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THE INFLUENCE OF THE SYNTHETIC MICROBIOMES ON THE CHARACTERISTICS OF BIODIVERSITY AND CARBON SEQUESTRATION IN THE SOIL

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Abstract

The aim of the research was to evaluate the role of the microbial composition of synthetic microbiomes for soil carbon evolution. The microbiomes inoculated into the soil (M1-M9) release exometabolites that influence the transport of nutrients in the soil and the dynamics of the energy potential. Thus, after the inoculation of microbiomes in the soil, the biosynthesized metabolites, the influence on some biological parameters and on the dissolved organic carbon, released after 60 days in the soil, were analyzed. Bacterial microflora growth rate of up to 54% was determined when using M4 and M5 microbiome. Microbiomes M1 and M5 containing bacteria with antagonistic characteristics (Bacillus sp.) and hyperparasitic fungi (Trichoderma sp.) caused up to 32% increases in the microflora. Biomass induced by microbiome M4 reached values up to 354mg C x kg^{-1} soil. Microflora from each microbiome influenced differently the distribution of the fluorescent dissolved organic carbon from soil fulvic subfraction.

Key words*: microbiome; exometabolites; microbial biomass; fulvic acids; biodiversity.*

INTRODUCTION

Uptake of carbon-containing substances from the atmosphere and soil sequestration by the microbial community play an important role in the C cycle, and their activity is considered essential in determining differences in soil C storage potential. Also, soil microbial community composition is crucial for the maintenance of ecosystem services, as their structure and activity regulate nutrient delivery and organic matter decomposition rates.

The nature of soil microbiome composition is defining for soil carbon evolution. By making interventions in interactions between processes such as rates of synthesis/decomposition of organic compounds with carbon, soil carbon storage and microbiome composition, the carbon cycle (C) can be influenced. Also, the extracellular microbial content of microbiomes constitutes a competitive advantage through which they can intervene in influencing the transport of nutrients in the soil, in the dynamics of the energy potential and in the manifestation of tolerance in conditions of climatic stress.

The synthetic microbiome can also intervene in protecting/extending the activity of soil processes, stimulating the qualitative-quantitative evolution of organic carbon, facilitating microbial mobility as well as ensuring the stability of soil aggregates. Microorganisms in the composition of the microbiome function metabolically different and, in this way, directly influence the evolution of processes in the soil. Microbiomes also play an important role in nutrient cycling, ecosystem functioning, ensuring soil fertility and in plant growth development. The use and evaluation of synthetic microbiomes in terms of the impact on carbon sequestration also frames the concept of sustainable improvement of soil management, by using methods that stimulate carbon sequestration, making changes in the composition of communities, inducing structural changes in organic matter (OM) and soil aggregates, as a result of the inter- and intraspecific relationships that are established.

Synthetic microbial communities are a way to preserve the characteristics of their natural counterparts and act as model systems based on interaction/function for controlled assessments of the role of ecological, structural, and functional characteristics of communities. Also, these communities depend on the degree of

microorganism dispersal, environmental selection and species sorting, with effects difficult to control or characterize (Leibold et al., 2004). The study of the soil microbiome allows finding out the complex relationships with the biotic and abiotic environment, highlighting the changes in the microbiome as well as the role of the microbial communities.

The extracellular content biosynthesized by microorganisms is also involved in the qualitative and quantitative processes or evolution of soil organic matter, in microbial mobility, increasing heterogeneity and stability of aggregates. The importance of these microbial secondary metabolites is also reflected in the liberating role of nutrients through the degradation of organic matter, in the identification of priority microbial activities, but also as a sensitive indicator of ecological changes. Their monitoring can be ensured by evaluating the biotic component of the soil. The impact on the possible edaphic ecosystem requires a deeper understanding of the relationships between the strategies used and the synthetic microbiome introduced into the soil (Lehmann et al., 2020; Fierer et al., 2021).

An increase in carbon sequestration rates, in cultivated soils, also intervenes in reducing atmospheric carbon concentrations, and attempts to promote soil carbon sequestration can provide direct benefits for its health and productivity, taking into account the importance of organic carbon concentrations for the sustainability of agriculture (Amelung et al., 2020; Bossio et al., 2020).

By manipulating microbiomes, it is possible to enhance the carbon sequestration capacity of soils. Consequently, new strategies appear necessary to accelerate the rates of soil carbon sequestration, especially in cultivated soils, where carbon stocks have been reduced by agricultural activities and where practically their increase has been achieved exclusively through direct intervention. Through new strategies to accelerate carbon sequestration in cultivated soils, both the mitigation of climate change and the improvement of food security are contributed.

