nitrogen-utilizing bacteria (amylolytic) – on starch-ammonia agar (SAA); Pedotrophic microorganisms – on soil agar; microscopic fungi – on agarized Chapek's medium supplemented with lactic acid; Oligonitrophilic microorganisms – on hungry agar. After the inoculation of nutrient media, they were incubated at a temperature of 28°C for a period ranging from 5 to 14 days, depending on the growth rate of a specific group of microorganisms. Microbial abundance was quantified as colony-forming units (CFU) per gram of arid soil. Soil moisture content was determined by the thermostatic weight method. The calculation of colony-forming units considered the soil moisture and the dilution factor of the soil suspension. The following coefficients were calculated: coefficients mineralization-immobilization (Cm-i), coefficients oligotrophy (Col), and coefficients pedotrophy (Cp) (Andreiuk, et al., 2001) in order to determine the direction of soil microbiological processes.

The coefficient of mineralization-immobilization of nitrogen was calculated according to the formula:

$$C_{m\text{-}i} = N_{SAA}/N_{MPA},$$

N_{SAA} – number of microorganisms, that grew on the starch-ammonia agar (SAA);
N_{MPA} – number of microorganisms, that grew on the meat peptone agar (MPA).

The coefficient of oligotrophy was calculated according to the formula:

$$C_{ol} = N_{HA}/(N_{SAA}+N_{MPA}),$$

N_{HA} – number of microorganisms, that grew on the hungry agar;
N_{SAA} – number of microorganisms, that grew on the starch-ammonia agar (SAA);
N_{MPA} – number of microorganisms, that grew on the meat peptone agar (MPA).

The coefficient of pedotrophy was calculated according to the formula:

$$C_p = N_{SA}/N_{MPA},$$

N_{SA} – number of microorganisms, that grew on the soil agar;
N_{MPA} – number of microorganisms, that grew on the meat peptone agar (MPA) (Andreiuk, et al., 2001).

The soil chemical characteristics of studied plots (0–30 cm) are given in Table 1. The soil organic carbon content was determined using the Tyurin method (FAO, 2021).

Soil type was determinate according to the WRB soil classification system (IUSS Working Group WRB, 2014). Amount nitrogen(NH_3-N) content was determined according ISO/TS 14256-1:2003.

The content of mobile P_2O_5 and K_2O was determined by of Machigins' method, according to DSTU 4114-2002. The method is based on removing mobile compounds of P and K from the soil with 1% ammonium carbonate solution (pH = 9.0) with a soil and solution ratio of 1:20 at 25 ± 2°C temperature. Shaking of soil suspension lasted for 5 minutes. Soil infusion in the solution lasted for 20 hours. Before determining mobile P_2O_5 and K_2O, soil extraction was discoloured with activated carbon. Phosphorus (P) content was determined by a colorimetric method. A flame photometer C-115M1 (2006, Ukraine) was used in order to determine potassium (K) content. Soil samples were prepared for analysis by drying (t = 25 ± 2°C) and sifting.

Research results of soil characteristic and number of microorganisms of the main ecological-trophic and taxonomic groups were presented as the average value of 4 replicates. Statistical processing of soil characteristic and number of main ecological-trophic and taxonomic groups of soil microorganisms was performed by dispersion analysis one-way ANOVA at a significance level of 95% using Microsoft Excel 2010. Relationships between soil microbial processes and soil organic matter was made using Pearson correlation analysis at a significance level of 5% ($p<0.05$).

3. Results

Research results of the soil microbial community under energy crops growing show that the number of microorganisms of the main ecological-trophic and taxonomic groups was high (Table 2). Soils of the control variant (grassland) had the lowest abundance values (by 1.2–3 times) for all the studied ecological-trophic groups of microorganisms. The soil under cultivation of switchgrass was characterised by the highest values of abundance of eco-trophic groups of microorganisms (except amylolytic bacteria) compared to the soil under cultivation of *M. giganteus*. The highest number of amylolytic bacteria was observed for *M. giganteus* – 12.79±1.34*10^6 CFU per 1 gram dry soil Table 2.).

