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CARBON STABILIZATION AND SEQUESTRATION PROCESSES IN SOIL THROUGH SYNTHETIC MICROBIOMES

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Abstract

The soil microbial community plays a crucial role in the carbon (C) cycle, acting as a primary driver of variability in the soil's carbon storage potential. The taxonomic composition, through its structure and level of activities, ensures the regulation of nutrient delivery and the rate of decomposition of organic matter. The paper presents the results of the research carried out to assess the role of soil inoculation with synthetic microbiomes (M1, M4, M5, M7) containing performing heterotrophs, in priming the processes responsible for carbon sequestration. Specifically, the study evaluated the influence of these microbiomes on soil glycoprotein level, exopolysaccharide (EPS) production, dissolved organic carbon (DOC) and microbial biomass content. Significant differences were observed among experimental variants, particularly in microbial biomass accumulation. Among the tested microbiomes, M5 demonstrated superior performance, inducing the highest increases in glycoprotein content (1.73 mg·g^{-1}), EPS (594 µg·mL^{-1}), microbial biomass (363.2 mg C·kg^{-1}), and the lowest DOC content (10.13 mg·L^{-1}). These results indicate M5's enhanced integrative capacity and compatibility with soil conditions, supporting its potential role in improving carbon stability and sequestration.

Key words: *dissolved organic carbon (DOC), exopolysaccharides (EPS), glycoprotein, microbial biomass, synthetic microbiome.*

INTRODUCTION

Generally, synthetic communities (SynComs) of microbiomes have a created, controlled composition, with low complexity, an aspect that is reflected in the reduced capacities to manipulate the native microbiota in soils. Their taxonomic composition is important for maintaining soil ecosystem services, because the structure and level of activities regulate the delivery of nutrients and the rate of decomposition of organic matter in the soil. Carbon sequestration, through microbial contribution, is influenced by factors such as the structure of the microbial community, by-products of microbial secondary metabolism, microbial biomass and soil characteristics. Created synthetic communities can present biodiversity, can contain species with different ecological niches and sensitivities to soil pressures, can survive/colonize complex soils, relatively rich in resources, much better. Some of the basic soil microorganisms play key roles, essential in community organization and/or performing specific functions for maintaining communities, so that in the creation of synthetic microbiomes it appears necessary to protect and even adopt such key residents in their composition (Cheng et al., 2022; Finkel et al., 2020). The degree of microbial involvement in the process of carbon stabilization/ sequestration in the soil depends on the complexity of the interactions. Microorganisms selected from the composition of synthetic microbiomes constitute essential components for the presence of soil organic carbon (SOC) and carbon sequestration in terrestrial ecosystems. Thus, synthetic microbiomes together with endemic microbiota mediate the impact of these factors on the transformation of SOC through decomposition as well as the biosynthesis of new microbial C (Rillig et al., 2019; Yue et al., 2020; Li et al., 2023). Synthetic microbiomes can transform primary C derived from decomposition into persistent microbial metabolic products that gradually accumulate in the soil as stable forms of C, such as soil protein bound to glomalin and amino sugars (Irving et al., 2021; Singh et al., 2024; Agnihotri et al., 2022; Feng and Wang, 2023). Inoculated microbiotas attach to organic matter, other organisms, or mineral substrates, aggregating into biofilms with cells embedded in biosynthesized extracellular polymeric substances (EPS), generating adaptability to variable conditions and diverse habitats. EPSs produced by new microbiota in soils restore ecosystem health through the properties of mineral-associated organic carbon formation, mineral particle aggregation, and water retention (Liang 2020; Xiao et al., 2021; Witzgall et al., 2021; Craig et al., 2022; Dong et al., 2022; Sokol et al., 2022; Zhang et al., 2022). Microbiota uses new organic matter as a source of energy and C for growth and biomass formation. Dissolved organic carbon in soil represents only a small fraction of total carbon, but is deeply involved in interactions with microorganisms, minerals and plants. The microbial processes responsible for their dynamics ensure biotic/abiotic degradation and aggregation on/in microparticles. Restoring soil quality and health are key elements in recent research and the use of synthetic microbiomes, a relatively ecological way to improve the carbon cycle. Inoculation of soil with beneficial microorganisms, grouped in different consortia, selected according to exometabolic qualities, integrative capacities and compatibility, can influence the stability and sequestration of biosynthesized C in soil organic matter (SOM). This research aimed to assess glycoprotein, EPS, DOC, and microbial biomass contents following the inoculation of synthetic microbiomes into Mollic Gleysol (Salinic), and to evaluate their potential as indicators of changes in organic carbon stability and sequestration promoted by these microbiomes during microbial restoration of nutrient cycles - particularly the carbon cycle.