The research aimed to identify the traits, respectively the microbial properties that can lead to changes in soil carbon dynamics, to the possibility of accelerating carbon sequestration, through the influence exerted by the composition of synthetic microbiomes and by capitalizing on their specific characteristics.

MATERIALS AND METHODS

Soil characteristics of Mollic Histic Gleysol (Salinic) (WRB) were: humus content 29.8%, organic C 17.3%, organic matter 51%, pH value 5.54, total nitrogen 1.740 mg·kg^{-1}, N-NO_3 2106 mg·kg^{-1}, P_{AL} 45 mg·kg^{-1}, carbonates 0.4%, microelements (Fe 23.259 mg·kg-1, Mn 144mg·kg^{-1}, Cu 55.3 mg·kg^{-1}, Zn 67.9 mg·kg^{-1}), clay content <0.002 of 38.6%.

In the experiment, carried out in control conditions at NRDISSAE, Bucharest, in summer 2023, the influence of the microbiomes composition M1-M9 on the soil biodiversity and dynamics of soil C was analysed after a period of 60 days from the application of 2 ml of inoculum/pot with concentrations between 3.26-5.42x10^3 ufc/ml for fungi and between 6.35-8.74x10^6 viable cell/ml for bacteria. The bioassay pots contained Mollic Histic Gleysol (Salinic) were inoculated and incubated at 27^{0}C, at a constant humidity of 60% of the soil field capacity and maintained for a period of 60 days, under the same controlled conditions. At the end of this period, soil samples were collected for analysis. Five replicates were used for each experimental variant.

The criteria for microbiomes selection and creation of synthetic microbiomes M1-M9 sought to ensure biodiversity and adaptability, microbial biomass production, increased exometabolic production and involvement in the modelling of organic fractions, the presence and rhizospheric activity, the predominance of fungal microflora, the presence of fungi endosymbionts and antagonists. Within each bio-system, the principle of using and grouping microbial isolates was respected in accordance with the biomass/metabolites/CO_2 production relationship. Thus, bio-systems M1-M9 contain combined microorganisms, between 7-12 isolates/microbiome.

Microbial communities belonging to microbiomes M1-M9 contain bacterial, fungal, diazotrophic and mycorrhizal microflora. Before inoculation, the microbial species are kept in pure cultures, on culture media (Topping, Czapek, LB, BHI, MRS, King, YEM,

MMN) at a temperature of 4ºC. The taxonomic study used the morphological criteria by optical microscope (MC 5.A) examination and measurements according to determinative manuals: Bergey & Holt (1994) for bacteria and Domsch & Gams (1970) for fungi, to assess microbial diversity.

Microbiomes M1-M9 contain microorganisms belonging to bacterial genera: *Pseudomonas, Bacillus, Azotobacter*, *Rhizobium, Acetobacter, Paenibacillus, Serratia, Thiobacillus, Streptomyces,* to the fungal genera *Chaetomium, Arthrobotrys, Cunninghamella, Myrothecium, Trichoderma, Torula, Rhizopus, Aspergillus, Penicillium, Stachybotrys, Mortierella, Cladosporium, Humicola, Micromonospora, Acaulospora, Glomus.*

The diversity indices used measured the evolution of the number and distribution, evenness of species in communities inoculated with microbiomes (abundance, evenness, Shannon-Wiener and Simpson indices).

The quantitative analysis of the microflora from the soil variants inoculated with the M1-M9 microbiomes were performed according to the method of soil serial dilutions. Culture media specific to microflora were used, respectively: Nutrient Agar (NA) for heterotrophic aerobic bacteria (Difco) and (PDA) for saprophytic fungi (Merck). Colonies developed after 4-7 days of incubation (27ºC) were counted and the results expressed as total number of bacteria (TNB)-viable cells $x10^6$ and total number of fungi (TNF)-colony-forming units (cfu) $x10^3$ reported per 1 gram of dry soil (Dumitru & Manea, 2011) .

Soil microbial biomass was determined according to the fumigation-extraction method. Soil samples were fumigated with $CHCl_3$ at 20°C for 24 hours, extracted with K_2SO_4 and filtered using cellulose ester filter. Soil microbial biomass carbon was calculated according to the Standard-SR-ER-ISO-14240-2-(2012), as average of five replicates/variant.

