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# THE INFLUENCE OF INOCULATION WITH SYNTHETIC MICROBIOMES ON THE EVOLUTION OF SOIL CARBON SEQUESTRATION UNDER THERMAL STRESS

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***Abstract***

*Synthetic microbial communities form networks of diverse microorganisms interconnected through a variety of interspecific interactions. Considering that positive interactions are favored in more stressful environments, assessments of the influence of complex synthetic microbial communities required a more precise identification, analysis of the evolution and amplitude of interactions. The effect of inoculation and the involvement of synthetic microbiomes in carbon sequestration/release was determined after evaluations of glycoproteins, exopolysaccharides (EPS) and dissolved organic carbon (DOC) content, under thermal stress conditions (35ºC and 45ºC). The Mollic Gleysol inoculated with microbiomes M5 and M7 had a DOC content in the rhizosphere at both temperatures below the level of the uninoculated control. Also, M7 induced the highest changes in glycoprotein content, which, after 14 days at 45ºC, reached 1.79 mg·g$^{-1}$. Under similar experimental conditions, M5 was highlighted by inducing the highest changes in exopolysaccharide content (587µg·ml$^{-1}$). Synthetic microbiomes (M1, M4, M5, M7) promoted beneficial interactions in soil after inoculation, strengthened beneficial microbial cooperation and improved organic carbon decomposition/cycling in the rhizosphere.*

***Key words:*** *dissolved organic carbon, exopolysaccharides, glycoproteins, microbiome, thermal stress.*

## INTRODUCTION

Carbon (C) in soil organic matter (SOM) is integrated into a wide range of organic compounds, from mobile, chemically reactive forms to stable, recalcitrant forms. They may be protected from decomposition and accumulate due to their chemical nature or physical stabilization, achieved by binding to particles and/or placement in aggregates, under specific microenvironmental conditions.

Among the compounds, some can be attributed to specific organic groups, such as certain lignins, tannins, waxes, and other compounds are produced exclusively by microorganisms, such as specific glycoproteins, exopolysaccharides, amino-sugars, ether-lipids, fatty acids (Six et al., 2006; Knappy et al., 2015; Lehmann et al., 2017; Guhra et al., 2019; Cheng et al., 2020).

For ecosystem functioning, the carbon content sequestered in organic matter (OM) is important for its feedback effect on soil quality, microbial activities, ensuring the maintenance of ecosystem services, including those involved in carbon stability/sequestration processes, soil aggregate stabilization, water retention, slow nutrient release, root exudates and soil-plant relationships (Heimann & Reichstein, 2008; Ding et al., 2015; Bradford et al., 2016).

Soil organic carbon (SOC) is deposited in any layer of the trophic profile, coming practically directly from plants or from components of the soil food web, which includes exudates, excretions, exometabolites, dead biomass residues and other organic compounds in particulate or dissolved form.

SynComs represent initially carefully selected microbial groups, designed with the aim of optimizing microbiome functions (plant growth, synthesis of disease resistance, a.s.o.) and achieving stability upon contact with native communities.

The microbial species used in the construction of SynComs also differ in terms of growth and adaptive capacities at different temperatures (Bárcenas-Moreno et al., 2009; Engqvist, 2018; Boyle et al., 2024; Zhao et al., 2024).

Assessments of the degree of sensitivity and growth rates of different species to temperature have been carried out in ecosystems with different microbiomes, to estimate the impact on the level of ecosystem functionality (Bowles et al., 2014; Hungate et al., 2015, Koch et al., 2018).

The presence of SynComs leads to changes in plant metabolic pathways, through the type and amount of exudated C compounds (organic acids, amino acids), more useful for symbiotic association or in secretion of specific compounds, for the recruitment of beneficial bacteria in the rhizosphere.

Under thermal stress, microbiomes induce changes in the profile of root exudates, enhance the synthesis of specific signaling compounds for plant adaptation, as well as the secretion of enzymes, determined by specific carbon compounds released by mycorrhizal roots (Li et al., 2017; Preece & Peñuelas, 2020; Goddard et al., 2021; Wu et al., 2022; Sharma et al., 2023).

