Carpathian Journal of Earth and Environmental Sciences February 2023, Vol. 18, No.1, p. 51 – 64; DOI:10.26471/cjees/2023/018/240

SOIL RESPIRATION AS MICROBIAL RESPONSE TO THE ENDOGEN INPUT OF BIO-SYNTHESIZED ORGANIC MATTER AND ITS IMPLICATION IN CARBON SEQUESTRATION

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Abstract: Active C, as a measure of the level of chemical oxidation of organic matter, reflects the carbon available to microorganisms. Soil respiration, as a direct way to estimate edaphic microbial activity, could be a measure of the fluctuations of carbon stocks in soils. To determine the ability of soil respiration to evaluate such fluctuations we used soils with an increased content of organic carbon, constant optimal conditions, to eliminate the disturbing factors, analyzed in a short period of time. The influence of the specific decomposition rates of these soils was assessed by both spot determinations of soil respiration and analyzing the flux of CO_2 from *ex situ* soil samples, under standard experimental conditions, to highlight carbon storage in such soils. Reference data can be accumulated through the analysis of these parameters, which compared with the results of quantitative/qualitative determinations regarding the changes in the content of microbial biomass, the content of fulvic sub-fractions, the fluorescence of dissolved organic material and the evolution of the content of siderophores, could be considered, by their own evolutions, as arguments in sustaining the use of respiration in the efficient estimation of carbon storage evolution in the soils. The analyses of these parameters were carried out in two phases, for comparing initial and final data of experiment (after 30 days). The soils had different levels of the respiration potential between phases. The level of soil respiration was reduced in time between 4.27-14.60%, in each soil. The CO_2 flux showed, in time, a continuous decreasing trend in both soils. In the case of Mollic Histic Gleysol (Salinic), the coefficient of determination has the value R^2=0.92 for the flux determined in the final phase. The levels of microbial biomass of both soils were increased significantly at the end of the experiment. In the case of Mollic Histic Gleysol (Salinic), microbial biomass increased from 456±23.12 µgC·g^{-1} to 514±24.57 µgC·g^{-1} soil. The fulvic sub-fractions A-D of both soils revealed significant accumulates of soluble organic compounds, with different molecular weights and complexity levels, after 30 days of incubations in standard conditions. The fluorescent components present in the water-extractable organic matter were highlighted by imagistic method. The highest degree of storages of newly bio-synthesized compounds of carbon was registered in organic matter of Mollic Histic Gleysol (Salinic). The intensity of siderophores biosynthesis increased over time, starting from an initial lower presence in the Mollic Gleysol (Salinic) (with Ø 11 mm halo), which were followed by an increasing of siderophores content and availability of iron, at the end of the experimental period. Accumulations of siderophores in the Mollic Histic Gleysol (Salinic) determined a Ø 31 mm halo diameter.

Keywords: Carbon sequestration, soil types**,** soil respiration, CO_2 flux, microbial biomass, fulvic acids, fulvic acids subfractions, fluorescence, siderophores, bio-synthesis

1. INTRODUCTION

Carbon (C) storage in soil is an opportunity in climate change mitigation actions. Soils can store up to three times the amount of carbon in the atmosphere, but as they warm due to climate change, microbial metabolic rates begin to increase, the decomposition of soil carbon compounds (CO_2) that is released into the atmosphere intensifies, practically constituting positive feedback. But even under these conditions, if the supply of organic matter continues to increase in the soil, either due to the activities of microbial

biosynthesis, plants growth, mineral availability, proper management, a.s.o., the soils can begin to actively and efficiently sequester atmospheric carbon. The balance that can be achieved between these two processes will ultimately define whether soils will be able to become a net source of carbon, influencing and exacerbating climate change, or whether they can become sinks themselves, with the potential to mitigate climatic changes (Tyc at al., 2017; Soong et al., 2019; Wang et al., 2020; Chabbi et al., 2022; Zhang et al., 2022). Such assessments require accurate data to determine soil C losses and calculate soil C balances. Ways to measure soil organic C accumulations and accurately assess how they change over time are still important challenges (Angst et al., 2018; Liang et al., 2019; Paustian et al., 2019; Smercina et al., 2021).

Generally, the problem refers to how soils, through regenerative cultivation, could intervene and influence climate change, actively transforming them into a huge carbon reservoir. Trying to develop carbon markets for such a reservoir relies especially on how to sample and measure soil carbon, repeatedly and very precisely. Healthy soils have a good C cycle, contributing to the continuous improvement of nutrient and water use efficiency, but also to the degradation mitigation (Haney et al., 2018; Jing et al., 2020). Common measurements of organic carbon in soils have been based on the loss-on-ignition (LOI) test of organic matter or the dry combustion method. As direct percentage measurements of total soil carbon, these methods are not sufficiently precise. In addition, in soils that also contain inorganic carbon, the analysis becomes more complicated, the determination of the percentage of organic carbon presupposing the determination of the difference between the measurement by dry combustion and the percentage of organic matter, measured by loss on ignition, of the same soil samples. Carbon credits are based on actual soil C mass, but also on bulk density, which varies with soil type, management, harvesting from a depth of 30 cm, time of sample collection, location monitoring from year 0 to year 5, amplifying the level of uncertainty, all of them with repercussions on the credit value. Studies have shown that there are analyzes that can be used as indicators of faster changings soil carbon content such as active C, 24-hour soil respiration and beta-glucosidase enzyme activity. Initial research has highlighted the sensitivity of these analyzes in assessing changes in the carbon cycle resulted from the microbial activities or the changes applied in soil management.