For separating water-soluble subfraction D, fulvic fraction of Mollic Histic Gleysol (Salinic) was extracted and fractionated by adsorption on activated charcoal, to following by serial elution with acetone, NaOH and finally in distilled water, followed by its migration on a ascendant chromatograms (Votolin et al., 2022).

Fluorescence of dissolved organic carbon in the fulvic sub-fractions of microbiome-inoculated soil was extracted, treated with fluorochromes, and its distribution revealed by specific ascending chromatography (Wang et al., 2021). The photographic images obtained under 350 nm UV illumination revealed the qualitative differences between the final phases of microbiome application, as well as aspects related to the different densities of the newly synthesized biochemical composition, the distribution of the material and its complexity, highlighted by the affinity for fluorescence.

RESULTS AND DISCUSSIONS

Microbial communities have been selected to constitute synthetic microbiome variants (M1-M9) that were inoculated into Mollic Histic Gleysol (Salinic). Due to their microbial carbon generating character, diversity indicators were used to assess the effects on biodiversity and soil carbon dynamics. Taxonomic composition and relative abundance in bacterial and fungal communities control soil and variants inoculated with synthetic microbiomes are presented in Figure 1 and Figure 2.

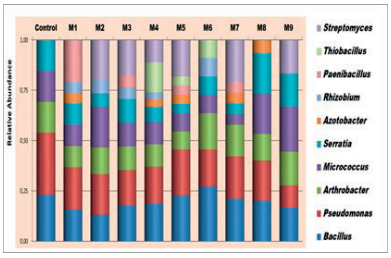

Figure 1. Taxonomic composition and abundance of bacteria in soil inoculated with microbiomes M1-M9

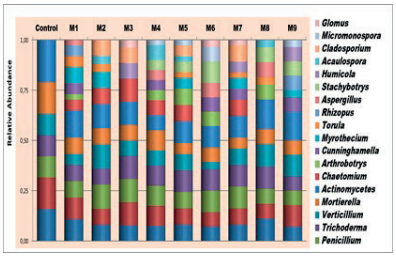

Figure 2. Taxonomic composition and abundance of fungi in soil inoculated with microbiomes M1-M9

From an ecological point of view, increased number of species favours community functionality and stability in the face of perturbing factors through complementarity mechanisms, such that after inoculation with M4 and M5 microbiomes, soil microflora had different abundance of component species, the more numerous individuals representing new inoculated species integrated in the communities of soil. They use more of the available resources, increasing the biosynthesis of exometabolites. These species (e.g. as those from genera *Bacillus, Pseudomonas, Streptomyces, Trichoderma, Penicillium, Chaetomium, Mucor,* in variants M4-M5) developed abundantly in soil compared with other inoculated or endemic species. Also, through selection mechanisms, individuals from different species originating from the M1-M9 microbiomes contribute differently to the overall functionality of the soil microbial community after inoculation, because they differ from each other in biosynthetic efficiency. As a result, the increased abundance in microbiome-inoculated soil likely stems from the richer content in adapted and efficient individuals belonging to certain species in the microbiome community.

Homogeneity (evenness) is an important factor in assessing maintaining functional stability and increasing productivity. In the analysed communities, it refers to the distribution of individuals by species (Table 1).

Table 1. Diversity indices and evenness of microbial communities in control and soils inoculated with synthetic microbiomes M1-M9

Microbiome Variants	Diversity indices		
	Shannon-Wiener index (H')	Evenness (ε)	Simpson index (D)
Control	2.447	0.916	0.910
M1	2.993	0.908	0.946
M2	2.883	0.903	0.941
M3	2.890	0.910	0.942
M4	3.054	0.922	0.950
M5	3.047	0.897	0.947
M6	2.921	0.894	0.943
M7	2.979	0.903	0.945
M8	2.825	0.907	0.938
M9	2.886	0.914	0.941

Thus, the homogeneity is high (ε=0.922) in the soil community inoculated with the M4 microbiome where almost all species have a similar distribution and the uniformity is lower (ε=0.894) in the soil community inoculated with the M6 microbiome, reflecting a more heterogeneous distribution by species. By intensifying the expression of the functional traits of the species, in the case of soil inoculated with M1-M9 microbiomes, compared to the control communities, they may be more resistant to stress and various disturbances, less susceptible than in the case of non-inoculated soil.