MATERIALS AND METHODS

Soil type and characteristics - The sampling site is the Danube Delta, the soil used represents a soil subtype of the soil type, Mollic Gleysol (WRB). The location, using the global positioning system (GPS), was at 45.034452° N latitude and 29.205589° E longitude for Mollic Gleysol (Salinic). The soil was collected in randomly extracted samples, divided into subsamples from the surface layer (0-20 cm), for incubation experiments or stored directly at 4°C, for subsequent soil characterization. The soil had the following parameters: pH value 6.46, organic C content 5.5%, organic matter content 32%, humus content 9.54%, clay content <1.002%.

The experiment - A laboratory experiment was conducted to evaluate the impact of inoculating synthetic microbiomes (M1, M4, M5, M7), previously selected and tested, on the dynamics of organic C compounds, the capacities to influence the processes of stabilization and carbon sequestration in Mollic Gleysol (Salinic). The influence of microbiomes was analyzed after a period of 120 days from the application of 10ml of inoculum/pot and the variants carried out had three repetitions each. Analyses were performed for a series of parameters considered potential indicators for monitoring the processes of stability and carbon sequestration, such as the content in glycoproteins, exopolysaccharides, dissolved organic carbon, water-soluble fulvic compounds.

Microbiomes - The selection and creation of the synthetic microbiomes used M1, M4, M5, M7 ensures the biodiversity, adaptability, integrative capacity and compatibility of the microbial species in their composition. The basic discriminatory criteria were the production of microbial biomass, exometabolites, the level of rhizospheric activity, fungal preponderance, the presence of ecto-, endosymbionts and antagonists. The microbiomes had 7-12 microorganisms, the component microbial isolates were grouped in relation to the biomass/metabolites/CO_2 relationship, and in the case of each microbiome the exometabolites influence on the biochemical parameters characterizing the soil organic matter was monitored.

Composition of synthetic microbiomes - The complex microbial composition of the M1, M4, M5, M7 microbiomes consists of bacterial, fungal, diazotrophic and mycorrhizal microflora. Synthetic microbiomes contain microorganisms belonging to soil microflora, to bacterial genera such as *Pseudomonas, Bacillus, Rhizobium, Azotobacter, Acetobacter, Serratia, Paenibacillus, Streptomyces*, and to the fungal genera *Trichoderma, Stachybotrys, Mortierella, Chaetomium, Arthrobotrys,* *Humicola, Cunninghamella, Myrothecium, Torula, Aspergillus, Penicillium, Penicillium, Boletus, Cladosporium, Glomus*. The selected microbial species are kept in pure cultures, in various culture media (Topping, Czapek, YEM, MMN, LB, BHI, MRS, King), at a constant temperature of 4°C.

The Glycoprotein content - Glomalin-related soil protein (GRSP), as a glycoprotein, was extracted using 1g of dry soil, granulation <2 mm, mixed with sodium citrate, pH 7.0. The mixture autoclaved at 121°C, 30min. was cooled and centrifuged at 5000 rpm, 10 min. resulting in a supernatant, typical of glomalin. The content was quantified colorimetrically using bovine serum albumin (BSA) as a standard and the method of Bradford (1976) for detecting color variations, and the glomalin content in the samples was determined using a microplate reader at 595 nm. Using specific fluorochrome, photographic images of glomalin on the surface of the aggregates were obtained.

Exopolysaccharide (EPS) quantification - The EPS soil sample analyzed by the phenol-sulfuric acid method involved extraction by ultrafiltration with a 10-500 kDa (MWCO) membrane, dissolution in ddH$_2$O, addition of phenol, concentrated sulfuric acid and glucose, used as a standard. The tube with the reaction mixture is left for 10 min. at ambient temperature, shaken and incubated in a water bath at 25°C for 20 min. and the absorbance of the yellow-orange color measured at 490nm, using a spectrophotometer (DuBois et al., 1956).

Dissolved organic carbon (DOC) - The soil samples were air-dried according to Ghani et al. (2003), deionized water was added and the suspensions obtained were subsequently sonicated with an ultrasonic power of 27W, for 10min, at a temperature below 30°C and under continuous stirring. After sonication, the samples were centrifuged for 10 minutes at 10°C, the suspensions filtered through a cellulose acetate membrane with Ø 0.45 μm. The samples were analyzed spectrometrically with UV-Vis, at spectral absorption at 254-400 nm, 1 cm quartz cuvette. The amount of DOC was calculated for substances with absorption in the UV range using the methods of Brandstetter et al. (1996).