Table 1
Description of the topsoil (0–30 cm depth) properties of the experimental plots

Properties	Parameters	Grassland (control)	Switchgrass (*Panicum virgatum L.*)	*Miscanthus (M. giganteus)*
Soil type		*Haplic Luvisol*	*Haplic Luvisol*	*Haplic Luvisol*
Soil organic carbon	%	3.58±0.04	5.82±0.02	4.82±0.07
NH_3-N	mg kg^{-1} dry soil	140±1.05	140±1.08	154±1.11
P_2O_5	mg kg^{-1} dry soil	1056.0±3.12	381.0±7.12	1095.0±4.03
K_2O	mg kg^{-1} dry soil	703±1.21	646±1.18	675±1.20
pH		6.23±0.01	7.20±0.04	6.47±0.02

Note: The data is an average result of 4 repetitions ± SD values. All differences are statistically significant

Table 2
The number of main eco-trophic and taxonomic groups of soil microorganisms

Variants	Bacteria using organic nitrogen (ammonifying), 10^6 CFU per 1 gram dry soil	Microscopic fungi, 10^3 CFU per 1 gram dry soil	Bacteria using mineral nitrogen (amylolytic), 10^6 CFU per 1 gram dry soil	Streptomycetes, 10^6 CFU per 1 gram dry soil	Oligotrophic bacteria, 10^6 CFU per 1 gram dry soil	Pedotropfic bacteria, 10^6 CFU per 1 gram dry soil
Grassland (control)	25.30±1.44	38.30±6.91	7.86±0.77	6.71±0.60	4.64±0.31	10.01±0.92
Switchgrass (*Panicum virgatum L.*)	76.00±6.07	96.00±17.28	10.16±0.28	9.72±0.12	5.56±0.68	13.64±1.74
M. x giganteus	31.86±2.20	48.40±8.71	12.79±1.34	8.35±0.95	5.17±0.59	11.21±0.49

Note: The data is an average result of 4 repetitions. Information presented as SD values. All differences are statistically significant.

Table 3
Coefficients of the direction of soil microbiological processes

Variants	Coefficient of oligotrophy (C_{ol})	Coefficient of mineralization-immobilization of nitrogen (C_{m-i})	Coefficient of pedotrophy (C_p)
Grassland (control)	0.15	0.31	0.40
Switchgrass (*Panicum virgatum L.*)	0.06	0.13	0.18
M. giganteus	0.13	0.40	0.35

Note: The calculation of the coefficients of the direction of soil microbiological processes was made on the basis of the average values of studied parameters (Table 2).

In order to assess the direction of soil microbiological processes in the soil and to analyse in more detail the possible changes in the structure of the soil-biotic complex under the growth of energy crops, the coefficient of mineralisation and immobilisation (Cm-i), the coefficient of oligotrophy (Col) and the coefficient of pedotrophy (Cp) were calculated (Table 3). This makes it possible to characterise the level of mineralisation processes (Cm-i), the supply of the soil with easily digested nutrients (Col) and the functionality of the soil microbiocenosis structure (Cp).

In all studied variants, the number of ammoniating bacteria was higher than other groups of microorganisms (Table 2), respectively mineralisation-immobilization coefficients, based on the quantitative ratio of trophic groups of bacteria, were less than 1 (0.13–0.40. Table 3.). The lowest Cm-i (0.13) was observed in soil under switchgrass growth, which shows the superiority of this crop compared to *M. giganteus* and a higher level of potential soil fertility.

The number of oligotrophic bacteria is obtained about 5 million CFU per 1 gram dry soil in all studied variants, which indicates the availability of organic substrate in the soil in sufficient quantity for microorganisms. The coefficients of oligotrophy (Table 3) were calculated for the studied variants in the range from 0.06 to 0.15. A low amount of easily digestible substances was found in the soils under switchgrass cultivation (Col=0.06).

The value of the pedotrophic factor in studied soils ranged from 0.18 to 0.40 (Table 3), which characterises the degree of assimilation of soil organic matter by microbiota. The highest level of organic matter assimilation was found in soils under grassland. The coefficient of pedotrophy in control soils (Cp=0.40) was higher almost in twice the level of compared to the soil under energy crops. For switchgrass Cp was 0.18, for *M. giganteus*–0.35 (Table 3).

Correlation analyses allow us to determine the relationships between the amount of Corg and the direction of soil microbiological processes (Figure 1–3). According to statistical analysis, coefficients (r) ranged from 0.32 to 0.89 (Table 4), which confirms statistically significant relationship between studied parameters.