Temperature effects on carbon content include increases in secondary metabolism, intensification of plant root exudation, accelerated microbial decomposition, transformations into organic carbon and water-extractable labile compounds.

Carbon dynamics can be mediated by microorganisms with specific thermal thresholds, where behavior evolves from rapid degradation to accumulation, at temperatures of 26-35°C and which even promote the retention of refractory dissolved organic matter. Temperature increases, also enhance dissolution, diffusion, exchange and increase in soil carbon concentrations, induce qualitative changes, generate reduced molecular weights, aromaticity and complexity. Soil temperature, in deeper layers, appears positively associated with carbon concentrations and, if temperatures increase, carbon availability increases and potential carbon losses accelerate, especially in temperate agroecosystems.

The study aimed the evaluation of four SynComs variants formed by selected microorganisms that actively contribute to the carbon cycle.

Inoculated into soil, they constitute sources of microbial C, which specifically influence the evolution of the microbiota through the synthesis of organic compounds in the soil and in the rhizosphere of plants, contributing to a possible subsequent stabilization of additional C in SOM, under thermal stress conditions.

## MATERIALS AND METHODS

**The experiment** analyzed the influence of the composition of synthetic communities (SynComs) of the microbiomes M1, M4, M5, M7 (Matei et al., 2025) on the storage/ sequestration of biosynthesized organic compounds in the soil, after the application of 10 ml SynCom inoculum/pot with concentrations ranging between $4.56\text{-}7.32 \cdot 10^3$ CFU $mL^{-1}$ for fungi and between $7.25\text{-}9.34 \cdot 10^6$ viable cells $mL^{-1}$ for bacteria, under termosensible tomato (*Solanum lycopersicum* L.) culture.

After a period of 60 days of vegetation, the biotest pots with Mollic Gleysol, tomatoes and inoculum were incubated under controlled conditions for periods of 3 days and 14 days, for a duration of 8h/day at temperatures of 35°C and 45°C, maintaining humidity.

Plant and soil samples have been taken from each culture pot for specific analyses, in three replicates

**The synthetic communities (SynComs)** correspond to the microbiomes M1, M4, M5, M7 containing selected bacterial and fungal heterotrophic microflora, with exometabolic efficiency and not genetically modified. Microbial strains selected were preserved in pure cultures on special culture media, Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi, at 4°C.

**Composition of microbiomes** - The inoculated SynComs contain microorganisms belonging to 9 bacterial genera including *Bacillus, Azotobacter, Rhizobium, Acetobacter, Paenibacillus, Streptomyces* a.s.o. and 16 fungal genera including *Torula, Stachybotrys, Humicola, Cladosporium, Micromonospora, Glomus*. The composition of SynComs M1, M4, M5, M7 includes microflora with the same genera. The specificity of each SynCom is given by the different metabolic capacities of the component strains.

**Glycoprotein content -** Glycoproteins were extracted from dried soil with sodium citrate at pH 7.0, autoclaved and centrifuged at 5000 rpm for 10 min. Using standard bovine serum

albumin (BSA), the glycoproteins content was evaluated colorimetrically. Bradford reagent was added to each cuvette and mixed by repeated inversion of the cuvette.

The mixture was incubated at room temperature for 5 min., and after the color change from brown to blue, the absorbance of the samples and the standard was measured spectrophotometrically at 595 nm.

The glycoprotein concentration of the samples was calculated by comparing their absorbance values with those of the standard curve (Bradford, 1976).

**Fluorescence of glycoproteins -** Fluorophores and fluorescence-based techniques were used to obtain images of glycoproteins on the surface of rhizosphere aggregates, under stress conditions.

Fluorescence of glomalin-related soil protein (GRSP) was revealed using thin soil sections to distinguish organic material from minerals, taking advantage of its characteristic fluorescence in the blue/green range.

**Exopolysaccharide (EPS) quantification -** Soil samples were analyzed for EPS content using the phenol-sulfuric acid, followed by ultrafiltration and succesive applications of 1ml phenol (5% in water), 5 ml sulfuric acid (95%) and 2ml glucose (80 mg $L^{-1}$).