Water-soluble fulvic subfraction - A separation of the water-soluble fulvic sub-fraction was achieved from Mollic Gleysol (Salinic) by extraction and subsequent fraction, by adsorption on activated carbon, serial elutions with acetone, NaOH and distilled water (Votolin et al., 2022).

Specific ascending chromatograms - To assess the changes occurring in the water-soluble fulvic sub-fraction, an adapted method of specific chromatograms was used. Through them, the contribution of exometabolites of the microbiomes formed images whose distribution pattern, shape, and dimensions highlighted the intensity of metabolic activities, variations in the composition of the fulvic sub-fractions, as well as changes in specific biochemical characteristics.

Dissolved Organic C Fluorescence - DOC from the water-soluble fulvic sub-fractions of Mollic Gleysol (Salinic) inoculated with microbiomes was treated with fluorochrome and the distribution revealed by specific chromatography (Wang et al., 2016). Photographic images were obtained under the influence of 350 nm UV illumination and revealed qualitative differences such as the distribution, density of biosynthesized components and their complexity, through the degree of affinity for fluorescence.

Microbial biomass - After 120 days of inoculation of synthetic microbiomes in bioassay pots, soil samples were collected, and the soil microbial biomass was determined by the fumigation-extraction method. Soil samples were fumigated with CHCl$_3$ for 24 h at 20°C, extracted with K$_2$SO$_4$ and filtered through cellulose ester. The carbon in the soil microbial biomass was calculated according to Standard-SR-ER-ISO-14240-1-(2012), as a means of three replicates.

Data analysis - Results were interpreted by one way analysis of variance (ANOVA). The value $p<0.05$ was considered statistic significant (Student test).

RESULTS AND DISCUSSIONS

Glycoprotein content in soil - Generally, the glycoprotein content of the soil is considered an indicator of the quality of organic matter, of soil fertility, and a series of recent studies have also highlighted the fact that its values correlate significantly with the content in the microflora. Considering these aspects, the influence of synthetic microbiomes M1, M4, M5, M7 inoculated in Mollic Gleysol (Salinic) on the evolution of the content of glycoproteins, respectively of glomalin-related soil protein (GRSP), was analysed, in terms of a potential impact on the stability and sequestration of carbon. Thus, in Mollic Gleysol (Salinic) inoculation with the M4 and M5 microbiomes determined a GRSP content of 1.35 mg x g^{-1}, and respectively 1.73 mg x g^{-1}. The glycoprotein contribution appears lower after the introduction of the M7 and M1 microbiomes, the glycoprotein content reaching 1.27 mg x g^{-1}, and respectively 1.12 mg x g^{-1}, in the analyzed soil (Figure 1).

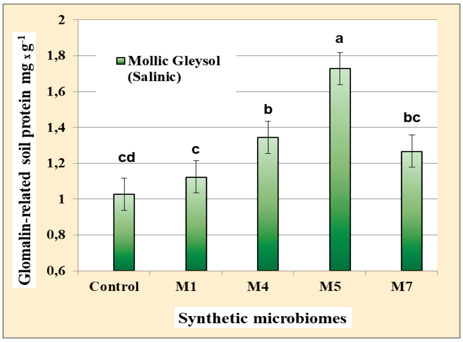

Figure 1. GRSP content generated by inoculating microbiomes in Mollic Gleysol (Salinic)

The GRSP content changed during the experiment, in relation to the analysed microbiome variant, presenting a stability and evolution similar to the intermediate assessments, an involvement in carbon dynamics, but also in the evolution of other carbon evolution indicators (soil respiration, microbial biomass) (Matei et al., 2015; Matei et al., 2017).

GRSP accumulations influence soil properties and compositional features, and for the stability of its optimal properties, GRSP could intervene through quantitative/qualitative adjustments, in guiding the way of managing them in the soil.