Correlation coefficients indicate that the control soil (Fig. 1) had a medium correlation between soil organic carbon content and all investigated soil microbial process coefficients (r=0.33–0.51). For soil under switchgrass (Fig. 2), the correlation coefficients showed a strong relationship between the Col and Cp coefficients (r=0.89; r=0.72 respectively). Correlation coefficient between Corg content and Cm-i indicates a medium relationship between the parameters studied (r= 0.32). The analysis of the

Table 4
Correlation coefficients (r) of studied parameters (coefficients of soil microbiological processes and soil organic carbon content)

	C_{ol}/SOC	C_{m-i}/SOC	C_p/SOC
Grassland (control)	0.45*	0.51*	0.33*
Switchgrass (*Panicum virgatum L.*)	0.89**	0.32*	0.72**
M. giganteus	0.63*	0.7*	0.7*

*r=0.3–0.7 – medium correlation; **r ≥ 0.7 – strong correlation.

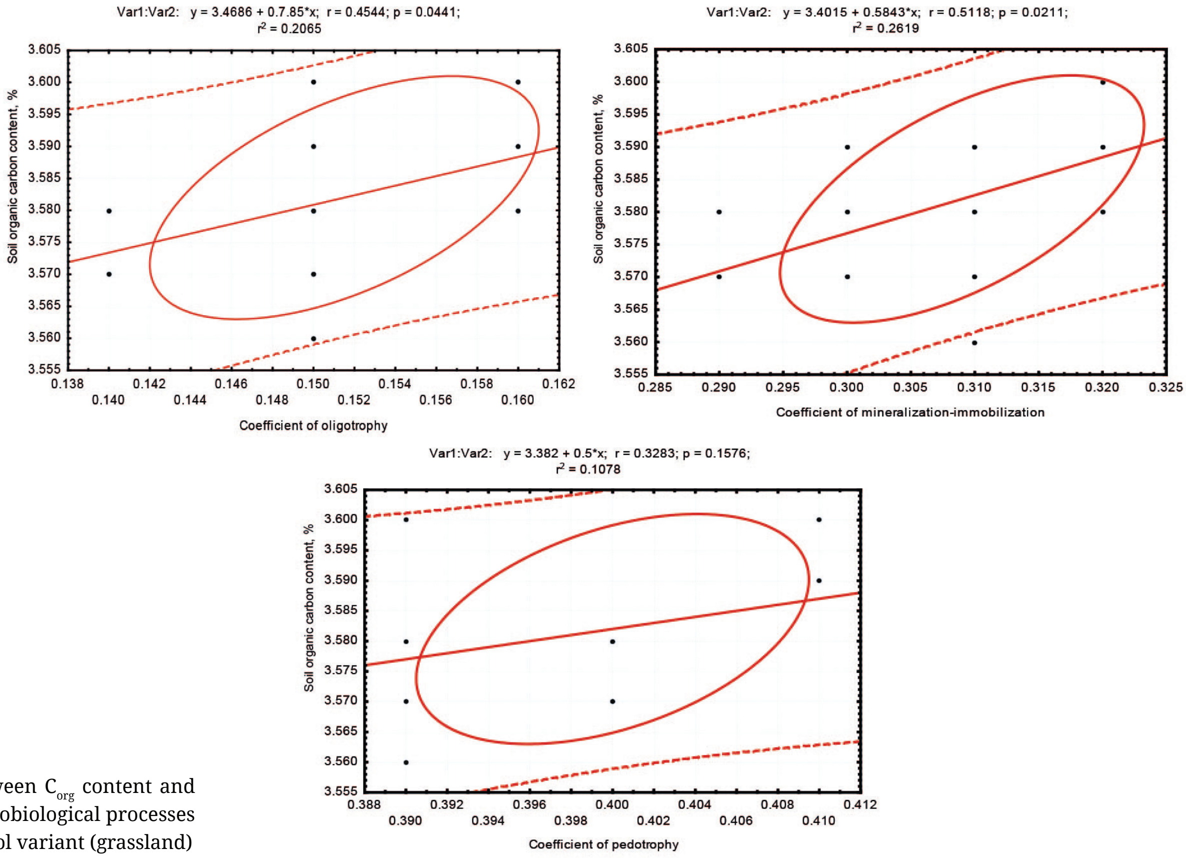

Fig 1. Correlation between C_{org} content and coefficients of soil microbiological processes (C_{ol}, $_{Cm-i}$, C_p) in the control variant (grassland)