Active C constitutes oxidizable C as a measure of the level of chemical oxidation of organic matter that correlates with total organic carbon and reflects the carbon available to microorganisms (Hemingway et al., 2018; Berthelin et al., 2022). Soil respiration for 24 hours is the direct way to measure edaphic microbial activity, under standard conditions and beta-glucosidase is, like soil respiration, a measure of the carbon cycle, a measure of the enzyme activities in soils, responsible for the biodegradation of the residues. In the researches on the effectiveness of evaluation of the types of measurements for assessing the health of the soils, these indicators were used at more than one hundred sites dispersed in North America (Oliveira et al., 2018). Relatively recently, the development of integrated soil carbon measurement and monitoring systems aimed to create new soil carbon measurement technologies that combine sensor-equipped instruments, that measure both soil carbon concentration and soil density in a non-destructive and cost-effective way, with an optimized spatial sampling algorithm and spectroscopy data, to be able to obtain rapid measurements of soil carbon stocks. Also, the information on the physico-chemical and biological properties of soils, organic matter content, pH, nutrients and soil structure were integrated through a visual evaluation, as well as by the content in earthworms, for a correct assessment of soil complexity (Sun et al., 2019; Smith et al., 2020). The soils used in our research contain carbon stocks (Mocanu et al., 2022). These soils are not degraded and represented the opportunity to see if there is a possibility to increase the C storage capacity of such organic soils, in a short period of time, based on microbial biosynthetic activity, under standard conditions, without supplementary carbon additions.

The aim of the research was to determine if the storage capacity will be influenced by the decomposition rates, if through spot determinations of soil respiration and analyses of the CO_2 flux from soil samples under experimental conditions, it is possible to highlight carbon storage in such soils. Through the analysis of these parameters, reference data could be accumulated and compared with the results of quantitative/qualitative determinations regarding the changes in the content of microbial biomass C and fulvic sub-fractions, of the fluorescence of carbon organic matter and the evolution of the content of siderophores, it could support the possibility of evaluation of carbon storage in the soils.

2. MATERIAL AND METHODS

2.1. Soil types and characteristics

Sampling sites from Danube Delta were selected in order to represent two different soil subtypes (typic and histic) of the same soil type, i.e. Mollic Gleysol (WRB). Their location, using the

global positioning system (GPS), are 45.034452° N latitude and 29.205589° E longitude for Mollic Gleysol (Salinic) and, respectively, 44.986804° N latitude and 29.242671° E longitude for Mollic Histic Gleysol (Salinic). Soil samples were taken from topsoil (0–20 cm), from the defined locations. The Mollic Gleysol (Salinic) has the following parameters: pH value 6.46, the humus content 9.54%, organic C content 5.5%, organic matter content 32%, total nitrogen content 1.177 $mg \cdot kg^{-1}$, $N\text{-}NO_3$ 266 $mg \cdot kg^{-1}$, P extracted by ammonium lactate (P_{AL}) 121 $mg \cdot kg^{-1}$ and K_{AL} 294 $mg \cdot kg^{-1}$, carbonates 0.2%, microelements content (Fe 30,250 $mg \cdot kg^{-1}$, Mn 336 $mg \cdot kg^{-1}$, Cu 39.8 $mg \cdot kg^{-1}$, Zn 80 $mg \cdot kg^{-1}$), clay content <0.002 of 16.7%. Mollic Histic Gleysol (Salinic) has the following parameters: pH value 5.54, the humus content 29.8%, organic C content 17.3%, organic matter content 51%, total nitrogen content 1.740 $mg \cdot kg^{-1}$, $N\text{-}NO_3$ 2106 $mg \cdot kg^{-1}$, P_{AL} 45 $mg \cdot kg^{-1}$and K_{AL} 380 $mg \cdot kg^{-1}$, carbonates 0.4%, microelements content (Fe 23.259 $mg \cdot kg^{-1}$, Mn 144 $mg \cdot kg^{-1}$, Cu 55.3 $mg \cdot kg^{-1}$, Zn 67.9 $mg \cdot kg^{-1}$), clay content <0.002 of 38.6%.

2.2. Laboratory experimental design and analyses

After removing visible plant debris, soil was collected from 3 different points, mixed, sieved (2mm) and divided into two halves. While half of the sample was introduced into the laboratory circuit for analysis of soil respiration, CO_2 flux, microbial biomass, chromatography of fulvic subfractions, fluorescence and siderophore content, the other half was immediately stored and maintained under standard conditions for carrying out the same type of analysis, after 30 days.

The intrinsic efforts of the specific microflora of the two soil subtypes in influencing the increase of the carbon storage capacity, in a short period of time, were analyzed.

The period covered 30 days, in which 200g of each type of soil were placed in bioassay pots, in the dark, in the incubator, in five replicates and the conditions of temperature, humidity and pressure were kept constant (27ºC temperature, 60% field water capacity, 0.98 atm normal atmospheric pressure).

The analyses of some parameters characterizing the microbial activity of the soils were carried out in two stages, at the beginning and at the end of the experiment, as follow:

2.2.1. Soil respiration

Soil respiration was determined by alkali absorption method.

The soils at 60% field capacity were incubated. The NaOH (0.1 M) solution in each jar was maintained for 24h at 25ºC. After 24 h, 0.5 M $BaCl_2$ and 3 drops of indicator were added into alkali and then titrated with 0.1 M HCl, in five replicates. The CO_2 absorbed from the soil was calculated according to Romanian National Standard - SR-ER-ISO-14240-1-(2012) - Soil quality - Determination of soil microbial biomass-Part 1: Substrate-induced respiration method.

2.2.2. CO_2 flux

Measurement of CO_2 flux in soil respiration was performed in closed enclosures, in opaque accumulation chambers to measure only the component derived from physiological and biochemical reactions in the soil, without any organic or inorganic carbon supplements. The chambers have incorporated sensors to measure CO_2, temperature and relative humidity inside the chamber, being absolutely sealed, with no internal mixing system, no moving parts. A CO_2 detector was used to measure the changes in CO_2 concentrations, using in the calculation all the determinations (720/sample), representing the automatic recordings made every 2 minutes by the sensor, during 24 hours. Flux rates (F) were calculated in ppm CO_2 according to CO_2 concentrations recorded by sensor, as specified in user manual. Polynomial regression was used as a common algorithm for flux estimation.

2.2.3. Soil microbial biomass carbon

Soil microbial biomass was determined according to the fumigation-extraction method with chloroform. Soil samples of 12.5 g (dry weight equivalent) were fumigated with $CHCl_3$ at 20°C for 24 hours, in five replicates, extracted with 0.5 M K_2SO_4 (30 min at 200 rpm), then filtered using a 0.45 mm cellulose ester filter. Soil microbial biomass carbon was calculated according to the Romanian National Standard - SR-ER-ISO-14240-2-(2012)- Soil quality- Determination of soil microbial biomass-Part 2: fumigation-extraction method.