The GRSP content in soils, evaluated in numerous recent studies, can be considered an indicator of the quality of organic matter in arable soils (chernozems, luvisols, cambisols, fluvisols, etc.) or under different crops (wheat, barley, rapeseed, alfalfa, clover, potatoes, beet, corn, etc.). In these studies, the results of long-term monitoring and different soil-climate conditions revealed that the GRSP content significantly correlates with the carbon content of soil organic matter, with the ratio of carbon content in humic and fulvic acid (CHA/CFA), with the clay content (Ø < 0.002 mm), with particles smaller than 0.01 mm, with the type and texture of the soil. The GRSP content was significantly higher in chernozems compared to other soils (luvisols, cambisols, fluvisols, etc.). The results of other studies did not reveal significant correlations between GRSP and pH $CaCl_2$, bulk density, porosity, plant-available phosphorus content, the amount of organic matter applied (straw, manure, compost) or the type of organic fertilizer. Research has shown that GRSP has a dual contribution to the storage of soil organic carbon (SOC) through its long residence time (6-42 years) in which it directly improves SOC, through its sticky nature that leads to the formation and stabilization of soil aggregates, both of which protect labile C from microbial decomposition. The persistent composition of microbial biosynthesized products allows them to gradually accumulate in the soil as stable forms of C. Also, the contribution of GRSP to SOC stocks is considered to vary between 5-25%, to which is added the biomass and the microbial necromass. It has also been observed in some experiments that arbuscular mycorrhizal fungi (AMF), which are part of some microbiomes, produce GRSP in close correlation with the intensity of root colonization. Infrared thermogravimetric analyses showed that GRSP is thermostable, contains Fe and can form flocs with soil particles and has high adsorption capacity (76-95%) for metals such as Cu, Zn, Pb, Cd (He et al., 2020; Wang et al., 2021; Hartmann & Six, 2022; Li et al., 2023; San-Emeterio et al., 2024; Singh et al., 2024; Philippot et al., 2024).

The fluorescence affinity of the GRSP structure was analysed after inoculation with synthetic microbiomes, revealing the presence of biosynthesized microbial organic compounds on the surface of the aggregates. The fluorescent components highlight the degree of formation and influence of new biosynthesized compounds on the aggregate structures, adjacent organic matter, in a short period of time (Figure 2).

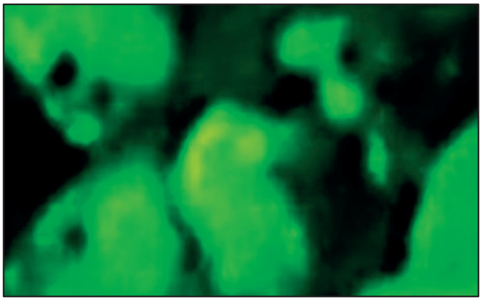

Figure 2. GRSP appear on the surface of aggregates as a green, fluorescent material, after M5 inoculation

The distribution of biosynthesized GRSP was different in the specific case of the type of microbiome applied to the soil. The organic component generated by endemic microflora of the un-inoculated soil allowed the comparison with the contribution of microbiomes and the possibility of preserving biosynthesized microbial C after inoculation in the soil. Through the dynamics of microbial processes and activities, through the composition of the extracellular organic component introduced into the soil, the direct impact on the organic component can be highlighted in the case of the M5 microbiome through the influence on the aggregate structures, generating organo-mineral protection.

Exopolysaccharides content in soil - Soil microbial exopolysaccharides (EPSs) are involved in the colonization and survival of microbiota, in the formation and stabilization of aggregates, in the enzymatic activity of organic matter, in the aggregation and degradation of minerals, in the water retention capacity, in the storage and capture of nutrients, in the sequestration and transformation of contaminants. The cells appeared incorporated in the self-produced extracellular biopolymeric substances, as a result of the strategy of adaptation and resistance to the conditions in Mollic Gleysol (Salinic). Their qualitative and quantitative content in the soil appeared possibly in close agreement or complementary to the composition of the exometabolites excreted by the endemic microbiota of the inoculated soil and thus helping the assessments regarding the improvement of ecological functions, through extracellular products. Thus, the biochemical protection offered to carbon compounds by the components of the synthetic microbiome M5, through the increased EPS biosynthesis capacity (594 µg x ml^{-1}), was more efficient than that offered by the components of the microbiome M4 (548 µg x ml^{-1}). This aspect perhaps suggests a more intense involvement in carbon sequestration/stabilization in Mollic Gleysol (Salinic), possibly through a direct adsorption of exometabolized organic molecules, as well as possibly through an intensification of aggregation with soil minerals. Also, the synthetic microbiomes M7 and M1 inoculated into the soil influenced the distribution of carbon compounds in the fulvic sub-fractions. Biochemical protection was provided less effectively by EPSs metabolites biosynthesized by the M7 and M1 microbiomes (487 µg x ml^{-1}, and respectively 436 µg x ml^{-1}), which generated a complexity and a reduced influence on the stability and sequestration of organic carbon in the soil (Figure 3).