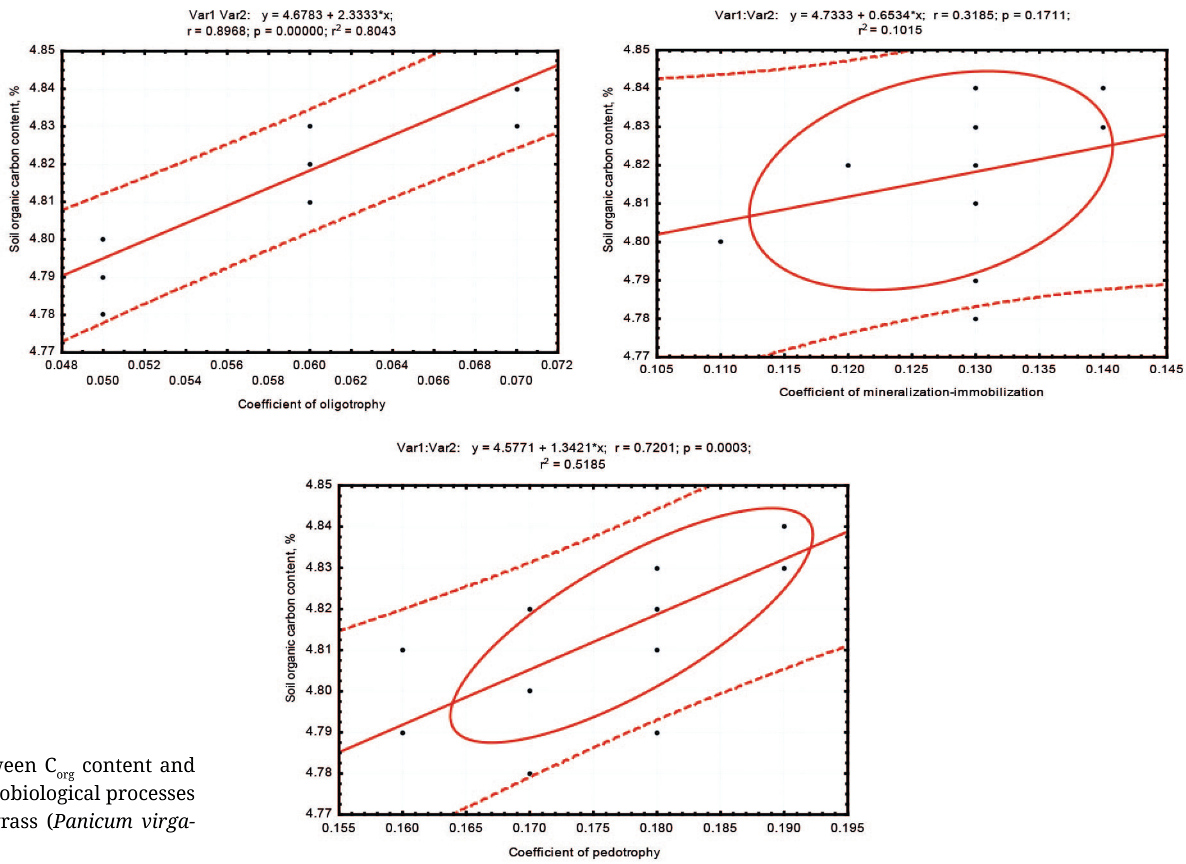

Fig 2. Correlation between C_{org} content and coefficients of soil microbiological processes (C_{ol}, C_{m-i}, C_p) in switchgrass (*Panicum virgatum L.*) cultures

