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Master's degree thesis

Development of a methodology to assess soil carbon stock stability in the presence of microbial activity

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Summary

Given that carbon is one of the most ubiquitous elements on Earth and plays a crucial role in the global carbon cycle, comprehending the fluxes of carbon among environmental compartments and identifying the factors influencing these fluxes is of fundamental importance. Moreover, the continuous emissions of CO_2 and greenhouse gases accentuate the significance of soil, already recognized as one of the Earth's largest carbon reservoirs, as a promising resource to sequester carbon dioxide from the atmosphere in the form of soil organic carbon.

With the paradigm shift from the conventional classification of soil organic matter based on humic substances to the emerging framework of the soil continuum model, innovative techniques have been under development to characterize this organic matter. Among these methods, the Rock Eval is a promising technique for quantifying soil organic matter and assessing its stability. This method involves a pyrolysis followed by combustion of a desiccated sample and was initially created for evaluating the petroleum potential of geological sources. In recent years, it has found application in studying the dynamics and persistence of soil organic carbon in the environment.

The core objective of this research project is to use the Rock Eval technique to establish a methodology for investigating the impact of various microorganisms on soil organic matter. This study represents the initial phase of a broader research aimed at producing microbial consortia able to increase carbon storage in soils. To achieve this, distinct microorganisms, both bacteria and fungi, were introduced into sterilized laboratory-prepared soils of varying textures, supplied with a readily available source of nutrients, and maintained at a constant temperature for one month. Samples were taken at the beginning and conclusion of the incubation period and analysed with the Rock Eval method for total organic carbon (TOC) and its stability. Furthermore an attempt was done to analyse the composition of the TOC in terms of Water Extractable Organic Carbon and Nitrogen, mineralogical and particulate fractions.

The results derived from the Rock Eval method revealed a decrease in the total organic carbon, as anticipated due to bacterial proliferation, followed by an enhancement in the thermal stability of the organic matter in certain samples. The methodology developed in this work can be applied to future investigations involving more complex soil types and microbial communities, supporting the identification of optimal microbial consortia to enhance carbon sequestration in soil.

1. Introduction

Soil organic matter and soil organic carbon have always been widely investigated due to their high importance in the carbon cycle, agriculture, and soil health. In the last years the characterization of soil organic carbon has shifted from the "humic substances" vision to the so-called soil continuum model, a new approach that focuses more on the total carbon and its forms in the environment. Furthermore the role of microorganisms in soil is strongly related to soil organic matter, and for these reasons, finding a methodology to study the qualitative and quantitative variations in soil organic carbon is always more important. This study aims to define a methodology to evaluate the quantitative variation of soil organic carbon and its thermal stability under different microbial consortia. This study was developed at SOIL lab of EPFL (Lausanne) and represents the first step of a research project conducted by the SOIL Lab in collaboration with the company YpHen. The results of this research will be useful, among other purposes, also to understand if the company's products have a positive impact on soil organic carbon stabilization.

1.1 The importance of carbon cycle

The carbon cycle is a fundamental process leading the transfer of carbon among various components, including plants, animals, microbes, minerals in the Earth, water bodies, and the atmosphere. Carbon, as the fourth most abundant element in the universe and fifteenth on earth (Jane B. Reece, Lisa A. Urry, Michael L. Cain et al, 2019), plays a crucial role in sustaining life on Earth. This cycle holds significant importance as it helps regulate the Earth's temperature by controlling the levels of carbon dioxide present in the atmosphere. In fact, CO_2 is connected to the greenhouse effect, and the recent constant release by humans in the atmosphere of CO_2 itself or of other greenhouse gasses is leading to the increase of temperatures and climate changes. ('Climate and the Carbon Cycle: Unit Overview', 2023). Human activities have a substantial impact on this cycle, primarily through the combustion of fossil fuels, alterations in land use, and the utilization of limestone for concrete production. These activities result in substantial carbon emissions into the atmosphere, leading to a rapid increase in atmospheric carbon dioxide levels (US Department of Commerce 2023).

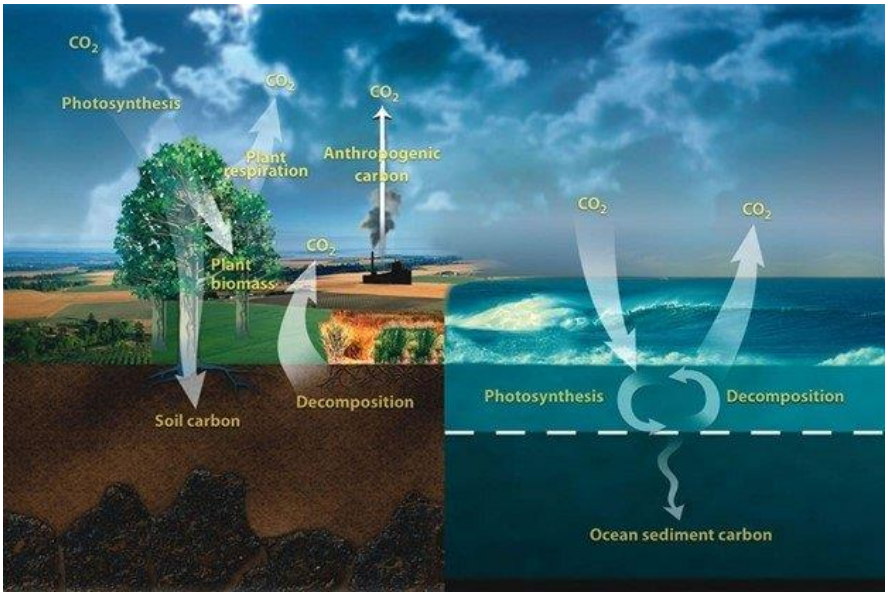

Figure 1: Representation of carbon fluxes among different environmental compartments('DOE Explains...the Carbon Cycle' 2023)