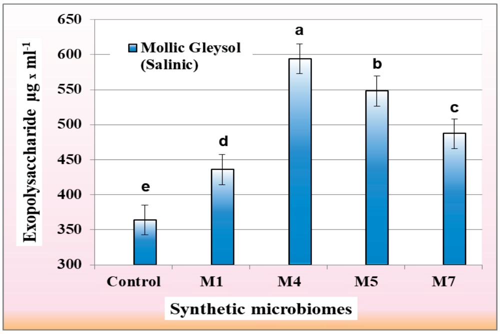

Figure 3. EPS content biosynthesized by microbiomes inoculated into the soil

The composition of EPSs is improved by the contribution of the new microbiota inoculated in Mollic Gleysol (Salinic) which intervenes in maintaining soil health by increasing the stability of aggregates and retaining extracellular enzymes to ensure the metabolic stability of the soil. The exopolysaccharide content biosynthesized in the soil by synthetic microbiomes, differed in the inoculated experimental variants, correlated with the changes in structure and complexity of the organic carbon content in the water-soluble fulvic sub-fractions of Mollic Gleysol (Salinic).

The bacterial microflora of the synthetic communities (SynCom) contributed substantially to maintaining the stability of C compounds in both macro-aggregates and micro-aggregates, and the fungal microflora had a strong impact predominantly on macro-aggregates. Thus, recent studies have highlighted the role of the *Bacillus* and *Pseudomonas* genera that secrete EPSs or their components in the formation of biofilms. The ability of microflora to improve soil aggregate formation is influenced in particular by the ability of the selected strains to secrete EPSs, the composition and biosynthesized structure of EPSs (Lehmann et al., 2017; Bérard et al., 2020; Cheng et al., 2020; Costa et al., 2020; Wei et al., 2024).

In studies on the biodegradation of polycyclic aromatic hydrocarbons in soils, bioremediation is effective because EPSs create bioavailability and accelerate their biodegradation, the dense matrix of EPSs being involved in the biodegradation of benzo-pyrene through external electron transfer, enzymatic catalysis, photo-degradation, etc. Also, quantitative quantifications, analyses of the composition and structure of EPSs have allowed estimates of the response of soil microbiota to stress, as well as the involvement of biosynthesized EPSs in soil functions and in ensuring its health (Flemming et al., 2023; Costa et al., 2020; Nkoh et al., 2020; Guhra et al., 2019).

Microorganisms in synthetic microbiomes can release stress-sensitive EPSs in difficult environments, supporting minimal nutrient concentrations and prosperity in oligotrophic conditions, constituting a resource of hydrocolloids, biodegradable and biocompatible biopolymers, bio-sorbents and soil improvers (Mahmoud et al., 2021; Kant et al., 2023; Ibrahim et al., 2022).

Studies on the taxonomic composition of microbiomes include bacterial and fungal species that produce EPSs and belong to the genera *Rhizobium, Pseudomonas, Bacillus, Azotobacter, Acetobacter, Azospirillium, Aureobasidium, Schizophyllum, Sclerotium, Botryosphaeria, Lentinus, Grifola*, etc. (Bourles et al., 2019; Deka et al., 2019; Luláková et al., 2019; Ali et al., 2020; Almansoory et al., 2020; Aksenov at al., 2021; Osińska-Jaroszuk et al., 2021; Jeong et al., 2022; Liu et al., 2022; Sagar et al., 2022; Balducci et al., 2023; Stoica et al., 2023; Liebmann et al., 2024).

The microbiota of microbiomes inoculated in Mollic Gleysol (Salinic) produce EPSs, as metabolic by-products or as extracellular polysaccharides, initially important in biofilm formation and microbial interactions.

Dissolved organic carbon content in soil - DOC, as a relatively labile fraction, actively participates in the soil in the processes of stimulating microbial activity and composition, co-metabolism, the passage of soil organic carbon into labile fractions, promoting the formation of aggregates, introducing stable components, inhibiting microbial activities, sorption of soil organic carbon and stimulating specific microorganisms involved in the C cycle belonging to the genera *Pseudomonas, Acidobacter, Acidimicrobium* etc.