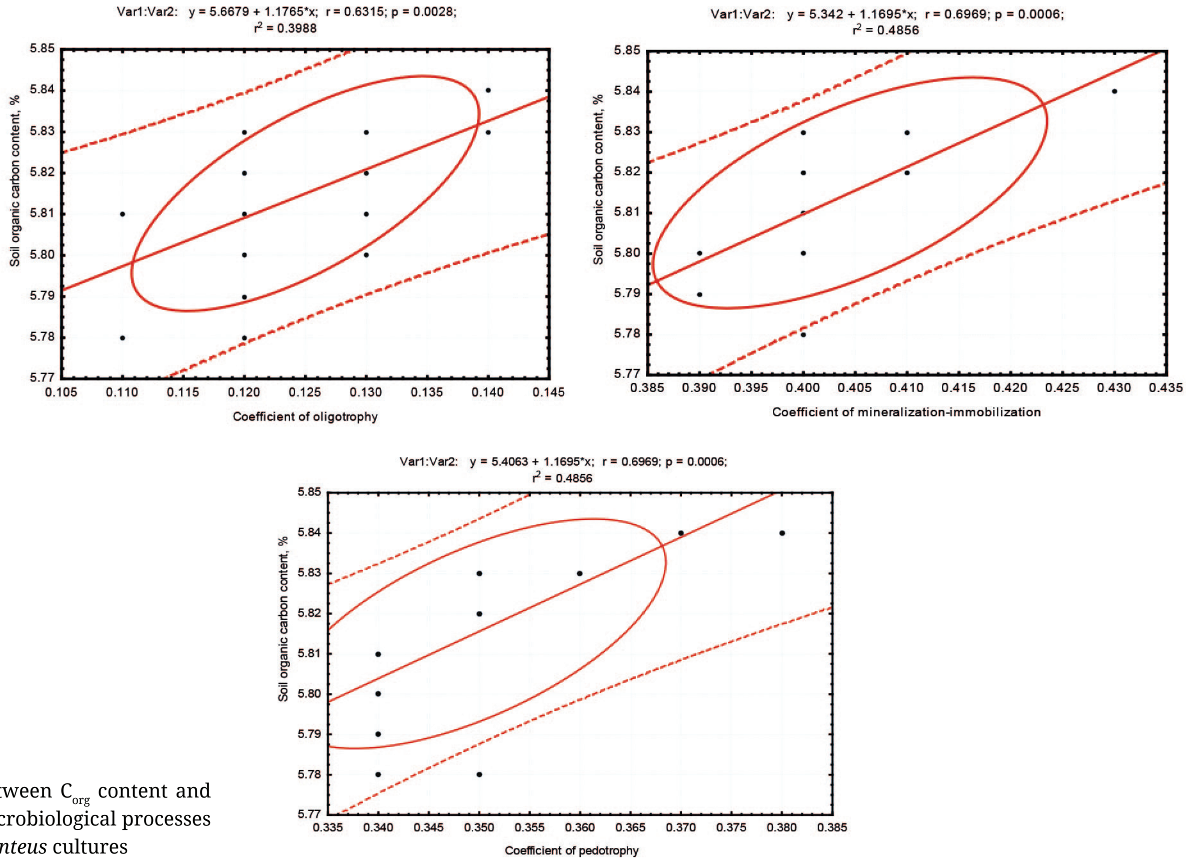

Fig 3. Correlation between C_{org} content and coefficients of soil microbiological processes (C_{ol}, C_{m-i}, C_p) in *M. giganteus* cultures

correlation coefficients of the soil under *M. giganteus* growth (Fig. 3) shows a strong relationship between the parameters of soil organic carbon content and Cp, Cm-i (r=0.7); a medium relationship between the organic carbon content and Col (r=0.63). Therefore, the analysis of the results showed a reliable relationship between soil microbiological parameters and soil organic carbon content during energy crop cultivation.

4. Discussion

Soil is a mixture of minerals, organic matter and unconsolidated substrates essential for sustaining life. It is a highly complex ecosystem that supports biodiversity, defined as the collective assemblage of organisms that inhabit it. Soil is home to a diverse range of plants, animals, micro-organisms and the abiotic environment around them. One gram of soil can contain approximately 1 kilometre of Fungal hyphae and more than 109 eubacteria and archaea cells, comprising tens of thousands of species, most of which cannot be cultured in the laboratory. Studying the taxonomy of soil microorganisms can help to determine soil quality and the status of the soil microbial community in the context of agricultural practices.

Microbiota is responsible for processes in soils, from the mobilisation of chemical elements to their accumulation (Patyka et.al., 2014). Especially in the context of global climate change, microbial studies of soils are essential for understanding the dynamics of geochemical, biochemical and biophysical interactions occurring at the Earth's surface. Looking at the taxonomic groups of studied microorganisms, we should pay attention to one of the most important ecological and trophic groups of microorganisms. They play an important role in formation the soil nitrogen balance and can assimilate atmospheric molecular nitrogen and convert it into a form of ammonia available to plants. All ecological-trophic group members can fix atmospheric nitrogen and enrich the soil with biological nitrogen.