As a measure of diversity, the Shannon index used can also be constituted as a measure of entropy, both of which have conceptual similarities, and therefore entropic and diversity indices share many of the characterization axioms, but do not mean that they are equivalent. Thus, the entropy indices are linear functions of abundance/probability, while the diversity indices used are non-linear and the average diversity of the number of microbial communities is not the average of their diversities. The adoption of this index assumed the consideration of appropriate conceptual differences, based on the evaluation of the real abundance of the different species present in the inoculated soil. In general, indices condense into one number relevant information on the diversity of an edaphic system, using complex data about that system (Davydov et al., 2016; Tucker et al., 2016; Kang et al., 2016; Buckland et al., 2017; Grabchak et al., 2017; Butturi-Gomes et al, 2017; Botta-Dukat, 2018). The use of this index depended on the way in which it is mathematically constructed, on the method of ecological interpretation, as well as on the context of the study, which assumed the observation of diversity changes induced by inoculation, the estimation of their evolution according to the level of biodiversity, the understanding of the interactions between biodiversity and trophic level reached, evaluation of the effect of change in diversity.

In this study we focused on species diversity in soil inoculated with different microbiomes because species diversity is considered the most common form of soil diversity analysis.

The values determined for the Shannon index in the bio-systems analysed varied between 2.447 for non-inoculated Mollic Histic Geysol (Salinic) and 3.054, respectively 3.047, for the same type of soil inoculated with microbiomes M4 and M5, respectively. In general, the inoculated microbiomes generated Shannon

index values above 2 and can be considered to have average diversity. Shannon diversity index values above 3, as in the case of the influence of the application of M4 and M5 microbiomes, are considered values of a high diversity.

In the case of applying the Simpson diversity index (D) in the analysis of diversity in Mollic Histic Geysol (Salinic) inoculated with microbiomes M1-M9, the highest value registered in the case of the soil inoculated with the M4 microbiome (D=0.950), which indicates an increased number so species and uniformity, respectively a good species diversity of the community. The lower values determined in the case of soil inoculated with microbiomes M2, M9 (D=0.941) and M8 (D=0.938), indicated a lower diversity of these heterogeneous communities. Compared to the Shannon-Wiener index, the Simpson index is an indicator of α-diversity and the rare species in the inoculated soil communities have a smaller role in the calculation of the index, in contrast to the common species that acquire a more important role. In general, the Shannon Index emphasizes the abundance component, sparse cover types, and the Simpson Index preferentially uses the evenness component and dominant cover types. The value of the presence of endemic species can be diluted by the evaluations of such indices. In general, endemic species have characteristic soil type distribution and specificity, and for indices, these endemic species become only a few more species in the microhabitat. Consequently, analysis of the endemic status was considered, in the case of artificial introduction of M1-M9 microbiomes. The results revealed that none of the introduced microbiomes significantly influenced the endemic soil microbial presence and dynamics.

The monitoring of synthetic microbiomes can be achieved not only based on changes in some abiotic variables (soil nutrients, pollutants, soil structure), but especially through biological indicators (where microbial strategies are used), represented by quantitative and qualitative assessments of microflora, biomass, carbon dissolved organic matter. The assessment of biodiversity in the soil was carried out by taxonomic tests based on the analysis and classification of different parts of the microbial material. The priority, in the case of studying the biodiversity of the soil inoculated with the M1-M9 microbiomes, included the determination of the newly formed microhabitats, the possible factors that intervene in vulnerability/favouring the increase of diversity, following the applied treatments, by changing the supply of energy and nutrients, the creation of microhabitats and the new biodiversity in the soil.

The microbial bio-systems active *in situ* were expressed by the presence and level of their activity in the soil, by the preponderant involvement of the fungal microflora in the circuits of major elements (carbon, nitrogen, phosphorus).

Biodiversity is important for the viability of edaphic ecosystems and its loss leads to functional degradation and a rapid evolution towards total collapse. Through human activities, this characteristic has worsened and reached critical values, due to high extinction rates of edaphic species and the generation of changes in the structure and functionality of the affected ecosystems. The molecular and ecological bases of functions at the microbial community level are reduced, as well as the stability, robustness of their functions, extent, diversity, structure, size, as community properties. The influence of these factors on synthetic community composition may vary in time and by ecosystem. The diversity is a challenge in the direction of relationships between species composition, function and community dynamics (Bunge et al., 2013).