Given its complex role in soil carbon dynamics, the evolution of the dissolved organic carbon content under the influence of inoculated microbiomes was analysed in the laboratory experiment in order to evaluate the stability and sequestration of carbon.

Thus, Mollic Gleysol (Salinic) inoculated with the M5 microbiome had a DOC content of 10.13 mg x L^{-1}. After inoculation of the M4 microbiome, at the end of the experiment, the dissolved organic carbon content determined was 11.89 mg x L^{-1} in Mollic Gleysol (Salinic). The highest dissolved organic carbon content was obtained after inoculation of the M7 and M1 microbiomes, 12.64 mg x L^{-1} and 14.06 mg x L^{-1}, respectively.

In the case of the M1 and M7 microbiomes, the dissolved organic carbon content in the soil decreased by 10.62% and 19.82%, respectively, compared to the control, and under the influence of the M5 microbiome, the dissolved organic carbon content decreased by up to 35.69%, compared to the control (15.76 mg x L^{-1}) (Figure 4). In the controlled environment, the variation of dissolved organic carbon concentrations did not depend on other disturbing factors such as the increase/decrease of average nitrate (NO^{-3}), sulphate (SO_2^{-4}), pollutants, climatic variations, etc., their magnitude depending on the specific influence of the microbiomes. The trends over the 120 days of DOC in the soil solution reflected the interactions between soil properties and the specific characteristics of the microbiomes.

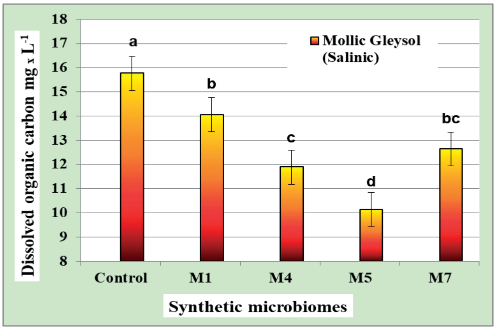

Figure 4. Dissolved organic carbon content in Mollic Gleysol (Salinic) inoculated with synthetic microbiomes

The amount of C stored in the soil depends on the metabolism of the microbial community and its physico-chemical structure, on the interactions or chemical absorption of carbon molecules to the mineral component, through which the accessibility of carbon can be modified. Extracellular enzymes produced by the synthetic microflora of the microbiomes may have a role in the production of consumable carbon compounds involved in the growth and activities of the microbiota. Through turnover, taxa from synthetic microbiomes contribute to the generation of necromass composed of melanins, chitins, hydrophobins, glycoproteins, lipids, β-glucans, etc., which enter the composition of DOC (Albright et al., 2020).

Recent studies have also identified in the early stages of soil decomposition of organic material, up to 42 bacterial and 9 fungal taxa, including protein compounds and tannins formed as a result of the process, which positively correlated with DOC concentrations. Changes in DOC concentration in the soil solution indicate the links of taxa with microbial protein consumption, and the observed correlations probably indicate their role as interconnecting factors between DOC concentration and microbial production, the decomposition of specific metabolites and carbon persistence (Campbell et al., 2022).

In some studies, DOC accumulation in soil has been influenced by the chemical profile of carbon generated by synthetic microbiomes acting on the substrate and on the endemic soil microbiota, such that some compounds and even groups of compounds can be predictive of increased soil DOC concentration and carbon storage (Thompson et al., 2019; Albright et al., 2020; Ghannam & Techtmann, 2021; Campbell et al., 2022).

Water-soluble fulvic sub-fractions - In the water-soluble fulvic sub-fractions were highlighted by fluorescence the changes in the composition structuring induced under the influence of the M1, M4, M5, M7 microbiomes inoculated in the soil. The results may suggest that the microbial carbon pumps in the analysed soil were stimulated differently depending on the microbiome, respectively the microbial composition and their metabolic and biosynthetic characteristics.