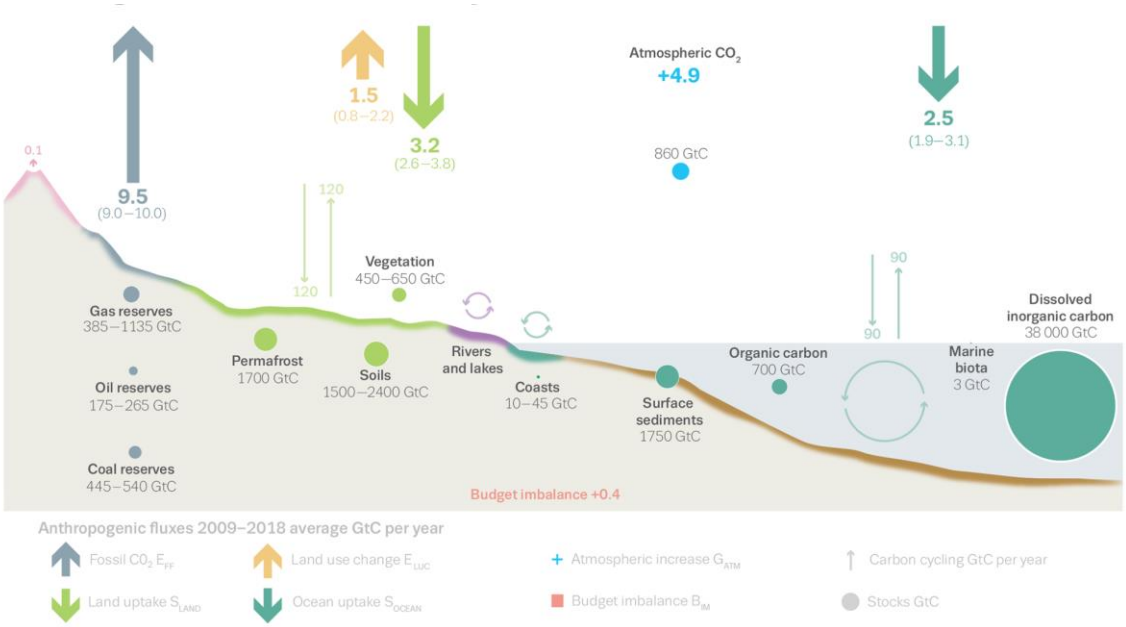

Figure 2: Carbon fluxes quantification (Friedlingstein et al. 2019)

The carbon cycle can be seen as composed of a short-term cycle, or biogenic cycle, involving the exchange of carbon between the atmosphere, land, and oceans, and a long-term cycle also known as the geological carbon cycle, involving the exchange of carbon between the Earth's crust, rocks, soil, oceans, and atmosphere (Horwath 2015).

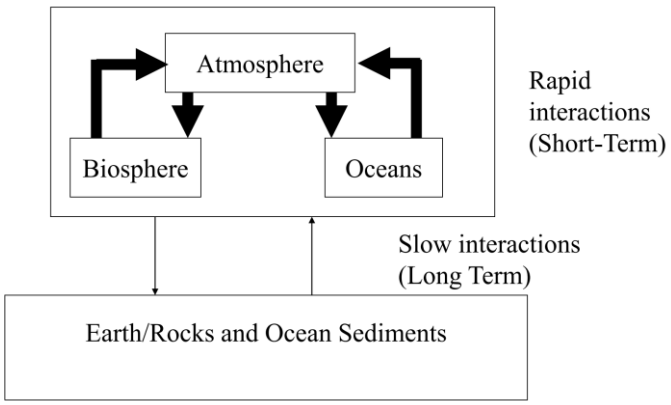

Figure 3: Carbon short and long-term cycle

Human activities have a significant impact on the carbon cycle. Some of the ways in which human activities affect the carbon cycle include:

- Burning fossil fuels: The combustion of fossil fuels, such as coal, oil, and gas, releases carbon dioxide into the atmosphere at a rate that exceeds its natural removal process. This leads to an increase in the concentration of carbon dioxide in the atmosphere ('What Human Activities Affect the Carbon Cycle?', 2023)
- Deforestation: involving the removal of trees, deforestation reduces the amount of carbon that can be absorbed by plants through photosynthesis, resulting in an increase in of atmospheric carbon dioxide levels ('7.4: Human Impacts on the Carbon Cycle', 2019)
- Land use changes: those alterations, such as converting forests into agricultural land, can also contribute to increase the amount of CO_2 in the atmosphere

- Cement production: The production of cement, which involves the use of limestone, releases carbon dioxide into the atmosphere('Carbon Cycle', 2023)
- Industrial processes: these activities affect the carbon cycle primarily through the release of carbon dioxide into the atmosphere. The burning of fossil fuels, such as oil, coal, and natural gas, releases stored carbon into the atmosphere, contributing to the greenhouse effect and global warming ('What Human Activities Affect the Carbon Cycle?', 2023)

Collectively, all human activities resulted in a 9.7 Pg CO_2 increase in the atmosphere in 2012 (Peters et al., 2013).