The mixture was left for 10 min, shaken and maintained at 25°C for 20 min, in a water bath. The absorbance was measured at 490 nm with a spectrophotometer (DuBois et al., 1956).

**Dissolved organic carbon (DOC) content -** Deionized water was applied to air-dried soil samples, the suspensions were sonicated with an ultrasonic power of 27W, stirred at 30°C for 10 min, then centrifugated at 10°C for 10 min. For extraction ultrapure water was used, ratio soil:extractant was 1:10, without changes in pH.

The suspensions were pre-filtered with a cellulose filter, additionally filtered by cellulose esters membranes with pore diameter of 0.45 μm and DOC absorbance was evaluated at 254-400 nm with UV-Vis spectrophotometer (Bolan et.al., 1996).

To establish the calibration curve, the absorbance was performed in relation to standard humic substances.

**Fluorescence of Dissolved organic C** - The water-soluble fulvic sub-fractions with DOC, after inoculation of SynComs, contained added fluorochrome and their arrangement in soil was evidenced by specific ascending chromatography (Wang et al., 2016).

**Data analysis** - The analysis of variance (ANOVA) and Student test were used for the results evaluation.

## RESULTS AND DISCUSSIONS

The experiment assessed the influence of inoculating Mollic Gleysol with selected SynComs (M1, M4, M5, M7) and evaluated their involvement in the initiation of processes responsible for C synthesis/ sequestration.

The influence of SynComs was estimated through changes in glycoprotein, exopolysaccharide and dissolved organic carbon content, under thermal stress conditions. The metabolically active microbial species in the analyzed SynComs acted by forming new organic molecules or by complexing existing ones.

Glycoproteins, namely glomalin-related soil proteins (GRSP), are closely associated with the amount of carbon and nitrogen reserves in the soil, heavy metal sequestration, aggregate stability and stress mitigation (Liu et al, 2022).

The processes of synthesis, accumulation, as well as the decomposition of GRSP in soils depend on both the interactions of SynComs with native soil biota and the processes in the rhizosphere of cultivated plants.

Generating new/modified metabolic pathways, SynComs intervene in changes regarding the type/quantity of glycoproteins present in the rhizosphere soil and in the activities of endemic biota, influencing the rates of decomposition/ synthesis of new organic compounds.

Among the SynComs tested, M7 demonstrated superior performance, generating large increases in glycoprotein content (1.92 mg $g^{-1}$) after 14 days at 35°C. Their level decreased by 6.7% (1.79 mg $g^{-1}$) at 45°C, under the influence of the same microbiome.

The variations in glycoprotein content in the rhizosphere area, after incubation for 3 days at 35°C, compared to 45°C, were only 2.3% under the influence of M7. SynComs M5 and M4 determined, after 14 days at 35°C, 1.56 mg $g^{-1}$ and 1.43 mg $g^{-1}$, respectively.

At 45°C, under similar conditions, decreases of 7.6% and 8.3%, respectively, in glycoprotein content were recorded. In the rhizosphere, after incubation for 3 days at 35°C, the decreases in glycoprotein content compared to 45°C were 4.4% under the influence of M5 and 5.7% under the influence of M4.

SynCom M1 induced after 14 days at 35°C a glycoprotein content of 1.17 mg·g$^{-1}$, a level that decreased by 11.7% at 45°C, but which remained 5.82% higher than the uninoculated control (0.97 mg·g$^{-1}$), under high thermal conditions (Figure 1).

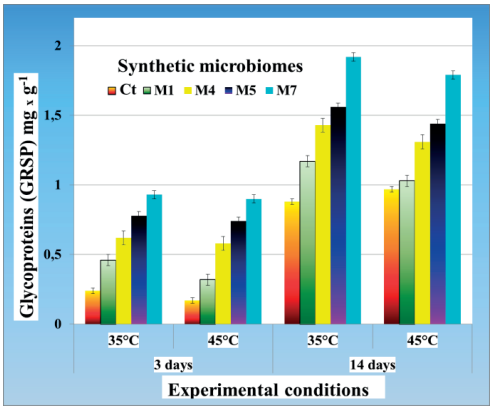



Figure 1. GRSP content generated by inoculating Mollic Gleysol with SynComs under two different periods of time and temperatures

The stable aggregates formed by the new glycoprotein input determined favorable changes in the stability of the soil structure, prolonging the persistence of sequestered carbon through the significantly modified qualitative/quantitative content that allows the establishment of a more stable reserve in the soil.