Thus, in Mollic Gleysol (Salinic) inoculated with the synthetic microbiomes, the extracted and isolated water-soluble fulvic sub-fractions were analysed by ascending chromatography in terms of the molecular distribution of the compounds, the formation of more stable molecular structures, of complex organic carbon compounds, highlighted by their fluorescence affinity. The analysed water-soluble fulvic sub-fractions presented the highest density of organic compounds, after 120 days from the inoculation with the M5 microbiome, in Mollic Gleysol (Salinic). In comparison, the synthetic microbiome M4 inoculated in the same soil had a water-soluble fulvic sub-fraction with organic carbon structures highlighted by the presence of dense areas with a lower molecular weight, as well as those with a lower density, represented by more complex compounds with a higher molecular weight. The stability of organic carbon (OC) compounds was lower in the analysed fulvic sub-fraction, after inoculation with M4, because the biochemical protection offered by exometabolites secreted by the components of the own microbiome was weaker compared to the stability of C compounds offered by the amount/diversity of exometabolites biosynthesized by the components of the M5 microbiome. In comparison, the synthetic microbiome M7 inoculated into the soil influenced the water-soluble fulvic sub-fraction by enriching it with new structures of biosynthesized organic C highlighted by the presence of dense areas dominated by compounds with lower molecular weight, the increase in the share of those with reduced density, respectively with higher complexity and molecular weight. The stability of organic C in the soil inoculated with M7 is increased compared to M1, but lower than that provided by the microbiomes M5 and M4. In addition, even under conditions of reduced protection of C compounds, compared to other microbiomes, the exometabolites of the M7 microbiome provide a higher efficiency for the stability of organic C, than that provided by the microbiome M1 (Figure 5).

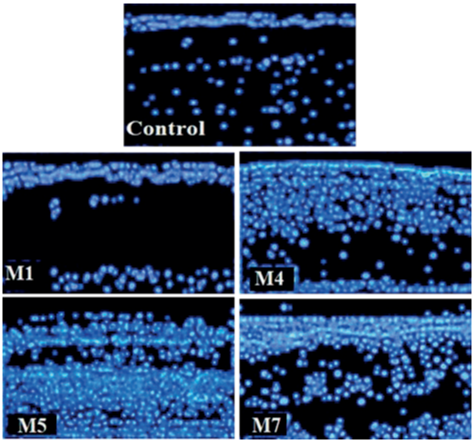

Figure 5. Water-soluble fulvic sub-fraction of soil after 120 days of microbiomes inoculation

Microbial biosynthesis of carbon in soil, for short periods of time and under standard conditions, generated labile substances, biosynthesized by endemic and inoculated microflora, extractable in water but also with fluorescence. To these was added the fluorescence of organo-mineral, humic and protein substances from the organo-mineral reserve of the soil. Monitoring their distribution and their visualization allowed estimates regarding the short-term influence of different microbiomes, with a biosynthetic role and possible options for enhancing C storage. Imaging, fluorescent compounds highlighted the degree of formation, distribution and organization of newly biosynthesized carbon, compared to uninoculated soil, under the influence of synthetic microbiomes. The distribution of biosynthesized carbon was specific to each type of microbiome applied to the soil. The dynamics of the processes and the composition of exometabolites introduced into the soil allowed highlighting the direct impact on the organic component. Biosynthesized exometabolites, detectable by fluorescence, revealed an abundance of fulvic materials of different molecular weights and degree of condensation, closely related to the number of species in the inoculum composition. Biosynthesized microbial C complements DOC in the soil, can stabilize on mineral surfaces, but if their concentration in the soil solution causes competition for sorption sites, desorption can also occur, with an impact on fluorescence. In addition, as a result of microbiota-mediated processes, the amounts of protons and the anion exchange capacity may increase in the soil, which allows a good highlighting of biosynthesized organic molecules.

Microbial biomass content in soil - The biostimulatory influence of selected synthetic microbiomes (M1, M4, M5, M7) on the evolution of total microbial biomass was analysed on Mollic Gleysol (Salinic).

The inoculated synthetic microbiomes contained different microbial species, present in different concentrations, compositions and ratios.

The biomass produced by the synthetic microbiomes inoculated in Mollic Gleysol (Salinic) reached values of up to 363.2 mg C x kg^{-1} soil (M5), after 120 days from inoculation and represented the experimental variant with an increase of up to 35.14% in the amount of microbial biomass. In the variants inoculated with the M4 and M7 microbiomes, the microbial biomass content increased by 25.28% (M7) to 31.28% (M4), reaching in its case a biomass of 352.8 mg C x kg^{-1} soil.

Under experimental conditions, biomass accumulations induced by inoculation showed variations ranging between 14.64-35.14% for the microbiome variants. The activity and diversity of the synthetic microbiomes correlated positively with microbial biomass. The biomass level reflected the intensity of activities generated by the inoculated microbiomes in the soil, maintaining constant conditions allowed the evaluation of their influence on the analysed parameter. The biomass accumulations reflected, in addition to the bio-stimulatory effect of the 4 synthetic microbiome variants, both the integrative capacities of the new communities of microorganisms introduced into the soil, and the functional compatibilities with the endemic soil microflora (Figure 6).