Canadell et al. 2010 identified four emerging effects of the modification of the carbon cycle as consequences of human activities in the literature:

- Natural carbon reservoirs on land and in oceans are becoming increasingly vulnerable, and their efficiency as carbon sinks may be decreasing, leading to enhanced carbon emissions from natural systems and faster atmospheric CO_2 accumulation
- The excess of anthropogenic emission of CO_2 is causing ocean acidification
- The methane cycle and its future dynamics are highly sensitive to climatic factors

Soil plays a crucial role in mitigation of greenhouse gas (GHG) emissions, as it constitutes more than two-thirds of terrestrial carbon reserves. Studies focusing on strategies to increase its capacity to capture carbon are in continue development (Rodrigues, Brito, and Nunes 2023). It has been estimated that the net uptake of carbon dioxide by soil corresponds approximately to one fifth of the annual anthropogenic carbon dioxide emissions due to the use of fossil fuels (Friedlingstein et al. 2019) and it has been proven that an increase in the temperature will decrease carbon storage in soils (Hartley et al. 2021).

Furthermore, changes in temperature and moisture levels affect microbial and biotic activity, resulting in alteration of the microbial decomposition of organic matter. Several studies have shown that an increase in air temperature accelerates the decomposition of organic matter, leading to higher carbon losses from soil and decrease in the carbon stocks.

1.2 Carbon in soil

Soil is a complex and dynamic natural material that plays a crucial role in supporting life on Earth and is a mixture of organic matter, minerals, gases, liquids, and organisms that together support the life of plants and soil organisms ('What Are Soils? | Learn Science at Scitable' n.d.).

Carbon in soil can be found both in organic and inorganic forms. Soil Inorganic Carbon (SIC) consists of primary and secondary inorganic carbonates. Calcium in surface soil reacts with atmospheric CO_2 and water, leaching into the subsoil and subsequently precipitating as secondary carbonates and sequesters atmospheric CO_2 directly to the soil.

Organic Carbon in Soil (SOC) could be separated into active or labile and passive or recalcitrant pools. Labile organic C pool has rapid turnover rate and can easily oxidize into the atmosphere; on the other hand, it fuels the soil food chain, hence influencing nutrient cycling, maintaining soil productivity. The recalcitrant pool of SOC is slowly used by microbes, contributing to the enrichment of the SOC stock and sustainability of the production system. Changes in C stock and the distribution of C into active and passive pools depend on crop management, type and biochemistry of organics added, crop residue management, climate, and soil environment (Majumder et al. 2018). Carbon buildup and stabilization in soil is a function of the quality and

quantity of the organic inputs applied. The application of different sources of different organic inputs has a significant effect on the manipulation of C stock and the allocation of C into different pools (Majumder et al. 2018).

Carbon is introduced into the soil through root exudates or by the decomposition of root or aboveground biomass. Within the soil matrix, carbon is present in various forms: as components of root or microbial biomass, in the form of bioavailable labile organic carbon, or in a more resilient, recalcitrant carbon state. The exit of carbon from the soil occurs through direct emissions or as a result of root or microbial respiration, with microbial-mediated soil respiration standing out as the primary contributor to terrestrial ecosystem CO_2 emissions. Additionally, carbon is lost from the ecosystem in the form of volatile organic compounds (VOCs) and methane (CH_4) (Jansson et al. 2021).

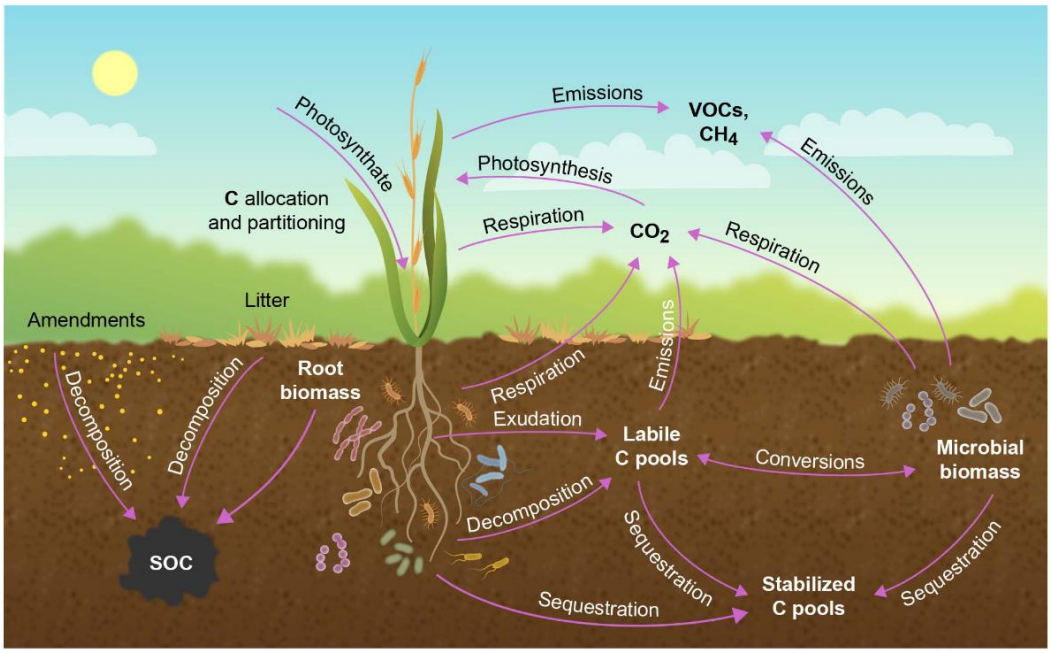

Figure 4: Carbon dynamic in soil (Jansson et al. 2021)

Soil Carbon sequestration occurs when the C intake in soil from the atmosphere is higher than the C released from the soil and microorganisms respiration. At the same time not only the sequestration is important, but also the stability of the new carbon sequestered, which depends on multiple different factors (Jastrow, Amonette, and Bailey 2007).