The presence of *Azotobacter* species in the rhizosphere, recently introduced through SynCom, enriched the rhizosphere in nitrogen, possibly causing a reduction in the oxidative enzymatic activity that decomposes glycoproteins.

Through reactions and involvement they can be considered indicators of the quality of organic matter, recent studies highlighting the fact that their level correlates with the ratio of carbon in humic and fulvic acids and with the ratio of C in SOM and microbial biomass.

Glycoprotein accumulations constitute an essential component of SOM composition. Glycoprotein content undergoes changes depending on temperature, exposure time, SynCom variant and their involvement in carbon cycling, soil respiration and microbial biomass (Pietikainen et al., 2005; Walker et al., 2018; Ye et al., 2019; Matei et al., 2024; Matei et al., 2025).

As a possible indicator of carbon accumulation, glycoprotein content changed during the experiment, varying according to time, temperature and type of SynCom inoculated. Glycoprotein accumulations were found in all SynComs up to 35°C and increased over time, later evaluations revealed significant decreases at 45°C and incubation for 14 days, the differences being generated by the composition of the SynCom type, within the thermal ranges.

Thus, the SynComs intervened in the quantitative/qualitative regulation of glycoprotein accumulations, generated changes in properties and compositional traits and improved soil properties.

They also significantly contributed to the storage of SOC, due to qualitative diversification and quantitative input, to the increase in the residence time of C and its stabilization in soil aggregates, protecting the labile organic compounds of C, generated by microbial decomposition.

The modified composition of the newly synthesized microbial compounds ensures the gradual accumulation in the soil, as stable forms of C (Liu et al., 2020; Holátko et al., 2021; Wang et al., 2021; Agnihotri et al., 2022; Guo et al., 2022; Yuan et al., 2022; Yang et al., 2024a; Zhou et al., 2024; Ling et al., 2025).

The biosynthesis of glycoproteins after SynCom inoculation differs qualitatively/quantitatively from the specific composition generated under thermal conditions. The glycoprotein component generated by the specific microbiota of SynComs was analyzed by fluorescence after an exposure time of 14 days, under thermal stress conditions.

Due to the dynamization of microbial processes/activities under these conditions, glycoprotein accumulations appear highlighted by fluorescence, especially at 35°C. The set of newly synthesized compounds under the

influence of SynComs appear on the surface of the aggregates as fluorescent clumps (Figure 2).

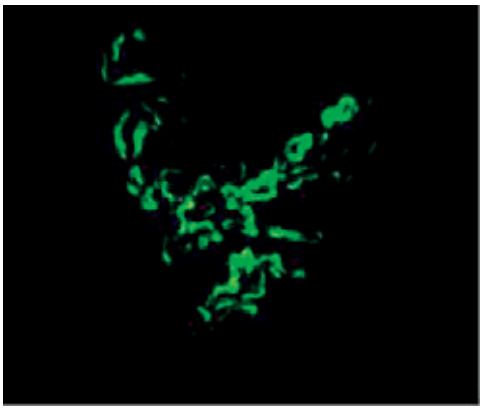


Figure 2. Glycoproteins GRSP appear as fluorescent clumps, after inoculation with the SynCom M7 and exposure at 35°C for 14 days

The direct impact of additional compounds biosynthesized by SynComs under thermal stress influences the structure of rhizospheric organic components and aggregates, generating a possible form of protection at the organo-mineral level.

Under the influence of SynCom M7, fluorescent components highlight the accumulations and distribution of the new glycoprotein complex at 35°C in aggregates, after 14 days of incubation under controlled conditions.

Under heat stress conditions, SynComs can also influence the evolution of soil glycoprotein content through glycosylation, enhanced synthesis of stress-protective glycans, and changes in symbiotic relationships.

Thus, SynComs act through modifications that promote the maintenance of functional stability, stimulation of metabolic activities, and adaptability by regulating their responsibilities regarding temperature and reactive oxygen species at the protein level.