2.2.4. Fractionation of fulvic substances

Fulvic fraction of soil organic matter was separated into subfractions A,B,C and D by partial adsorbtion on activated charcoal and serial elutions of the absorbed material with acetone, distilled water and NaOH (Votolin et al., 2022).

2.2.5. Specific ascending chromatograms

Information on the quantitative/qualitative changes of organic matter stocks and the degree of complexity reached was obtained by using specific

ascending chromatograms adapted method (Mukadam et al., 2021).

These allowed, through analytical separations of the fulvic sub-fractions, the formation of images whose pattern of uniformity, shape, size and color could directly highlight the intensity of microbial activities and the variation of composition of labile organic fractions, with specific chemical characteristics, in a short period of time.

2.2.6. Fluorescence of dissolved organic C

The dissolved organic carbon was extracted (in both phases) from soils treated with fluorochromes and their distribution was revealed by specific ascending chromatography (Wang et al., 2021).

Thus, photographic images were obtained under UV illumination of 350 nm, which revealed the qualitative differences between the initial and final phases of each soil subtype, as well as the aspects related to the density of the newly synthesized biochemical composition, the complexity and material distribution according to their molecular weights and fluorescence-based affinity.

2.2.7. Estimation of siderophores in soil

For the semi-quantitative estimation of the siderophores content, the initial and final samples of both soils were placed in medium with low iron content, incubated at 28°C, for 7 days, then the cell mass was separated by centrifugation at 10,000 r.p.m., for 15 min.

The supernatant was concentrated and an organic solvents mixture was used to extract the siderophores. Serial purifications follow to remove traces of phenol, after which the supernatant is mixed with the CAS test solution, in which iron is bound to HDTMA, a blue dye, according to the method of van Bergeijk et al., (2022) adapted.

Un-inoculated medium was used as reference. The positive test indicates a change in color from blue to orange and is quantitatively proportional to the diameter of the halo formed around the well.

2.2.8. Statistical analyses

Data, graphically expressed as mean (n = 5) values ± SD were subjected to analysis of variance (ANOVA), compared by Student's t test. The p value <0.05 was considered statistically significant.

3. RESULTS AND DISCUTIONS

3.1. Soils respiration

The biosynthesis and microbial degradation of soil organic matter (SOM) are fundamental processes for ensuring a balance for carbon (C) in the soil, because its biochemical processing intervenes both in the stabilization of C within the organo-mineral complexes and in the loss of C as atmospheric CO_2, due to microbial respiration.

Generally, organic input, temperature and humidity are considered the main factors involved in the control of C dynamics in the soil, because they can intervene in the stimulation/inhibition of microbial activities, but also in C mineralization, through the quality and quantity of organic matter (OM) introduced into the soil, by the availability of water and energy necessary for microbial processes (Bünemann et al., 2018).

Under standard conditions and without an organic input, the soils analyzed had different levels of respiration potential between the initial phase (I), before incubation, and the final phase (F), after being kept in the dark for a period of 30 days, under conditions of relatively constant temperature and humidity (Figure 1).

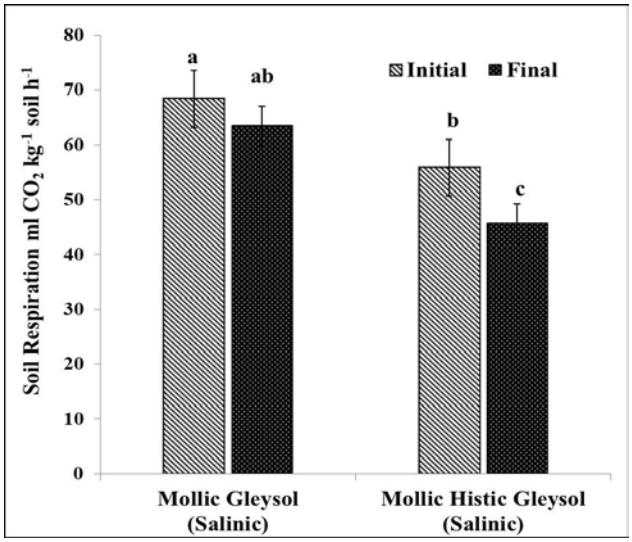

Figure 1. The soils respiration levels in initial and final experimental phases

Thus, in the case of Mollic Gleysol (Salinic), the initial level of soil respiration was 68.35 ml CO_2 kg^{-1} soil h^{-1} and at the end of the experiment 65.43 ml CO_2 kg^{-1} soil h^{-1}, which represented a 4.27% decrease of respiration level.

The Mollic Histic Gleysol (Salinic) had an initial level of soil respiration of 55.82 ml CO_2 kg^{-1} soil h^{-1} and at the end of the experiment of 47.67ml CO_2 kg^{-1} soil h^{-1}, which represented a decrease with 14.60% of the respiration. The decrease of soil respiration level at the end of the experiment is significant compared to the initial moment, in the case of the Mollic Histic Gleysol (Salinic).

Studies carried out over periods longer than one year contain more information regarding changes

in soil respiration, under the conditions of intake of residues (Feng et al., 2017; Hemingway et al., 2019; Cîșlariu et al., 2020; Tang et al., 2020; Yasin & Mirici, 2020; Noormets et al., 2021). On the contrary, studies carried out for short periods of time, on different agricultural soils, in a controlled environment are relatively scarce and they highlighted in particular the fact that the ability to store new C in the soil is influenced by the availability of inorganic nutrients. Also, there are only few studies on the short-term response of microbial respiration to different levels of the fresh organic matter in soils relatively different regarding their organic content. The respiratory activity of the microflora appears maximized under optimal conditions of temperature and humidity, without a consistent physico-chemical protection of the organic matter (He et al., 2022).

This parameter showed a linear evolution to an additional supply because, compared to the microbial biomass, it can increase independently of the availability of nutrients.