Soil microbial community (SMC) plays a major role in carbon cycling and its activity has been considered as the main driver of variation in the potential stock of carbon in soils. The composition of the SMC is crucial for preserving soil ecosystem services, as its structure and activity also regulates the turnover and delivery of nutrients, as well as the rate of soil organic matter (SOM) decomposition. The SMC not only regulates the rate of C decomposition, but also influences the formation of soil aggregates and the impact of agronomic practices, primarily mediated by short and long-term dynamics of SMC (Bhattacharyya et al. 2022).

Moreover Kallenbach, Frey, and Grandy 2016 demonstrated that SOM accumulation is driven by distinct microbial communities rather than clay mineralogy, where microbial-derived SOM accumulation is greatest in soils with higher fungal abundances and more efficient microbial biomass production. Alterations in SOM chemical composition influence its stabilization potential, interaction with clay minerals and potential responses to anthropogenic disturbances.

Grandy and Neff (2008) argued that soil carbon turnover, its variation across ecosystems and its response to perturbation can be understood through the interaction of three components:

1. the deposition of chemically distinct compounds in soil from plants (Spaccini et al., 2000; Wang et al., 2004)
2. The conversion of these plant-originated substances by bacteria and fungi through the use of external enzymes, as well as the absorption and re-release of carbon compounds when microorganisms die (Fierer and Schimel, 2003; Waldrop and Firestone, 2004)
3. the physical redistribution and stabilization of carbon in soils which includes transport, aggregation of soil particles and adsorption mechanisms (Jastrow et al., 1996; Mikutta et al., 2006; Grandy and Robertson, 2007).

1.3 Stabilization pathways of C in soil, C dynamics in soil (biochemical alteration and physicochemical protection)

Even if different points of view can be found in the literature regarding soil organic carbon stabilization pathways, most of them recognizes that C stability in soil depends not only on the chemical formula and stability, but also on other external environment factors.

According to Lützow et al. (2006) SOM is stabilized mainly through two mechanisms:

- The formation of Mineral-Associated Organic Matter (MAOM), which is the result of interactions between the SOM molecules and the mineral soil component forming bonds (stronger thanks to ligand exchange or weaker through Van der Waals forces)
- The formation of aggregates which constitute a protecting environment for SOM by altering its physical accessibility for uptake (Bhattacharyya et al. 2022)

Similarly two major mechanisms has been described to stabilize SOM: (bio)chemical alteration and physicochemical protection. With (bio)chemical alteration, SOC is transformed by biotic and abiotic processes to chemical forms that are more resistant to decomposition and more easily retained by sorption to soil solids. With physicochemical protection, the biochemical attack of SOC is inhibited by organo-mineral interactions at molecular to millimetre scales. Stabilization of decomposable SOC can occur via sorption to mineral and organic soil surfaces, occlusion within aggregates, and deposition in pores or other inaccessible locations. Soil structure controls and indicates the SOC stabilization capacity of a soil, one potential option for reducing SOC turnover and enhancing sequestration, is to modify the soil physicochemical environment to favour the activities of fungi (Jastrow, Amonette, and Bailey 2007).

Kopittke et al. 2020 also showed that newly added organic matter can form organo-mineral associations not only thanks to N-rich microbial metabolites attached directly to mineral particles, but also forming associations with already existing mineral-bounded organic matter.

However, current knowledge of OM evolution and sequestration in soils (Schmidt et al. 2011; Lehmann and Kleber 2015) suggests that, all the processes can be related to two main mechanisms. First, in the organic soil horizons, the preferential decomposition of immature biopolymers leads to the relative increase in refractory SOM compounds. Conversely, in organo-mineral and mineral soil horizons, the increased contributions of the refractory SOM compounds should be linked to an increased mineral protection (i.e. adsorption on mineral surfaces) of progressively decomposing organic compounds (D. Sebag et al. 2016; Lehmann and Kleber 2015).

In soils where clay and silt concentrations are high, physical protection should dominate C stabilization mechanisms. Evidence for this includes the close relationship between soil C concentrations and both clay content and aggregation (Six et al., 2002; Shaver et al., 2003), as well as the fact that the turnover times of physically protected C may be an order of magnitude greater than particulate C (Balesdent, 1996). The stabilization of different forms of C varies across size classes, but the molecular structures can be altered by N fertilization, tillage and, very likely, other ecosystem alterations that modify microbial communities and their access to substrates. (Grandy and Neff 2008).

Clay mineralogy is a key parameter for chemical protection of SOC. Clay can be seen as composed by two main building blocks, silica tetrahedron and aluminium octahedron, that can be spatially disposed with different combinations ('Soil Chemistry/Clay Minerology', 2023). It is possible to identify the so called:

- 1:1 clay, composed of one tetrahedral sheet and one octahedral sheet, like kaolinite
- 2:1 clays, constituted by two tetrahedral sheets on either side of an aluminium octahedral sheet as montmorillonite and vermiculite