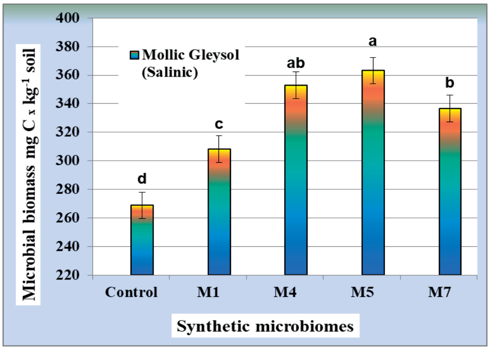

Figure 6. Microbial biomass of the Mollic Gleysol (Salinic) inoculated with synthetic microbiomes

In inoculated microbiomes, the fungal microflora, in addition to biosynthetic activity, is subsequently transformed into necromass, constituting the main microbiological contributing factor for the creation of stable soil organic matter in a much higher proportion, compared to the bacterial necromass originating from microbiomes. Also, the mycorrhizal fungi in the composition of synthetic microbiomes contribute significantly to the sequestration of carbon in the soil by transferring photosynthates to intraradicular hyphae, then to extraradicular hyphae and subsequently, to their release into the soil matrix, involving in obtaining these results the amount of hyphal biomass produced and the stabilized soil aggregates. Microbial growths, in the initial phase of decomposition, contain mainly bacterial taxa of the phylum Proteobacteria and fungi of the phylum Ascomycota, which is a particularly useful aspect regarding the involvement in the formation of soil organic matter (SOM). The relationships between natural/synthetic microbial communities in soil, organic matter and external factors manifest their impact on carbon storage, on the variability of available carbon in the soil. Synthetic communities (SynCom) can intervene to improve the microbial composition of soils and present themselves as a sustainable solution in restoring soil qualities.

Thus, synthetic microbiomes are not only participating elements in the soil, but have a facilitating role in the optimal functioning of ecosystems through interventions on: increasing the content of organic matter, through secondary metabolism compounds, organic nitrogen fixation, increasing the availability and acquisition of nutrients, inhibiting pathogens, secretion of extracellular biopolymers through which they bind soil particles into aggregates, creating stable structures, improving soil fertility, and plant growth (Asiloglu et al., 2021; Wang et al., 2021; Hartmann & Six, 2022; Zhang et al., 2022; Hao et al., 2024).

CONCLUSIONS

Synthetic microbiomes selected for their increased exogenous metabolic capacities for biosynthesis of organic compounds, compatibility, viability, intervened in the stabilization and sequestration of carbon in the soil.

Comparative studies and experimental data validated, through process indicators, the role of synthetic microbiomes with performing heterotrophs in priming soil processes and in carbon sequestration.

The analysed water-soluble fulvic sub-fraction in the soil inoculated with synthetic microbiomes allowed highlighting the different molecular distribution of the biosynthesized ones, the formation of more stable molecular structures of organic carbon, through changes in the distribution and molecular weight.

In the analysed soil, the endemic microbial communities as well as those from the inoculated microbiomes underwent quantitative growths, aggregated into biofilms in which the cells appeared incorporated in self-produced EPS substances, as a strategy of adaptation and resistance into the soil conditions.

The biosynthesized EPSs content determined by laboratory analyses correlated with the changes in the structure and complexity of the organic carbon content in the fulvic sub-fraction of the soil.

The GRSP content varied in relation to the type of inoculated microbiome, presented a stable evolution, influenced the carbon dynamics and correlated with other indicators of carbon evolution.

The determined biosynthesized EPSs content varied in the case of the microbiomes and correlated with changes in the complexity of the organic carbon content.

GRSP, EPSs and DOC content in the soil varied significantly depending on the type of inoculated microbiome and were sensitive in detecting the amounts of bio-generated carbon after inoculations, as potential indicators of the evolution of carbon stability/sequestration.

The efficiency of synthetic microbiomes was validated by their specific contribution to the differential increase in biomass accumulations in the soil.

The results demonstrated that microbial carbon pumps in the soil were stimulated differently in the edaphosphere depending on the composition and characteristics of the microbiomes.

The results of the comparative study on the efficiency of selected synthetic microbiomes in carbon stability/sequestration processes highlighted the M5 and M4 microbiomes, for the composition and functional efficiency in the newly formed microbial communities.

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