Soil microbial communities are essential in maintaining soil quality. They are an important part of the ecosystem (Jessup et al., 2004; Dıaz de Otalora et al., 2021), and underground generators of soil benefits, too (Matei et al., 2019). There are differences between the compositions of the microbial communities in the experimental sites compared to the conventional systems, between the increases of microbial biomass or between the levels of their activities that appear much intensified (Frac et al., 2018; Fenster et al., 2021; Lujan Soto et al., 2021). Also, the aboveground activities of these soils can have an influence on the components of artificial microbiomes, as well as on the bacterial and fungal microflora of the underground ecosystem (Hermans et al., 2017; Banerjee et al., 2019).

Different studies have shown that synthetic microbiomes improve soil fertility through the

capabilities of intervening in biogeochemical cycles, C storage and providing greater plant diversity through soil microbial processes.

Microbial activity in the rhizosphere has an impact on soil hydrophobicity, offering the possibility of increasing efficiency by indirectly manipulating soil microbiomes, changing management practices or directly by modifying endemic microbial communities, respectively by introducing appropriate microorganisms with a functional regenerative role (Matei et al., 2016a; Hu et al., 2018; Hartman et al., 2018).

The sequestration of soil carbon involves a faster accumulation than its loss over time. Thus, multiple approaches (e.g., through rooting depth, through cover crops) have attempted to alter this balance in agricultural systems, but one can intervene in increasing soil carbon sequestration capacity and through a direct manipulation of microbiome composition soils by introducing synthetic microbiomes. Through this approach or in combination with other types of approaches, the potential of microbial activities that control the net flux of carbon within soil systems can be harnessed.

The soil microbiota controls the processing rates of organic carbon inputs as well as biochemical stabilization through reactions with mineral surfaces. It can also intervene in the transformation of organic carbon into soluble or gaseous forms of carbon that can leave the system, form stable micro-aggregates with a diameter below 250 μm containing clays, sesquioxides and microorganisms (Totsche et al., 2018). Microbes ensure the protection of soil carbon reserves from mineralization processes or the reduction of losses in the form of organic carbon particles, through erosion (Hartmann et al., 2022).

In general, the soil microbiome is particularly complex and its multiple specific contributions to carbon dynamics are little appreciated (Fierer et al., 2017). The processes of stabilization over time and carbon retention in soil are complex and highly variable in time and space, with relatively little estimated results, due to biotic and abiotic interactions (Lehmann et al., 2015).

Perhaps research in the field will also focus on evaluations of the functional contributions of synthetic microbiomes in soil, not just on compositional changes. Microbial communities can contain functional variations, given similar compositions, or, on the contrary, compositionally different microbial communities can have similar functional potential due to functional redundancy (Castaneda et al., 2017; Louca et al., 2018).

Strategies aimed at sequestering carbon cause changes in the soil environment so that different physicochemical properties of soils support microbial communities with different functional profiles. Microbial functions for soil and understanding how these functions change is also essential in estimating the overall impact on the soil ecosystem (Bahram et al., 2018)

The functions change with depth, so that the functions for carbon sequestration, for the metabolism of nutrients, nitrogen and phosphorus are lower than in surface soils. Investigating changes in active microbial communities deep in the soil profile after the introduction of synthetic microbiomes can improve the impact of regenerative practices (based on them) on soil health (Rchiad et al., 2022).

Analysis of biodiversity in soil inoculated with synthetic microbiomes provide some examples of how biodiversity indicators convey information about what is happening with synthetic microbiomes in a soil, as well as support for the development of ways to control the activities and processes involved in ensuring his health. The quantitative assessment of the edaphic heterotrophic bacterial and saprophytic fungal microflora in Mollic Histic Gleysol (Salinic) was carried out after 60 days from inoculation with M1-M9 microbiomes. In all experimental variants, after the introduction of selected synthetic microbiomes over the resident microflora, a stimulation of their numerical growth was found. Thus, the bacterial microflora showed growth rates of up to 54%, at 60 days after inoculation, in the case of using the M4 microbiome (344.32×10^6 viable cells x g^{-1} dry soil). In the non-inoculated variant, the increase within the same time period was 3.12% (19.9×10^6 viable cells x g^{-1} dry soil). The introduction of the M1 microbiome into the soil caused quantitative increases in the fungal microflora, in the final of period compared to the initial values (respectively from 45.36×10^3 cfu x g^{-1} dry soil to 86.34×10^3 cfu x g^{-1} dry soil, in the case of the M1 microbiome). Also, the quantitative increases of the fungal microflora

60 days post inoculation were significant compared to the initial values, after the introduction of microbiomes M4, M5 and M7 and non-significant, in the case of microbiomes M6, M8 and M9 (Figures 3 and 4).