A number of recent studies demonstrated that SOC dynamics involved a control exerted by more complex interactions (Luo et al., 2017; Haaf et al., 2021). Thus, it is considered that soil compounds of microbial origin could be stabilized through organo-mineral interactions. C dynamics of such compounds could be controlled in soils, inducing different potentials of the C stabilization, in close dependence by the weathering rates and different contents of SiO_2, Fe and Al (Doetterl et al., 2018).

Temperature and humidity variations, as environmental variables, could directly intervene on microbial metabolism and the microbial respiration-growth relationship, in favor of respiration (Hursh et al., 2017; Yan et al., 2018; Ye et al., 2019).

3.2. CO_2 flux of soil respiration

Quantifications of carbon dioxide (CO_2) fluxes from soils can play a key role in monitoring and possibly estimating their tailored contribution to global warming processes (Houghton & Nassikas, 2017; Pries et al., 2017; Guttières et al., 2021).

The automated cameras continuously measured the CO_2 fluxes of the two soils, with a relatively high frequency (every 2 min), over a 24-hour period. No significant fluctuations in the evolution of flux values were observed, which suggests the constancy in the development of biochemical processes, a certain tendency to maintain the equilibrium ratio between absorption and net release of CO_2. Determinations were performed in the dark to obtain reliable CO_2 fluxes, air fluctuations were eliminated and internal and external pressure gradients were maintained. The estimated CO_2 fluxes for the two soils represented the evolution of the rates of change of the CO_2 inside the closed chambers. Exponential regression of the CO_2 concentration data was used to adequately analyze the samples over a relatively short time span. The chambers were operated under the control of a system of automatic and simultaneous recording of CO_2, temperature and relative humidity data. The CO_2 released and evaluated in the closed automatic chambers came mainly from the respiration of active microorganisms and from the heterotrophic decomposition of organic matter from the analyzed soil samples. The use of automated cameras allowed continuous measurement of the evolution and net changes of CO_2 in soil samples, measurements that could be useful in evaluating the changes in the composition of annual soil carbon stocks.

The present studies focused on fluxes measured in closed chambers with samples from the topsoil layer of soils with an increased level of microbial activities. The evolution of the CO_2 fluxes recorded for the two soils depended on the level of microbiological activities and on the involvement of other factors such as molecular diffusion, CO_2 diffusivity from the soil samples, the difference in CO_2 concentration between the air in the chamber and that in the porous spaces of soils, due to pressure variations.

The evolution of the CO_2 for the two soils is presented in the Figure 2. These are models of evolution of CO_2 flux, in standard conditions of relative humidity and temperature. In the case of Mollic Gleysol (Salinic), the evolution pattern of the CO_2 in the closed chambers is observed in the initial phase and at the end of the experiment (Figure 2a). Thus, in the initial phase, the flux evolution reached the maximum measurable value (>10000 ppm) in 6 hours and the measurements in the final phase, after the 30 days of soil incubation, showed a decrease in the flux level, compared to the initial phase. The maximum measurable value of the CO_2 flux was reached after 13 hours, under the same standard conditions of temperature and humidity as in the initial phase. Then, the CO_2 flux remained elevated both due to a weak net absorption of CO_2 into the Mollic Gleysol (Salinic) and possibly due to an increase in the CO_2 gradient between soil and air. The coefficient of determination calculated for the flux was R^2=0.94 in the initial phase and respectively, R^2=0.92 in the final phase. In the case of Mollic Histic Gleysol (Salinic) the evolution pattern of the CO_2 in the closed chambers can be observed in both phases of the experiment (Figure 2b). In the initial phase, the CO_2 flux evolution measured 24 hours reached a

maximum of 6638 ppm and in the final phase, after the 30 days of soil incubation, the flux evolution continues the decreasing trend of CO_2 value (reaching a maximum of 4564 ppm). The coefficient of determination for the flux determined in the initial phase was R^2=0.91, and respectively, R^2=0.92 in the final phase. The experimental model was based on the study of the endogenous impact, in a determined period of time, of the biochemical processes on the CO_2 fluxes from the two soils.

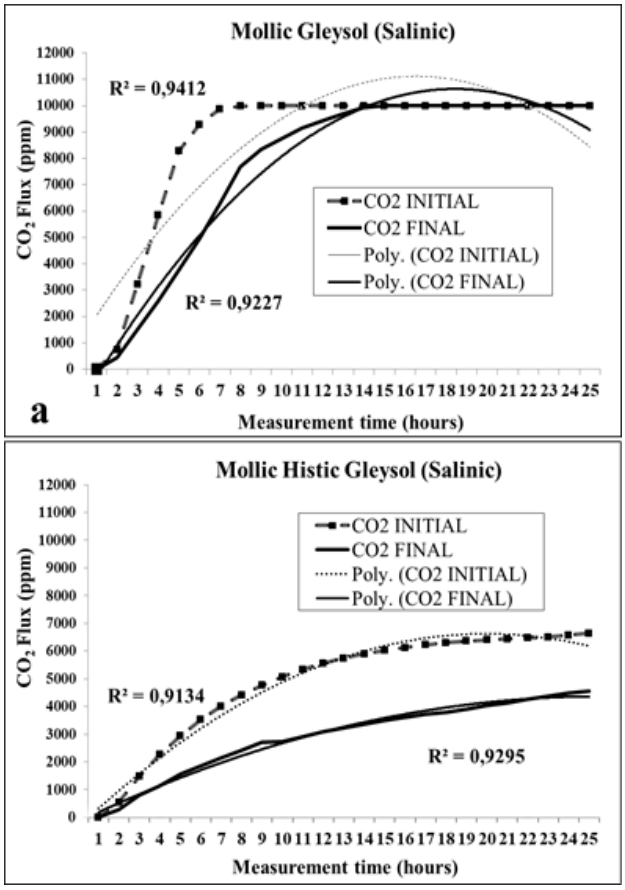

Figure 2. Analysis of CO_2 flux in Mollic Gleysol Salinic (a) and in Mollic Histic Gleysol Salinic (b)

The results represent simulations that captured the evolution of the CO_2 under the short time experimental conditions, in concordance with long time evaluations data from literature (Oliveira et al., 2018; Devi & Singh, 2019; Soong et al., 2021).