SynComs inoculated into Mollic Gleysol produce large amounts of EPS, through the component strains, which form and accumulate as a protective film around the roots, inducing an increase in the ability of tomatoes to tolerate heat.

Thus, through EPS, soil health is improved by increases in aggregation capacity, water retention in the rhizosphere zone, hormonal regulation, root growth and thermal tolerance.

Under experimental conditions, the performances of EPS-producing SynComs were analyzed and, among them, SynCom M5 induced, in the tomato rhizosphere, the highest increases in EPS content at 35°C after 3 days (552 $\mu g \cdot ml^{-1}$) and 612 $\mu g \cdot ml^{-1}$ after 14 days, compared to the uninoculated control variant (527 $\mu g \cdot ml^{-1}$). At 45°C, after 3 days of incubation, EPS content decreased to 543 $\mu g \cdot ml^{-1}$ and after 14 days to 587 $\mu g \cdot ml^{-1}$ (Figure 3).

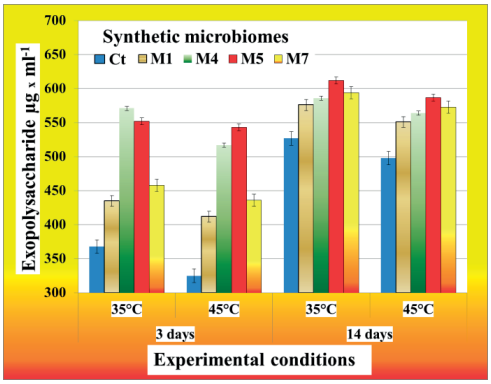



Figure 3. EPS content, after SynComs inoculating, generated in Mollic Gleysol under two different periods of time and temperatures

The results suggest an intense involvement in carbon sequestration/stabilization in the Mollic Gleysol, possibly due to the direct adsorption of new exometabolized organic molecules and the intensification of aggregation with rhizosphere minerals.

Thus, the biochemical protection offered to carbon compounds by SynCom M4 components is expressed by an increased capacity for EPS biosynthesis at 35°C, more efficient than that offered by SynCom M5 at 3 days.

At 45°C, SynCom M4 offered weaker protection than M5 and the exopolysaccharide content decreased after 14 days of incubation to 564 $\mu g \cdot ml^{-1}$. SynComs M7 and M1 produced, after 14 days at 35°C, an EPS content that increased by 12.7% and 9.4%, respectively, compared to the uninoculated control.

At 45°C, the EPS content decreased, under the influence of these microbiomes, in both

exposure periods (3 and 14 days), but remained at values higher than the uninoculated control, under increased thermal conditions.

SynComs M7 and M1 synthesized EPSs that had a lower efficiency in sequestering carbon in the soil (436 $\mu g \cdot ml^{-1}$ and 412 $\mu g \cdot ml^{-1}$, respectively), under experimental conditions.

The new EPS compositions from the tomato rhizosphere express the result of co-generation by SynComs and native soil microbiota, compositions that intervene, through the induced changes, in the modeling of the ecological functions of the soil.

Thus, in the soil, EPSs represent carbon-rich molecules, produced by soil microorganisms, which form rich sources of C added directly to the SOM reserve. In the Mollic Gleysol, EPSs synthesized in the rhizosphere by the SynComs microbiota intervene in the improvement of the quality/sequestration of C achieved through the formation of microaggregates from soil particles and organic matter.

By generating these more stable structures, the newly formed aggregates can, in turn, protect the newly synthesized C, sequestering it in the soil for longer periods of time, ensuring cohesion, preventing erosion and subsequent C losses. The SynComs used, through the structure of the communities, influenced both the evolution of EPSs in the rhizosphere, under thermal stress, and the selection, through direct mechanisms, of the native EPS-producing biota.Also, the production, composition and functional properties of EPSs in inoculated soil can be significantly influenced by temperature, which can constitute a stress factor involved in triggering the increase in EPS synthesis and ensuring microbial survival (Sandhya & Ali, 2015; Nouha et al., 2018, Cania et al., 2019; Silambarasan et al., 2019; Asgher et al., 2020; Yang et al., 2022; Yang et al., 2024b).