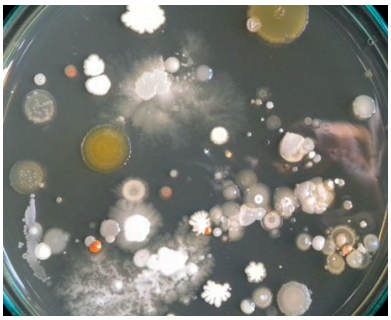

Figure 3. Soil bacterial microflora after 60 days from inoculation with microbiome M4

Figure 4. Soil fungal microflora after 60 days from inoculation with microbiome M4

The microbiomes M1 and M5 containing within the community bacterial microflora with antagonistic characteristics (*Bacillus* sp.,) and hyperparasitic fungi (*Trichoderma* sp.,) influenced the quantitative increases of the microflora. Also, the microbiomes M4 and M7 containing free-living microorganisms and symbionts with an important role in the nitrogen cycle (*Azotobacter* sp., *Rhizobium* sp.) and fungal endosymbionts with a role in the phosphorus cycle (*Glomus* sp.), influence the growth with up to 30% of the soil microflora, after 60 days from inoculation. The influence of the presence of the free diazotroph *Azotobacter* on the quantitative growth of the microflora could be due to the biosynthesis of a series of biologically active substances, to its biodegradative capacity on aromatic compounds, as well as to the role played in ensuring the mobility of metals in the soil. Also, the species of *Rhizobium* present in the microbiome community, as an endosymbiont, after inoculation in the soil obtains nutrients from the plant, produces nitrogen in the inoculated soil, through biological fixation, and mycorrhizal fungi (*Glomus* sp.) improve nutrient absorption and stimulate biodiversity.

Microbial biomass of soil inoculated with M1-M9 microbiomes correlated well with the level of microbiome activity and diversity. Thus, the biomass level directly reflected the intensity of the activities of the microbiomes in Mollic Histic Gleysol (Salinic), and the identical culture conditions allowed the evaluation of the influence of the inoculated microbiomes on the evolution of the biomass (Figure 5).

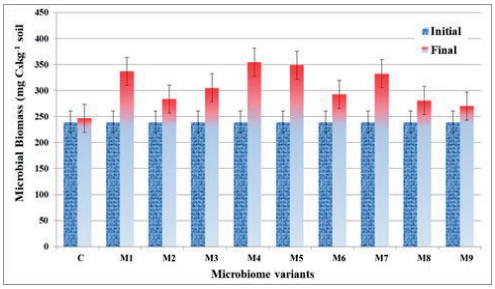

Figure 5. Microbial biomass of the soil inoculated with microbiomes M1-M9

Under experimental conditions, biomass accumulations showed variations between 12-48% for the 9 variants of inoculated with microbiomes. The biomass produced by microbiomes M1, M4, M7, after introduction into the soil, reached values of up to 354 mg C x kg^{-1} soil (M4) after 60 days from inoculation and represented the experimental variants with up to 48% increase in the amount of microbial biomass. In the variants inoculated with microbiomes M2 and M6, the microbial biomass content increased by 18.56% (M2) to 22.14% (M6), reaching a biomass of 272 mg C x kg^{-1} soil. The amount of biomass accumulated had the lowest level in the case of Mollic Histic Gleysol (Salinic) inoculated with the M9 microbiome, where the biomass increase was of only 12.81% compared to the initial determinations. Biomass accumulations partially reflect the degree of integration into the edaphic community of introduced bacterial and

fungal microflora, the functional and ecological compatibility with endemic microflora. Increasing the microbial biomass in soils by inoculating synthetic microbiomes can be an opportunity to create complex reservoirs of microbial life on the basis of which the effect of microbial monocultures of some performing microbial strains, the vulnerability of the edaphic bio-system to pathogens and extreme conditions can be avoided (Wagg et al., 2019; Averill et al., 2022; Mishra et al., 2022). Increasing and exploiting the dimensions of microbiomes in soils generates the necessary potential for the transformation/control of these bio-systems, as well as for microbiome monitoring and conservation. Attempts to increase biodiversity, microbial biomass, especially in managed soils, aim to restore or improve the composition of microbiomes with functionally and productively diverse bacterial and fungal microflora. Microbial biomass ensures the immobilization, mineralization of nutrients and the formation of soil aggregates, and during its quantitative development, the increase in carbon content (C) can provide between 1-3% of soil organic carbon (SOC) (Seita et al., 2012; Wu et al., 2020). Thus, the carbon in the microbial biomass can be considered as part of the active, unstable fraction, concentrated especially in the surface area of the soil and participating in the formation of SOC, alongside the passive fraction with long-term stability (Alvarez et al., 2016). Variable relationships are also thought to exist between microbial biomass carbon and soil physical and biogeochemical properties. The synthetic microbial bio-systems from experiment were active and caused increases in microbial biomass in the inoculated variants, the species in the microbiomes being grouped in accordance with the biomass-metabolites-CO_2 relationships.