Predictions of achieving soil carbon balance remain uncertain, as long as the mechanisms and potential of C sequestration are not well known. The balance between soil C inputs and outputs would ensure the quality of both source and sink for atmospheric CO_2. Thus, CO_2 emissions (even in the case of minor changes in the size of the CO_2 flux from the soil) can significantly influence the atmospheric CO_2 level (Ge et al., 2017). So, the flux of CO_2 is considered a sensitive indicator for the C cycle, for soil capacity of C sequestration. The flux of CO_2 from soils can be strongly influenced by variations in the biosynthetic capacity of soil microbial communities, by the quantity/quality of endo- and exogenous organic inputs, by changes in environmental conditions, as well as by different management practices.

3.3. Soil microbial biomass

The capacity of edaphic microorganisms to determine the formation of biomass expresses the efficiency of the way in which microbial C is utilized, efficiency dependent on a set of factors that include the physiological state of the microorganisms, substrate availability, biological and nutritional stoichiometry, humidity, temperature (Walker et al., 2018).

To sequester carbon dioxide, autotrophic and heterotrophic soil organisms incorporate CO_2 into various organic carbon products. The ecological sequestration of CO_2 can be achieved by appropriate use, by cyanobacteria, proteobacteria, mycobacteria, sulfur-, non-sulfur bacteria of different cycles (reductive pentose phosphate, reductive citric acid, reductively acetyl-CoA pathways a.s.o.). Biomass is the product obtained in the soil from atmospheric CO_2 following these complex biochemical reactions, in which renewable bioenergy is blocked, thus representing an advantage over other renewable energies.

The biomass produced is an alternative way to store captured CO_2, achieved by involving different species in the transformation of CO_2.

In experimental conditions, without organic input, the microbial biomass of the analyzed soils had different levels between the initial phase, before incubation, and the final phase, under constant temperature and humidity conditions.

Thus, in the case of Mollic Gleysol (Salinic), the initial level of soil microbial biomass was 258±11.32 μg $C \cdot g^{-1}$ soil and at the end of the experiment 282±12.6 μg $C \cdot g^{-1}$ soil, which represented a significant quantitative increase of 9.3% of the amount of biomass. The Mollic Histic Gleysol (Salinic) had an initial level of soil microbial biomass of 456±23.12 μg $C \cdot g^{-1}$ soil and of 514±24.57 μg $C \cdot g^{-1}$ soil at the end of the experiment, which represented a significant quantitative increase with 12.7% of the amount of microbial biomass as compared to the initial amount. The levels of microbial biomass of both soils, determined at the end of the experiment, significantly increased as compared to the initial moment are illustrated in Figure 3.

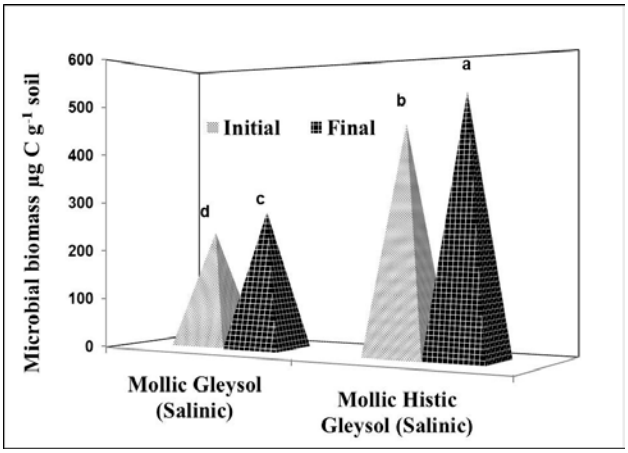

Figure 3. The microbial biomass of soils in initial and final exprimental phases

The benefits of CO_2 captured in biomass by soil microbiome include high rate of bio-fixation, reducing of atmospheric CO_2, production of a wide range of additives, avoidance of genetic augmentation difficulties, its use in bioprocessing, in ensuring rapid growth without competition and nutritional deficiency.

Generally, sustainable strategies promoting C sequestration in the soils were associated with variable levels of organic substrates and assume the establishment of microbial selection methods in the soil, both for the valorization of these substrates and for the establishment of priorities, between respiration and growth processes. Studies have shown that soil C content does not increase proportionally to the constant addition of new amounts of organic compounds, although it promotes microbial biomass. Difficulties regarding C accumulations could be due to changes in the efficiency of microbial C use, changes accentuated by the insufficient content of inorganic nutrients for biomass synthesis (Liang et al., 2017; Powlson & Neal, 2021).

The efficiency of microbial C use in soils expresses the balance between anabolic and catabolic reactions carried out by the C metabolism of decomposers. Thus, in soils with increased efficiency of microbial C use, an increased availability for labile C is also observed and the energy in these soils is provided by an extensive C substrate used for cell maintenance but also sufficient to promote growth and division processes.

The increased efficiency of microbial C utilization can also be determined due to a reduced content of recalcitrant substrates or a decrease in extra- and intracellular catabolism. In addition, the content and proportions in which nutrients occur are strictly controlled in soils. Sulfur (S), phosphorus (P), nitrogen (N) nutrient requirement of microbial biomass is another controlling factor of microbial C use efficiency. Also, the stabilized organic fraction in soil contains stoichiometric ratios similar to the nutrient ratios found in the biomass of soil microorganisms, per 1000 units of C, compared to the much different nutrient ratio of plant residues. The quality and availability of organic substrates, also as environmental variables, intervene in the control of the microbial growth rate, respectively the biomass (Adingo et al., 2021).

3.4. Subfractionations of fulvic substances

CO_2 stored in the soil can be used to produce organic compounds (polymers, bio-reactants, a.s.o.) by microorganisms, that are both natural filters and producers of biomolecules (carbohydrates, proteins, lipids). Secondary metabolites resulted from biological processes, come from multiple photosynthetic or non-photosynthetic pathways and they can also be bio-synthesized by various edaphic species (bacteria, fungi, yeasts, algae), in order to fix atmospheric CO_2.