Our results for a number of ecological-trophic groups of soil microorganisms demonstrate that the cultivation of energy crops can contribute to an increase in number of the most important groups of soil microorganisms, which consistent with other researches. Bourgeois et.al. (2015) confirm that *M. giganteus* has an initial impact on the soil microbial community. This effect is manifested by a 20% increase in bacterial diversity and a 10% increase in fungal diversity. These observed benefits may be due to the release of fresh organic matter from litter decomposition and root exudation. The absence of tillage and pesticide application, which are detrimental to soil microflora, contributed to this favourable outcome. In conclusion, our research suggests that M. giganteus cultivation is a promising beneficial practice for enhancing the regeneration of soil microbial diversity and for the remediation of contaminated soils. Our results on the direction of soil microbiological processes are related to other studies (Kane et al., 2023) and confirm that *M. giganteus* consistently increases microbial diversity and carbon use efficiency, thereby promoting soil organic matter accumulation across sites. Researchers such as Mao et al. (2012) and Kane et al. (2023) observed an increase in the relative abundance of key plant growth promoting microbes such as mycorrhizal fungi and bacterial nitrogen fixers. Our results of soil microbial abundance confirm the research results of He et al. (2017) which found that long-term switchgrass cultivation resulted in greater beta diversity variation across soil depths and more complex interactions among microbial taxa, although it did not significantly affect soil microbial community structure. Switchgrass cultivation significantly increases microbial OTU richness and shows increased variation in beta-diversity across the soil depth profile. In addition, Ruf et al. (2020) proposed that during the growth cycle of energy crops, plants enhance microbial abundance and activity by providing carbon through root exudates and root turnover. Studies have shown that cover crops increase microbial biomass by 24 to 51% compared to bare soil, depending on the indicator used. On average, they increase bacterial abundance by 15% and fungal abundance by 19%, thereby improving the fungal-to-bacterial ratio. All these studies highlight the relevance of research into soil microbiological processes under energy crop production.

Soil microbiological processes determine the quantitative characteristics of soil organic carbon content. The values obtained for the coefficients of mineralization-immobilization (Table 3) indicate that the processes of soil degradation are less active than the processes of synthesis of organic matter. This confirms the high level of potential soil fertility under energy crop cultivation. Oligotrophic and pedotrophic microorganisms develop intensively on soils due to their trophic specificity and lack of competition. The coefficient of oligotrophy indicates a high supply of easily digestible nutrients to the soil microbiota of the studied variants. The highest coefficient of pedotrophy in control soils indicates that under energy crops, the assimilation of organic matter by microorganisms, which may have the for increasing soil fertility. Research by Kane et al. (2023) also demonstrate that bacterial diversity and microbial carbon use efficiency show a positive trend over the time in positively correlation with soil organic matter content.

The growth and cultivation of *M. giganteus* also contributes to soil carbon sequestration, providing benefits for land restoration and climate change mitigation. Energy crop cultivation can accelerate microbial carbon turnover by stimulating soil microbial activity and facilitating the formation of soil organic carbon (SOC) (Ruf et al., 2020; Fu et al., 2022). High rates of absolute SOC mineralization indicate soil health, which is primarily driven by increased microbial activity. Soil microbes play a critical role in decomposing biomass in energy crops, thereby enhancing soil fertility through increased nutrient release for microbes and plants (Kane et al., 2020). Microbial regulation primarily controls soil microbial carbon turnover through complex biochemical reactions catalyzed by diverse enzymes (Jastrow et al., 2007), even in soil systems where CO_2 production occurs through non-microbial pathways (Wang et al., 2017). Notably, the rate of SOC mineralization is unaffected by input biomass's type, size and structure (Brookes et al., 2017). As a result, energy crops have the potential to act as ecosystem engineers, improving soil biological and chemical properties to transform highly disturbed soils into systems that resemble less disturbed soils over time (Zhang et al., 2022).

5. Conclusions

Research results of the functional composition of the soil microbiota in the *Haplic Luvisol* soil and climatic conditions of Ukrainian forest-steppe zones have shown a significant abundance of main ecological and trophic microbial groups indicating sufficient nutrient availability for soil microbiota and high levels of organic matter assimilation. Soils under energy crops growth (switchgrass (*Panicum virgatum L.*) and *M. giganteus*) had better microbiological indicators compared to the grassland (control) indicating an enrichment in organic matter and an increase in potential soil fertility. Soils under switchgrass had the highest microbiological parameters. Statistical analysis showed the correlation between soil microbiological parameters and soil organic carbon content during energy crop cultivation. We conclude that energy crops can act as ecosystem engineers, improving soil biological and chemistry properties so that highly disturbed soil become more similar to less disturbed systems over time, supporting the sustainability of soil ecosystems. Obtained results are particularly relevant in the context of climate change, the spread of soil degradation processes and the need to restore disturbed soil ecosystems.

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