The dissolved organic carbon compounds from the soil inoculated with the M1-M9 microbiomes were analysed because they represent the organic matter extractable in water, and their fluorescence characteristics were used because they allow the highlighting of active but also labile reserves of biosynthesized organic matter (Matei et al., 2016b; 2023). For example, free-living diazotrophs and other species of soil microbiota produce water-soluble compounds that can be of various colours, dark brown, yellow-green to purple, green with yellow-green fluorescence, and blue with blue-white fluorescence. The process of biosynthesis of such fluorescent compounds is carried out at high metabolic rates and they are involved in the protection of enzyme systems.

The process of microbial carbon biosynthesis in soil, under certain standard conditions and for short periods of time, had an effect on SOC through labile, water-extractable and fluorescent substances biosynthesized by endemic and inoculated microflora.

In addition, the fluorescence of protein, organo-mineral and humic substances, present in the organo-mineral reserve of the soil, is added. By visualizing their presence and monitoring their distribution, some of the short-term effects of various microorganisms with a biosynthesizing role, from the analysed soil, on the composition of the organic matter extractable in water and the possible options for intensifying C storage were elucidated.

Extracts from the water-soluble sub-fraction of fulvic acids were used in order to highlight the most significant fluorescent components of organic matter (OM) extractable in water, components that can accumulate and integrate into organic matter, as a result of microbial biosynthesis carried out in short term.

The fluorescent components integrated into the structure of water-extractable organic matter from the soil inoculated with synthetic microbiomes were analysed and each of the sub-fractions related to the soil inoculated with microbiomes M1-M9 highlighted the presence and level of association of biosynthesized microbial organic compounds.

The fluorescent components highlighted (by imagistic technique) the relative degree of formation and storage of newly biosynthesized carbon in the structure of the organic matter, compared to the composition of the initial organic component, in a short period of time (60 days). The distribution of this biosynthesized carbon was highlighted in the specific case of each type of microbiome applied to the soil (Figure 6).

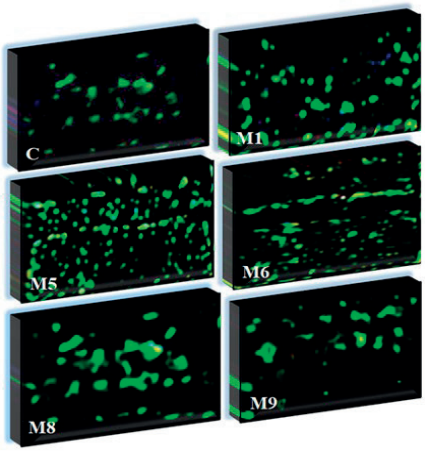

Figure 6. Fluorescent components of Mollic Histic Gleysol (Salinic) after 60 days from inoculation with M1-M9 microbiomes

The sensitivity of the fluorescent components biosynthesized by the microbiomes M1-M9, but also their complexity, was compared with the initial spectrum of the endogenous organic component of the non-inoculated soil, which allowed highlighting the storage possibilities of biosynthesized microbial C, in Mollic Histic Gleysol (Salinic), after inoculation. Through the dynamics of the processes and the level of microbial activities and also by the composition of the extracellular organic component introduced into the soil, it is possible to highlight the direct impact on the organic component, to estimate the efficiency of the mineral protection of the deposited carbon, as well as on the biotic processes of synthesis/decomposition in soils, which appear as relatively complex, correlated and interdependent phenomena. After the inoculation of the M1-M9 microbiomes, the dynamic changes produced in the architecture, composition, concentration of biosynthesized C and of the dissolved organic matter (DOM) were highlighted by means of fluorescence characteristics (Wang et al., 2016; Fox et al., 2017; Arai et al., 2018).