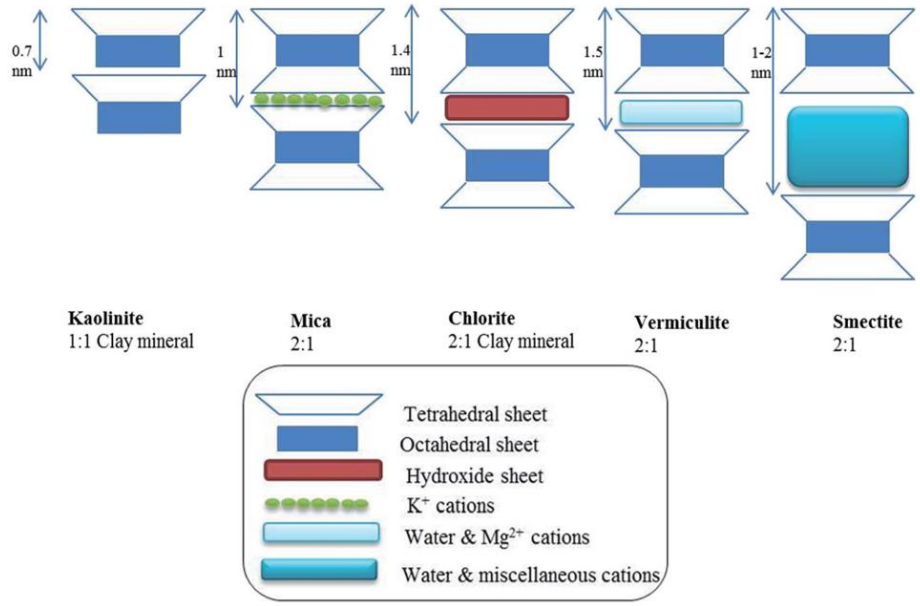

Figure 5: Clay composition (Ghadiri, Chrzanowski, and Rohanizadeh 2015)

Jastrow, Amonette, and Bailey (2007) shows that 2:1 clay minerals provide higher surface-charge density than 1:1 minerals, providing more sites for complexation with organics.

According to a more recent vision soil organic carbon persistence can be understood as a result of functional complexity derived from the interplay between spatial and temporal variation of molecular diversity and composition (Lehmann et al. 2020).

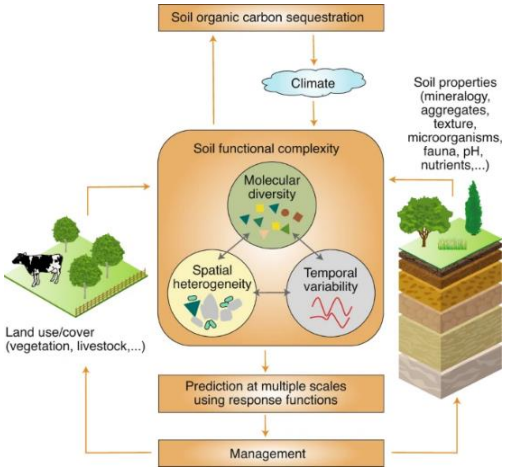

Figure 6: Integration of molecular, spatial and temporal complexity with management and prediction of soil carbon sequestration (Lehmann et al. 2020)

1.4 Soil organic carbon classification: the new soil continuum model

The traditional approach to the study of soil organic matter involved the first step of separating the organic and inorganic components, in order to allow subsequent further studies of the organic matter. This organic matter extraction involves the addition to the soil sample of a sodium hydroxide solution, characterized by a very high pH equal to 13, which allows the ionization of most oxygen-containing functional groups and enhancing the water solubility of organic compounds (‘International Humic Substances Society (IHSS). What Are Humic Substances?’ 2023). With the addition of protons to the solution, a dark solid forms, commonly referred to as 'humic acid’, while the remaining organic matter that stays soluble after reacidification is termed 'fulvic acid'. The substantial portion of organic matter that does not react to the treatment, either due to a lack of ionizable functional groups or protection from the alkaline treatment by minerals, is designated as 'humin' (Schnitzer 2001).

However this extraction procedure is characterized by different conceptual problem, identified by Lehmann and Kleber (2015):

a. The extraction is always incomplete, with an extraction rate around 50-70%, although the use of the alkaline solution leads to the extraction of soil fractions not meant to be “humic substances” such as living biomass.
b. The high pH of the used solution leads to an exaggerated chemical reactivity rather than isolates the compounds
c. The definition of humic substances results as synthesis product without a link to the alkaline extraction is incorrect.

As consequence of the individuation of those problems Lehmann and Kleber (2015) identified a new view stating that organic matter exists as a continuum of organic fragments continuously processed by the microbial community in molecules of smaller size. This breakdown of molecules results in the decrease of the size of the primary plant material and a consequent increase in polar and ionizable groups, characterized by higher solubility in water. This leads to a higher mobility in the soil water, but at the same time to an higher reactivity towards mineral surfaces and incorporation into aggregates, giving a higher protection against further decomposition. This new view, represented in Figure 7 and Figure 8, and compared with the traditional view:

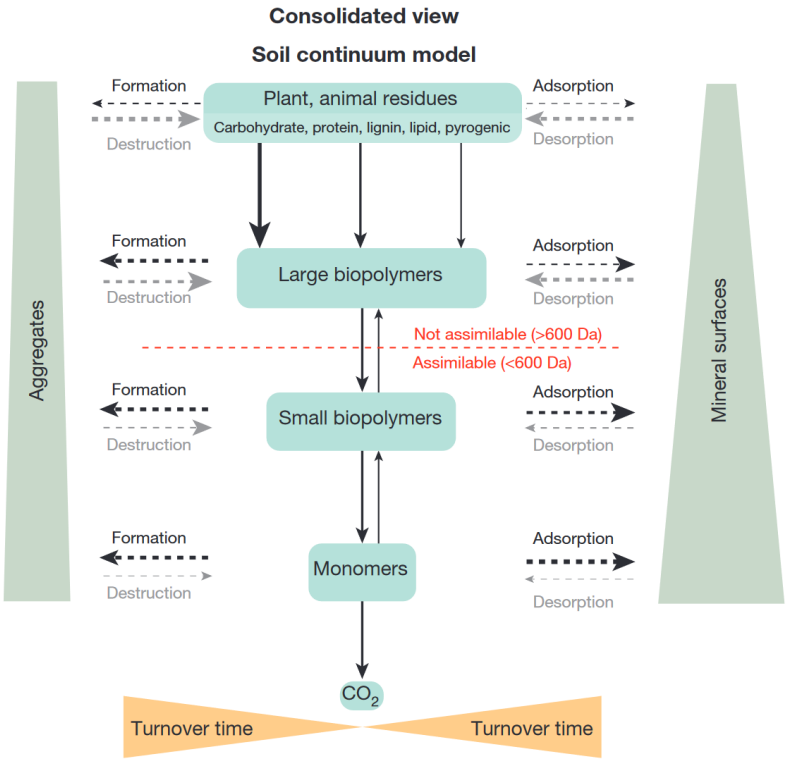

Figure 7: Comparison new and traditional view (Lehmann and Kleber 2015)

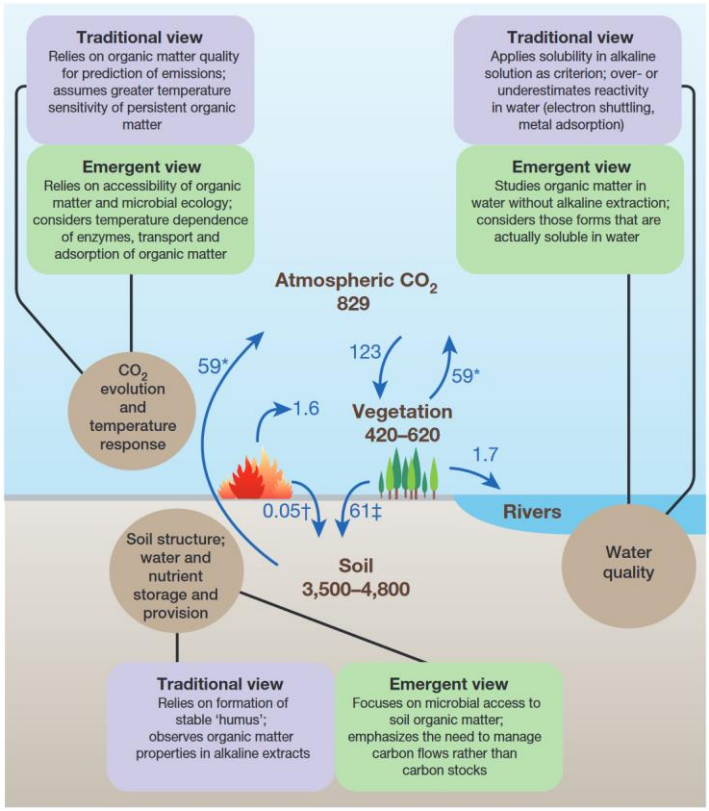

Figure 8: Comparison new and traditional view (Lehmann and Kleber 2015)

Despite the changes in the paradigm the concept of stability of organic carbon in soil remains one of the main points of soil organic matter study. The traditional classification of soil organic matter shifted from the concept of humic substances to the soil continuum model (SCM), with the division between particulate organic matter (POM) and mineralogical-associated organic matter (MAOM), different in term of their formation, persistence, and function (Lavallee, Soong, and Cotrufo 2020).

With this new vision soil organic matter is classes are identified only by their dimensional characteristics:

- POM between 53 μm and 2 mm, generally characterised by light fragments, relatively undecomposed
- MAOM smaller than 53 μm, which consist of single molecules or microscopic fragments of organic material (directly leached or microbial transformed)
- Dissolved organic carbon (DOM), also called Water extractable organic carbon (WEOC), being smaller than 0.45 μm.

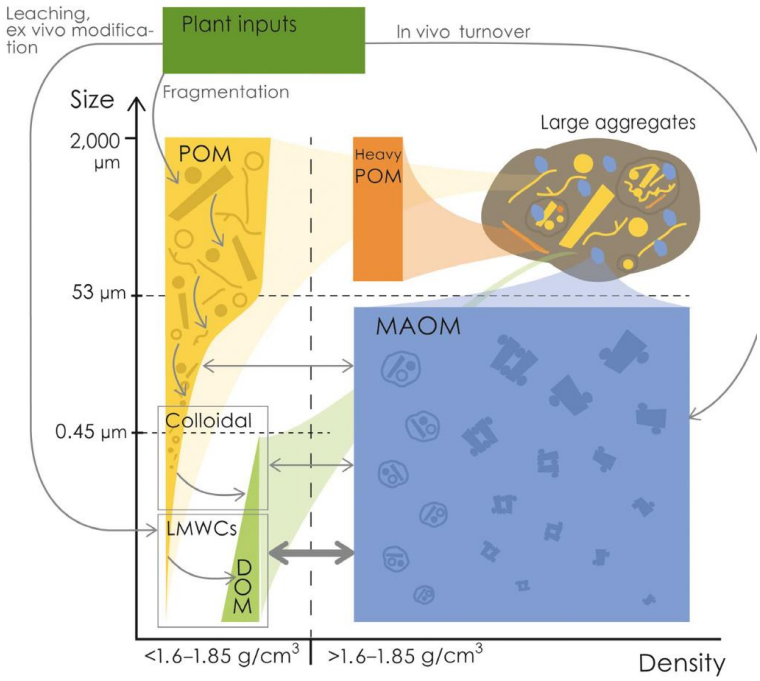

Figure 9: SOM component (Lavallee, Soong, and Cotrufo 2020)