For the composition of native and synthetic soil microbiota, temperature is one of the critical environmental signals that influences the production of EPSs with a protective, survival and modeling role of soil properties (More et al., 2014; Op De Beeck et al., 2021).

The soil structure can undergo changes induced by EPSs produced under thermal stress through actions similar to soil particle binding agents, which lead to increases in stability, water retention and nutrient availability. Also, the qualitative/quantitative evolutions of EPSs, under thermal stress, intervene in the stimulation of microbial activities, with microbial processes showing a remarkable variation in terms of temperature sensitivity, as well as in the clearer expression of their membership in a community.

The evolution of dissolved organic carbon (DOC) content under the influence of SynComs inoculation was analyzed in order to evaluate the stability/sequestration of C in the Molic Gleysol, taking into account its complex role in soil C dynamics.

Inoculation of SynComs M5 and M7, at 35°C and 3 days of incubation, determined in the rhizosphere of tomatoes a low DOC content of 2.82 $mg \cdot L^{-1}$ and 3.43 $mg \cdot L^{-1}$, respectively, both below the DOC level determined in the uninoculated control variant of 5.64 $mg \cdot L^{-1}$. DOC assessments at 45°C, in the rhizosphere, after inoculation with SynComs M5 and M7 were 3.17 $mg \cdot L^{-1}$ and 3.92 $mg \cdot L^{-1}$, respectively, compared to the uninoculated control (6.87 $mg \cdot L^{-1}$).

In the case of SynComs M4 and M1, incubated for 3 days, the DOC content at 35°C was 3.62 $mg \cdot L^{-1}$ and 3.48 $mg \cdot L^{-1}$, respectively, and at 45°C DOC increased by up to 18.76% and 20.26%, respectively, for SynComs M4 and M1, compared to their level reached at 35°C.

The Mollic Gleysol inoculated with SynCom M5 at 35°C and 14 days of incubation had a DOC content of 14.22 $mg \cdot L^{-1}$ and when inoculated with SynCom M7, under the same experimental conditions, the DOC determined was 15.29 $mg \cdot L^{-1}$, both quantitative values being below the DOC level analyzed in the uninoculated control.

DOC evaluated at 45°C, in the rhizosphere, after inoculation with SynComs M5 and M7 was 16.24 $mg \cdot L^{-1}$ and 17.66 $mg \cdot L^{-1}$, respectively, compared to the uninoculated control (22.28 $mg \cdot L^{-1}$).

In the case of inoculation with the M4 and M1 synthetic microbiomes, the DOC content in the soil at 35°C decreased by 12.32% and 21.27%, respectively, compared to the uninoculated control (17.84 $mg \cdot L^{-1}$) and at 45°C the DOC in the soil increased by up to 28.65%, under the influence of SynCom M1 (21.70 $mg \cdot L^{-1}$), compared to the DOC content at 35°C (Figure 4).

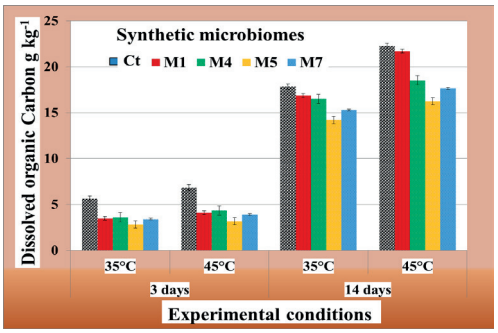



Figure 4. DOC content in Mollic Gleysol generated by inoculation with SynComs under two different periods of time and temperatures

Incubation of vegetation pots at temperatures considered as heat stress influenced the intensity of microbial activities in the soil, so that SynComs intervened in accelerating processes in the rhizosphere, making C use more efficient by enhancing sequestration and reducing free DOC.

The new communities of microorganisms in the rhizosphere act preferentially on more easily degradable compounds, either generating more DOC or participating in the construction of more stable aggregates, thus quantitatively reducing their presence.

The applied thermal stress had an effect on the DOC content in the inoculated rhizosphere also through the induced changes in the composition of root exudates, representing sugars, organic acids, metabolites, a.s.o., which constitute the main sources of DOC.