Due to the ability to capture carbon, extreme incorporation rates, tolerance to maximum CO_2 stress, adequate use of limited nutrients, tolerance to fluctuations in thermal parameters and of H^+, OH^-, microorganisms occupy the first place in carbon sequestration (Luo et al., 2017; Kaya et al., 2018).

The ascending chromatograms of the fulvic sub-fractions A, B, C and D of the two types of soil in the experiment highlight the evolution and qualitative changes induced in the structure of the organic matter.

The qualitative changes at the level of the fulvic sub-fractions allowed the highlighting of changes determined in the composition of the fulvic fraction, in a short period of time (30 days), changes that could not be revealed by classical analytical determinations. The accumulations of organic material in the fulvic sub-fractions, at the end of the experiment, represent the expression of bio-synthesized secondary exometabolites and express the biosynthesis potential of the microbiome specific to each type of soil. By means of the chromatograms, it was possible to distinguish different characteristics, from the point of view of the specificity of the type of microbiome, as well as indications of the progress of complexation and of the probable effect of the biotransformations induced on their organic substrates by the microorganisms.

The biosynthesis and biotransformation of organic matter specific to each type of soil studied can also be considered effective in retaining carbon, because it (in dissolved form) will quickly react with the minerals in the organic substrate for stabilization.

Figure 4 shows the ascending specific chromatograms of the fulvic sub-fractions A, B, C

and D from both soils in the initial phases (a and c) and final phases (b and d).

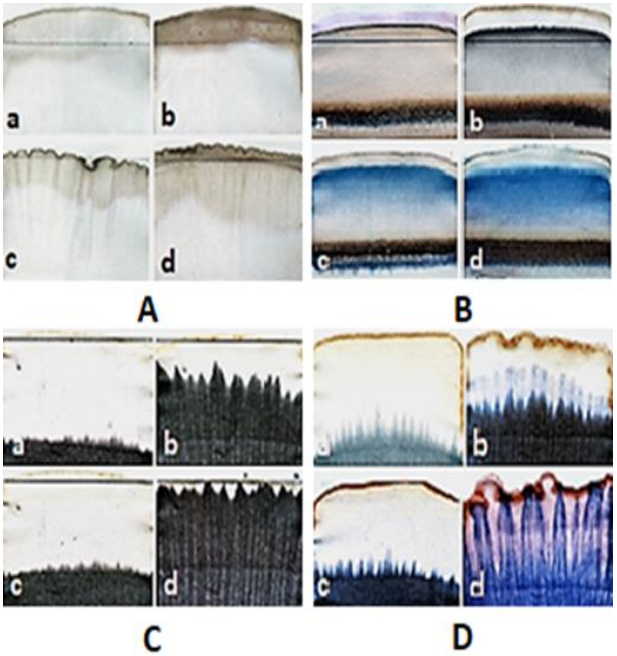

Figure 4. Ascending chromatograms of fulvic sub-fractions A, B, C and D in a) initial and b) final stage for the Mollic Gleysol (Salinic) and c) initial d) final stage for the Mollic Histic Gleysol (Salinic)

In Sub-fraction A of Mollic Gleysol (Salinic) (Figure 4a initial and Figure 4b final), respectively of Mollic Histic Gleysol (Salinic) (Figure 4c initial and Figure 4d final), the elution chromatograms showed, after absorption of the extract of the fulvic fractions on activated carbon, the accumulations of organic compounds soluble in acidified water, with relatively low molecular weights, such as carbohydrates and amino acids, much better highlighted in the final phase of the experiment, in the case of both soils. In Mollic Histic Gleysol (Salinic), the structuring of the compounds of the fulvic sub-fraction A, on the background of a richer and more diversified organic substrate, appears (in both phases) better highlighted than in Mollic Gleysol (Salinic).

In sub-fraction B, the elution chromatograms showed, in the case of both soils, some accumulations of organic compounds soluble in organic solvents, with high molecular weights, of the type of phenolic glycosides, tannins or insoluble ethers, much better highlighted, in the final phase of the experiment. In both soils, the structuring of the compounds of the fulvic sub-fraction B is observed, but for the Mollic Histic Gleysol (Salinic), these processes and biotransformation of the diversified organic substrate appear more intense.

In sub-fraction C, in the same way, the chromatograms highlighted, in the case of both soils, some accumulations of water-soluble organic compounds, such as polysaccharide compounds, mono-carbohydrates (glucose, galactose, xylose, ribose), fractions of uronic acids containing glucuronic acid, amino sugars rich in nitrogen, well highlighted after 30 days, in the final phase of the experiment, in the case of both soils.

In sub-fraction D, the elution chromatograms revealed a strongly pigmented content, in the case of both soils, of some accumulations of organic compounds soluble in alkaline water, with relatively high molecular weights which, after dialysis, contain mainly colored compounds such as amino sugars rich in nitrogen, pentose and organic compounds with phosphorus, well highlighted in Mollic Histic Gleysol (Salinic), in the final phase of the experiment.

During the 30 days of incubation, microbial activity become more intense, nutritional variety is constantly increasing and humic structures begin to stand out. At the end of the experiment, the biological processes appear more diversified and with increased intensity. The nutritional variety increases and the tendency is to accumulate melanic organic structures. Chromatograms of fulvic sub-fractions A, B, C and D were used to highlight the structuring of fulvic compounds, respectively the formation of condensed, short-acting and highly mobile compounds. The thickness and strong coloring of the analyzed external areas of the chromatograms suggest an intense biosynthesis of mobile organic compounds. At the same time, through the biotransformation of the endogenous organic substrates of the soils, in a short period of time, the initiated changes can be observed on the chromatograms by reducing the presence of mobile compounds. On the other hand, intensification of association reactions with other compounds indicates the formation of complexes with inorganic constituents and the evolution towards compounds with increased insoluble character. Also, if the concentration of fulvic compounds is high in the extract, thick migration fronts and dark external areas appear, as observed in the chromatograms of sub-fractions A and partially in sub-fractions B and D of both soils.