Thus, the fluorescence variations of the C compounds soluble in water would also be due to a possible increase in the lability of the analysed soil carbon, to the fixation in the organic complexes of the biosynthesized compounds simultaneously with the improvement of the activities and accumulations of microbial biomass. Biosynthesized organic components (proteins, amino acids, fulvic acid-like substances a.s.o.) detectable by fluorescence revealed an abundance of the composition consisting mainly of fulvic materials with different molecular weights, aromaticity and condensation, with the increase in the number of species in the microbiome. Also, if the biosynthesized compounds would influence soil acidity, the dispersion of fluorescent colloidal associations and aggregates, desorption of organic materials, the release of C compounds and the reduction of the fluorescence level could occur. Basically, the biosynthesized microbial C supplements the dissolved organic carbon in the soil and can also stabilize on mineral surfaces. Through electrostatic interactions, based on the compression of the charged layer around an ion, they manifest themselves until the repulsive forces are overcome by the attractive forces. Also, if the concentration of the soil solution ends up causing competition for the sorption sites, they can desorb, causing variations in the fluorescence level, due to the release/adsorption of the constituents.

In general, water soluble biosynthesized C compounds, constitute the most active and also the most mobile fraction of soil organic matter. Also, any increase in organic matter content influences soil health parameters (e.g. available water capacity).

In addition, soluble organic C compounds also play a critical role in many soil processes due to their mobility and reactivity at the soil-water interface.

Monitoring the variations and origin of dissolved organic matter is important in soils because they are factors of biotic functioning in various bio-systems, in the cycle of nutrients and their transport, the solubilisation of organic pollutants, the mitigation of ultraviolet radiation and the control of the bioavailability of metals.

Highlighted variations in dissolved organic carbon were due to characteristics of microorganisms in the composition of microbiomes that functioned metabolically differently and directly influenced soil processes.

Through the specific content of extracellular metabolites released, the microbiomes (M1-M9) inoculated into the soil influenced the dynamics of the energy potential.

Synthetic microbiomes also produce exometabolites that increase organic C retention in various soils, but can also cause a decrease in organic C retention in other soils. These additional inputs of labile organic carbon from microbial secondary metabolism activate C stocks and accelerate microbial decomposition of SOM (Lehmann et al., 2015).
For particularly complex soil conditions, ways can be created in which synthetic microbiomes can be used to promote soil carbon sequestration.
Microbial bio-systems M1-M9 from experiment were realized and selected according to the qualitative/quantitative composition and characteristics of exometabolic compounds.
Microorganisms in synthetic microbiomes are active players in the C cycle in the soil, and these microbial carbon pumps are to practically realize the microbial production of a set of organic compounds as well as their subsequent stabilization.
The composition and use of microbiomes appears important for the evolution of soil C. There are links between the composition of a microbiome and rates of synthesis /decomposition of other compounds, as well as increases in soil C storage.
The extracellular microbial content constitutes a competitive advantage through which microbiomes can influence nutrient transport, energy potential dynamics and tolerance to stress in the soil.
Also, through their intervention you can protect/prolong: the activity of soil processes; stimulating the qualitative and quantitative evolution of organic C; facilitating microbial movements; ensuring the stability of soil aggregates. Also, microbiome composition influences soil processes only if the microorganisms differ in how they function metabolically.

CONCLUSIONS

Soil inoculation with synthetic microbiomes M1-M9, selected for the biosynthesis characteristics of organic compounds, allowed obtaining experimental data regarding their influence on the evolution of soil biodiversity;
The soil inoculation with synthetic microbiomes M1-M9 influenced the rate of quantitative growth of microflora, biomass accumulation and biosynthesis of soluble organic C compounds from the fulvic subfraction, according to the level of their exometabolic activities.
Bacterial and fungal microflora showed significant growth rates compared to the initial values of uninoculated Mollic Histic Gleysol (Salinic), and in the case of inoculation of M4 and M5 microbiomes reaching increases of up to 54%, 60 days after inoculation.
The microbiomes M1 and M5 including bacteria with antagonistic characteristics, hyperparasitic fungi contributed to increases of up to 32% of the total soil microflora.
The microbiomes M4 and M7, which contain diazotrophs and mycorrhizal fungi, stimulated increases of up to 30% of total of counts of soil microflora.
The microbial biomass produced after the inoculation of soil with artificial microbiomes showed an increase of up to 48%, reaching values of up to 354 mg C x kg^{-1} soil (M4), at the end of the experiment.
Microflora from each microbiome influenced differently the distribution of the fluorescent dissolved organic carbon from each water soluble fulvic subfraction of Mollic Histic Gleysol (Salinic).

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