Thus, when temperatures increase, both the production of DOC by photosynthesis and the decomposition of organic matter undergo accelerations, causing their concentration in the soil to increase.

Possibly, thermal stress causes a photosynthetic stimulation, with tomatoes producing more organic compounds than they can process, generating their release in the form of DOC. Also, the high temperatures applied determined the growth and development of microorganisms in the rhizosphere, promoting an increase in the rate of decomposition of OM and the release of a greater amount of DOC into the environment (Campbell et al., 2022).

When the applied incubation temperature was 45°C, the photosynthetic capacity of tomatoes may have been oriented towards a hypersynthesis of organic compounds, far beyond their utilization capacities, causing their release in the form of DOC. Inoculation with SynComs enriched the rhizosphere with metabolites related to plant growth, stress inhibition and the structure of the overall DOC composition.

The inoculated SynComs promoted beneficial interactions, developed beneficial microbial cooperation and improved the dynamics of organic C in the rhizosphere.

Various studies have analyzed the impact of temperatures on DOC characteristics, investigated the ways in which high temperatures accelerate the decomposition processes of organic C for an increase in DOC production (Kalbitz et al., 2000; Smreczak & Ukalska-Jaruga, 2021).

Thus, the influence of temperature variations on the level of degradation, mineralization and DOC characteristics, interactions between microbial communities and DOC was evaluated (Liu et al., 2019; Garcia et al., 2022; Wang et al., 2022).

Soils contain natural organic matter (NOM) with a role considered essential in shaping their composition and microbiology and, through the amount and compositional specificity of NOM, influencing the types and concentrations of other compounds. In addition, temperature has been identified as a significant factor influencing DOC (Zhao et al., 2023; Huang et al., 2024; Ren & Cai, 2025).

To adapt to high temperatures, the lipid composition of the microbiota changes to express heat shock proteins and stabilize cell membranes. Warming intervenes in increasing the potential level of respiration of the heterotrophic saprophytic soil microbiota, enhancing $CO_2$ emissions, reducing SOM deposits necessary to maintain soil structure and water retention capacity (Karhu et al., 2014; Bond-Lamberty et al., 2018; Cavicchioli et al., 2019; Jansson & Hofmockel, 2019).

Under elevated temperatures, the application of SynComs inoculation resulted in an intensification of DOC production, through increases in metabolic activities, accelerated SOM decomposition, etc., which generated temperature-dependent dynamics.

Other effects of temperature on DOC include accelerated decomposition and microbial

transformation into particulate organic carbon (POC), water-extractable labile compounds, a microbiota with specific thermal thresholds and behavior that evolves from rapid degradation to accumulation at high temperatures (26-35°C), promoting the retention of refractory DOC.

Soil carbon fractions also suffer significant decreases in labile C, recalcitrant C, as elevated temperatures enhance the conversion of old, protected organic matter (SOM) into labile, soluble forms (DOC), accelerating the overall rate of C renewal in the soil.

Using UV illumination, images revealed differences between the uninoculated control and the inoculated variants, at different time (3 and 14 days) and temperature (35°C and 45°C) intervals (Figure 5).

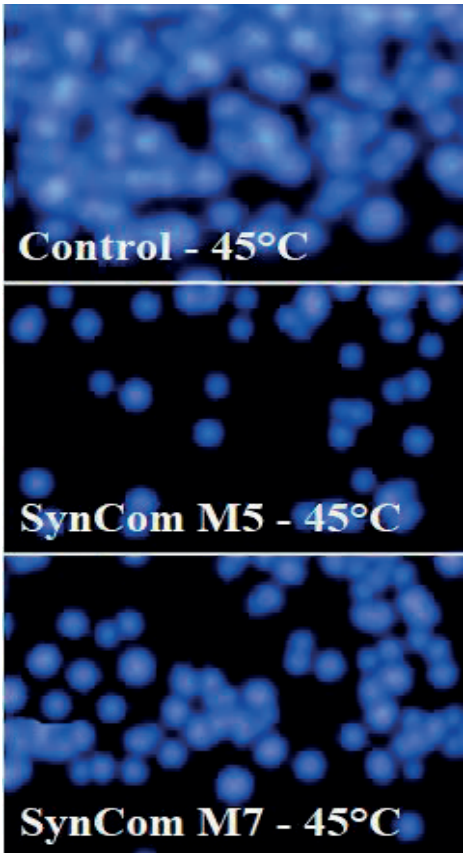



Figure 5. DOC from soil inoculated with SynComs M5 and M7, at 45°C after 14 days

The qualitative differences were generated by the specific composition of SynComs and were reflected, function by their affinity, in the aspects related to the level of accumulations of newly synthesized organic compounds.