Through the processes of biosynthesis and biotransformation, the weight of these mobile compounds in the composition of sub-fractions can change, become considerably lighter, more fragmented or disappear. Also, the fulvic sub-fractions A, B, C and D highlight the formation of humic substances, mainly acid-base, with nutrient content and low complex organic content, but in which colloidal substances are present. The formation of complex humic substances is initiated, as highlighted in the chromatogram of the fulvic sub-fraction D-d (final) at Mollic Histic Gleysol

(Salinic), more than in the chromatogram of the similar fulvic sub-fraction D-b at Mollic Gleysol (Salinic).

The chromatograms of the fulvic sub-fractions C-D show the progressive increase of the mineral diversity in the two soils, relatively integrated in the organic material, a rich content of protein material, especially in the Mollic Histic Gleysol (Salinic). Its structural complexity increases and the level of microbial activity is the highest in the chromatogram of the final fulvic sub-fraction D of Mollic Histic Gleysol (Salinic). One can observe the intermediate processes of accumulation and integration of the biosynthesized compounds in the composition of the less organized organic substrate. The links between the different components can be evidenced, in the case of both soils and they appear aggregated in the solution. The accumulations of humico-fulvic compounds are not sufficiently integrated. The increase in intensity of microbial activities maintains the accumulation and integration of biosynthesized organic material in the soil organic substrate, in the final phase. Mainly, the stabilization of microbial detritus on mineral surfaces determines the formation of stable organic matter in the soil (Li et al., 2019; Just et al., 2021). Also, analyses revealed that organic compounds linked to minerals come mostly from products of microbial metabolism, they appear layered on the clay particles and, due to the microbial residues with a content rich in proteins and polysaccharides, are additionally stabilized. Thus, it was confirmed that the microbial way of their formation is the most important mechanism for a long-term storage of C (Poeplau et al., 2018).

3.5. Fluorescence of dissolved organic carbon

The carbon compounds that come from the two soils analyzed represent the organic matter extractable in water and was used because it can be an indicator of active but also labile reserves of soil organic matter (SOM) (Musadji et al., 2019; Gao et al., 2022).

Microbial carbon biosynthesis in these soils, under standard conditions and short periods of time, could have an effect on SOM through the labile, biosynthesized, water-extractable, fluorescent components, to which is also added the fluorescence of humic, proteinaceous, organo-mineral substances, present in the soil. Visualizing their presence and distribution was useful in elucidating the short-term effects of different microbial bio-synthesizers from analyzed soils on the content and composition of water-extractable organic matter and on the possibilities of C storage. Fluorescence matrices used extracts from the soils to highlight the significant fluorescent components of water-extractable organic matter, accumulated in the organic matter structure, following the microbial bio-syntheses in short-term.

The usual spectrophotometric methods used for determinations of quantitative changes induced by microbial synthesized C compounds, in short period of time (30 days), were not able to provide significant results in evidencing the influence on the organic matter of the soil (SOM).

But the fluorescent components present in the water-extractable organic matter of the analyzed soils and, in particular, their related fractions showed significant associations with the level and quality of microbial bio-synthesized compounds from the two soils (Figure 5).

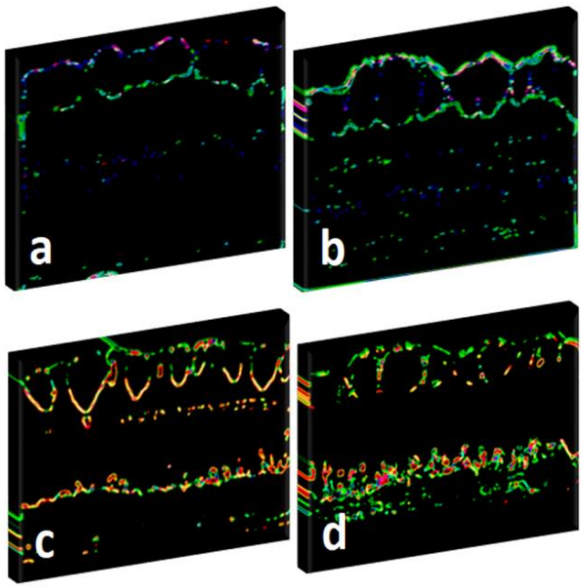

Figure 5. Fluorescent components of Mollic Gleysol (Salinic) at a) initial and b) final phase and of Mollic Histic Gleysol (Salinic) at c) initial and d) final phase

Thus, the fluorescent components highlighted imagistic the storage degree of newly bio-synthesized carbon in the organic matter, compared to the initial endogenous organic component, in the short period of time analyzed and its distribution, depending on specific characteristics for each type of soil. Also, the complexity and sensitivity of the newly bio-synthesized fluorescent components were compared to the endogenous organic component, with their initial spectrum, so highlighting the storage possibilities of biosynthesized microbial C from the two soils.

The microbial activity through the extracellular organic component determines the direct impact on the effectiveness of the mineral protection of the stored carbon and the biotic processes of its synthesis or decomposition in soils appear in real terms as complex interdependent and correlated phenomena (Liu et al., 2019; Rinot et al., 2021**)**.

3.6. Estimation of siderophores in soils

Acting as complexing ligands, siderophores were associated with the cycling of a wide range of biologically important micronutrients in soil, with implications for microbiome dynamics, organic matter and C stocks (Chen et al., 2020)

The synergistic interactions between the weak/strong organic ligands in the analyzed soils present different binding and transport affinities. Conditions and the physico-chemical characteristics influence the exchange of metal ions between the ligands.

The established standard conditions try to avoid the uncertainties regarding the stability points of the metal-ligand complexes, at the level of relevant interfaces in soils, respectively those between microorganisms and soil aggregates.

Studies have shown that the data obtained on interactions of high-affinity siderophores for Fe, always refer to a selected standard state.

Thus, our analysis regarding the stability, influence and evolution of the metal-ligand complexes depended on the environmental conditions in which they were carried out. Microorganisms in the Mollic Gleysol (Salinic) and Mollic Histic Gleysol (Salinic) need iron to ensure vital processes, for biosynthesis and cell growth.