The distribution of extracted organic carbon compounds DOC was highlighted based on fluorescence affinity. DOC showed different densities of organic compounds in the soil, after 14 days of incubation at 45°C, in the case of inoculated SynComs.Of these, SynCom M5 induced the lowest DOC content, at 45°C, after 14 days of incubation and the organic carbon structures appear well highlighted through less dense areas, with a lower molecular weight, compared to the DOC structures that appear under the influence of SynCom M7.

After 14 days of incubation at 35°C, the density differences are less evident and, in the case of SynComs M4 and M1, including the control, the differences are imperceptible.

After inoculation with SynComs M5 and M7, the stability of organic carbon compounds in DOC at temperature increased in the carbon subfractions, the biochemical protection offered by the composition of the newly secreted exometabolites was more intense and diverse, compared to the other inoculated SynComs. The images reveal the changes in the distribution and organization of newly biosynthesized DOC under the influence of SynCom M5, under thermal stress, compared to the uninoculated control.

It can be deduced that the stability of organic carbon in the soil inoculated with SynComs M5 and M7 also increased, compared to that provided by SynComs M1 and M4.

But, even under conditions of reduced protection of C compounds, SynComs M1 and M4 ensured better stability of organic C. compared to the uninoculated control.

The trend demonstrated by DOC under the influence of SynComs M5 and M7, after the 14 days of intermittent exposure at 45°C, is more pronounced than at 35°C, under the influence of the same synthetic microbiomes, which perhaps reflects a reaction model to the interactions between soil properties and the biochemical characteristics specific to SynComs.

The amount of C stored in the soil appears dependent on the metabolism of SynComs, the physico-chemical structure, interactions and chemical absorption of carbon molecules in

relation to the mineral component and their specific influence modifies the accessibility/stability of C.

Thus, microbial C biosynthesized by SynComs enriches DOC in the inoculated soil, can stabilize on mineral surfaces or in the soil solution, where it can compete for sorption sites.

Under controlled conditions of thermal stress, under the influence of the analyzed SynComs, labile substances were produced by both the inoculated synthetic communities and the native ones in the soil, to which substances from the organo-mineral reserve were added. The analyzed parameters, such as the content of glycoproteins, EPS and DOC, can be used as indicators for estimating the efficiency of carbon use under the conditions of increasing the supply of extracellular compounds on the long-term carbon storage and the dynamics of carbon capture/emission processes in the soil.

## CONCLUSIONS

SynComs selected for their exometabolic capacities intervened in the organic C dynamics, favoring the stabilization and sequestration of carbon in the soil, under thermal stress conditions.

In the soil, inoculated SynComs and native microbial communities, stimulated by their presence, enhanced the synthesis of GRSP and EPS, as a strategy of adaptation to stress conditions.

Inoculated SynComs and native communities influenced by their presence reduced the synthesis of DOC, as a strategy of resistance to thermal stress.

The content, quality and influence of additionally biosynthesized/ accumulated GRSP, EPS and DOC in the soil varied significantly depending on the type of SynCom.

Under heat stress conditions, the content of GRSP, EPS and DOC biogenerated after SynComs inoculation varied significantly between the two temperature ranges

Depending on the composition and characteristics of SynCom, the results obtained under heat stress conditions highlighted that soil microbial carbon pumps acted/functioned differently in the rhizosphere to compensate/stimulate the synthesis of GRSP, EPS and DOC.

Comparative evaluation of SynComs regarding influence on carbon stability and sequestration under heat stress conditions were observed at M5 and M7 in terms of the effect on functional efficiency of the new soil microbiota.

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