Basically, the role of siderophores biosynthesized by microflora in the soils consists in mobilizing insoluble soil Fe (III) and also can form complexes with other metal ions (Fe^{2+}, Mg^{2+}, Ca^{2+}), present in soil substrates (Senthilkumar et al., 2021; Roskova et al., 2022). The stability of newly biosynthesized organic matter in soils is possibly controlled by sorption/desorption mechanisms mediated by Fe(III) present in it, mechanisms by which it controls the stabilization of organic C. Thus, iron become a key regulator, both in neutral environments, where it precipitates as Fe(III) hydroxides, and in acidic environments, where Fe (III) is soluble. The siderophores synthesized by the microflora stabilize organic C by means of Fe (III), forming complexes between the OH functional groups and Fe oxides. Through these complexes, the binding of labile organic compounds to Fe (III) is achieved, which contributes to their preservation and the sizes of the organic fractions intervene in establishing the flocculation/coating mechanisms involved.

Our findings regarding the biosinthesized siderophores are in concordance with data from literature confirming the importance of microbial exometabolites and Fe(III) complexes in soil organic matter stability (Souza et al., 2017; Giannetta et al., 2019; Kleber et al., 2021; Song et al., 2022).

In the competitive environment specific to the soils studied, initially there is a deficit of soluble iron evidenced by the diameters of the wells, respectively 11 mm in the Mollic Gleysol (Salinic) (Figure 6a) and 17 mm in the Mollic Histic Gleysol (Salinic) (Figure 6c), in which the increased microbial activity causes a greater biosynthesis of siderophores, so that the iron increases its availability in the substrate of this soil.

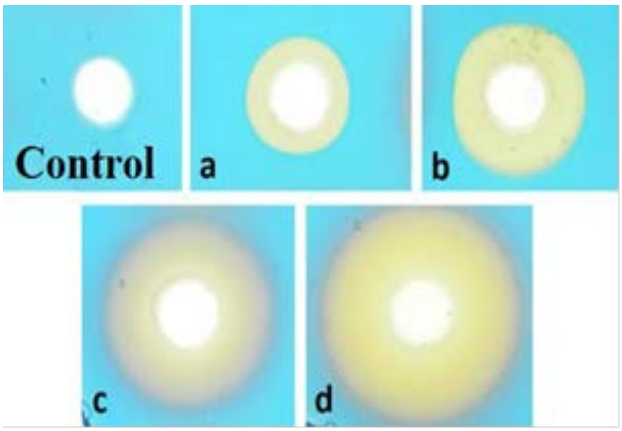

Figure 6. Siderophore concentration in Mollic Gleysol (Salinic) at a) initial and b) final phase and in Mollic Histic Gleysol (Salinic) at c) initial and d) final phase

The intensity of siderophore biosynthesis increases over time (30 days), in both soils, starting from a lower presence, especially in Mollic Gleysol (Salinic), the initial phase. An obvious increase follows, generated by an ample biosynthesis of siderophores by the specific microbiome developed in each soil, which, at the end of the experimental period, determines the diameters of the wells of 19 mm in Mollic Gleysol (Salinic) (Figure 6b) and respectively, 31 mm in Mollic Histic Gleysol (Salinic) (Figure 6d).

The increases in diameter reflect the induction and intensification of siderophore biosynthesis, the increase of microbial activity and the availability of iron in the substrate, especially in Mollic Histic Gleysol (Salinic).

Possibly, the functional groups of the biosynthesized siderophores progressively interact with iron in soils substrates, through the negatively charged oxygen atoms, but sometimes it can be replaced by nitrogen, potassium or magnesium, also coming from the substrate, a situation that causes distortions with implications on the reduction of affinity for Fe (III), of the capacity of microbial growth, biosynthesis and the capacity of soils to sequester C (Chen et al., 2020).

Over time, it is also possible that the higher chelating structures (hexadentate and tetradentate) become predominant and more stable, as the siderophores cause the entropy change by binding to

iron. Siderophore production by soil microflora provides sufficient amounts of iron needed for its survival, growth and activity in soils, with impact on C dynamics.

4. CONCLUSIONS

The soils had different levels of respiration potential between phases, before and after incubation period and in the final phase, the level of soil respiration was reduced in time with 4.27% in Mollic Gleysol (Salinic) and 14.60% in Mollic Histic Gleysol (Salinic).

The simultaneous evolution of CO_2 flux showed a continues decreasing trend in both soils, after the incubation in standard conditions, but more significant to Mollic Histic Gleysol (Salinic).

The levels of microbial biomass of both soils determined at the end of the experiment, after incubation period, were significantly increased, compared to the initial moment.

The ascending specific chromatograms of the fulvic sub-fractions A, B, C and D in both soils revealed, at the end phase of experiment, significant accumulates of soluble organic compounds, with different origins, molecular weights and complexity levels, obtained in only 30 days of incubation under standard conditions.

The fluorescent components present in the water-extractable organic matter of both soils evidenced associations with the level and quality of microbial bio-synthesized compounds, highlighting imagistic the degree of storages of newly bio-synthesized carbon, in a short period of time, compared with initial endogenous organic content, as well as their distribution, with specific characteristics for each soil subtypes.

The intensity of siderophore biosynthesis increased over time, starting from a lower presence, especially in Mollic Gleysol (Salinic) (Ø 11mm), followed by an increasing generated by an induced specific biosynthesis of siderophores, developed in each soil and an availability of iron in the substrates, by which, at the end of the experimental period, siderophores accumulations determine Ø 19 mm halo diameter for Mollic Gleysol (Salinic) and Ø 31 mm halo diameter for Mollic Histic Gleysol (Salinic).

Acknowledgements

The authors acknowledge the financial support received from the German Federal Ministry for Economic Affairs and Climate Action (BMWK), through The European Climate Initiative (EUKI) and the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH for the "Ecological resizing through urban and rural actions & dialogues for GHG mitigation in the Lower Danube Plains & Danube Delta EDAPHIC-BLOOM DANUBE" Project and from the Grant of the Romanian Ministry of Research, Innovation and Digitization, project number 44 PFE /2021, Program 1 – Development of national research-development system, Subprogram 1.2 – Institutional performance – RDI Excellence Financing Projects.

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Received at: 30. 09. 2022
Revised at: 10. 11. 2022
Accepted for publication at: 12. 11. 2022
Published online at: 14. 11. 2022