The main distinction between MAOM and POM lies in the level of protection they receive from decomposition. MAOM is protected against decomposition thanks to its connection with soil minerals, whereas POM lacks this protection. MAOM protection occurs through various mechanisms, such as the formation of chemical bonds between soil organic matter and mineral surfaces, as well as its confinement within micropores or small aggregates measuring less than 50-63 μm. These mechanisms collectively make SOM less accessible to decomposers and their enzymes, as described in studies conducted by Lehmann and Kleber 2015, Kögel-Knabner et al. 2008 and Totsche et al. 2018.

A further classification of soil organic matter can include also density characteristics, leading to the definition of light and heavy POM, that may be useful in order to understand the general POM dynamics in comparison to the MAOM one (Cotrufo 2015).

1.5 Rock Eval: a new method to analyse soil organic matter

Over the years, several methods have been developed for the study of soil organic matter, both in a quantitative and qualitative way (Chenu, Rumpel, and Lehmann 2015):

- Fractionation methods, chemical or physical: these methods aim to subdivide the complex continuum of SOM to identify and characterize its constituents and to isolate functional pools.
- Characterization methods, can include wet chemical methods for analyses of biochemical SOM constituents and Spectroscopic methods to characterize functional groups: the objective is the molecular characterization of SOM composition, essential because most pedological processes operate at the molecular scale. Those methods allow to extend the understanding of SOM and its components origin and transformation processes
- Visualization methods: allow to gain morphological information on SOM to have better understanding of its spatial heterogeneity, dynamics or roles. Those procedures allow to locate SOM within its natural environment and study its relationship with mineral soil constituents, its accessibility to decomposers, or its contribution to soil structure.

Among all the methods available, particularly interesting is the Rock Eval method, which allows for both quantitative analysis of the carbon stock in soil and qualitative studies related to various indices. These indices can be obtained either directly from certain instrument measurements or as a result of data computation.

Born in 1970s at the French Institute of Petroleum (IFPEN), the Rock Eval method was developed initially to study the total organic carbon and oil potential of petroleum source rocks ('IFPEN | Rock-Eval® : Thermal Analysis of Rocks and Soils' 2023). This approach allows to quantify the amount of free hydrocarbons and the one potentially released after maturation (Behar, Valérie, and Penteado 2001a). After the first optimizations of this process in the petroleum field, the Rock Eval method also began to be used for the study of total organic carbon and mineral organic carbon in soils, demonstrating its relevant application in this field (D. Sebag et al. 2006; Malou et al. 2020; Hazera et al. 2023; D. Sebag et al. 2016; Disnar et al. 2003).

The principle of the Rock Eval method is to measure the release of carbon compounds from the gradual thermal degradation of the sample, exposed to a programmed increase of temperature ('IFPEN | Rock-Eval® : Thermal Analysis of Rocks and Soils' 2023). In particular, in the latest version used of this method, Rock Eval 6, the sample undergoes to:

- A first step of pyrolysis, performed in an inert atmosphere (helium or nitrogen), where the sample is heated under non isothermal condition up to 650°C;
- To reach the total oxidation of the sample the pyrolysis is followed by a combustion under artificial air (20% N_2, 80% O_2) of the residual carbon, reaching 850°C (D. Sebag et al. 2006; Behar, Valérie, and Penteado 2001b; D. Sebag et al. 2016).

Through the whole process the amount of CO and CO_2 released are measured on line with a infra-red cell (IR). Furthermore, during the pyrolysis step a Flame Ionization Detector (FID) measures the release of hydrocarbons (HC). The two combustion chambers, the IR cell and the FID are combined in one instrument outlined in Figure 10.

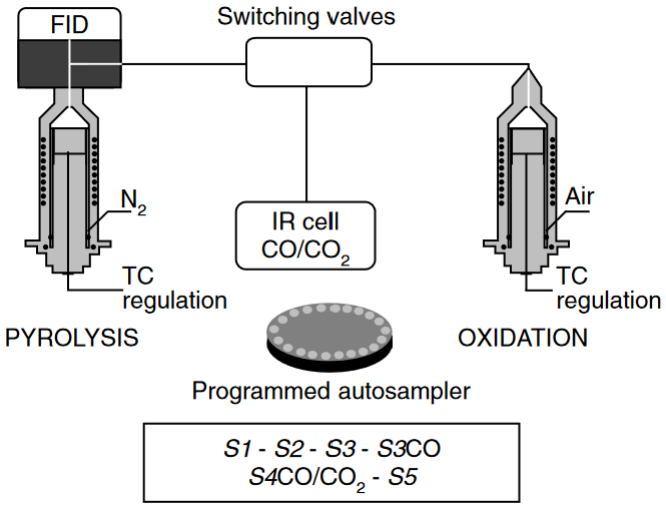

Figure 10: Rock Eval 6 complete analysis, serial process

The values of HC, CO and CO_2 emissions can be plotted over the temperature obtaining the following important curves for each sample as represented in Figure 11:

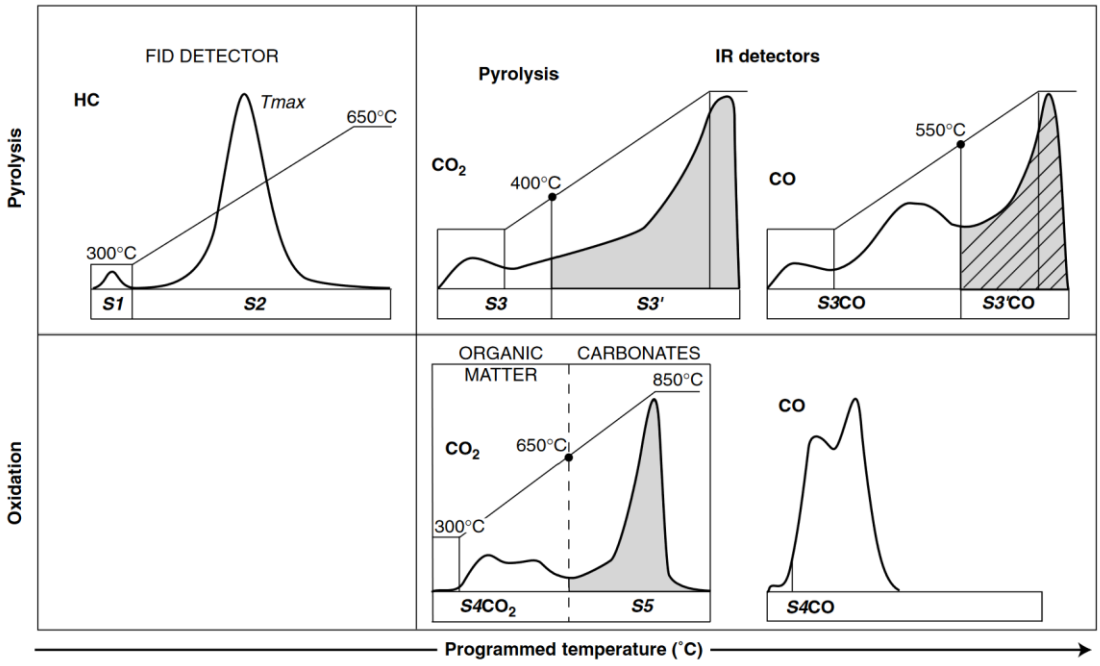

Figure 11: Curves resulting from Rock Eval method (Behar, Valérie, and Penteado 2001b)

Those curves can be split into different temperature range, as shown in Figure 11, and the integral of these curves are helpful to obtain different information. These integral are referred as S, meaning with S the integral of a specific part of the curve. The signal corresponding to the emission of HC is divided into two parts:

- S1, which is the integral of the part of the curve below 300°C, and usually represents the thermovaporized free hydrocarbons present in the sample
- S2 integrates the part of the curve registered after reaching 300°C until the end of the pyrolysis, represents the hydrocarbons released from the cracking of sedimentary organic matter (Behar, Valérie, and Penteado 2001b).

Also the signal measured from the IR detectors are split as follows (Behar, Valérie, and Penteado 2001b; D. Sebag et al. 2006; Lafargue, Marquis, and Daniel 1998):

- CO measurements in the pyrolysis phase are separated in two parts:
    - S3CO is the part of the curve measured until a minimum in the emission of CO is reached, this area represents the release of CO linked to organic matter
    - S3'CO integrates the second part of the curve till the end of the step, and is a consequence of the reactivity CO_2 released during the thermal decomposition of carbonate
- CO measurements during the oxidation phase corresponds to S4CO area, knowing that all the carbon measured as CO is of organic origin
- CO_2 measurements in the pyrolysis phase can be split into:
    - S3, which integrates the curve below 400°C, being the CO_2 with organic origin
    - S3', which integrates the curve after 400°C until the end, corresponding to a mineral origin
- CO_2 measurements in the oxidation phase is separated into:
    - $S4CO_2$ corresponds to the integral of the curve between 300°C and the temperature at which a minimum is reached, usually between 500°C and 720°C, with an organic origin of the carbon
    - S5 is the remaining area of the curve after the minimum, corresponding to an inorganic origin of the carbon

Starting from these areas, the Total Organic Carbon (TOC) is calculated with the following equation (Behar, Valérie, and Penteado 2001b):

$$TOC\ (\%) = \frac{\left[0.83 * (S1 + S2) + \frac{12}{44} * (S3 + S3' + S4CO_2) + \frac{12}{28} * (S3CO + S3'CO + S4CO)\right]}{10}$$

With the same method also the Mineral Carbon is equal to :

$$MinC = \frac{\left[\frac{12}{44} * S3' + \frac{12}{28} * \frac{1}{2} S3'CO + \frac{12}{44} * S5\right]}{10}$$

Another important parameter obtained directly from the pyrogram is the so called TpkS2 or Tps2, which corresponds to the temperature at which the peak of HC release occurs during pyrolysis (David Sebag et al. 2022). This parameter is connected to the thermal maturity of the organic matter in the sample, higher is the TpkS2 higher is the thermal stability of the compounds (D. Sebag et al. 2006).

Table 1: Parameters acquired with the Rock Eval 6 (Behar, Valérie, and Penteado 2001b)

Acquisition parameters	Detector/Oven	Unit	Name
S1	FID/Pyrolysis	mg HC/g rock	Free hydrocarbons
S2	FID/Pyrolysis	mg HC/g rock	Oil potential
TpS2	–	°C	Temperature of peak *S2* maximum
S3	IR/Pyrolysis	mg CO_2/g rock	CO_2 organic source
S3'	IR/Pyrolysis	mg CO_2/g rock	CO_2 mineral source
TpS3'	–	°C	Temperature of peak *S3'* maximum
*S3*CO	IR/Pyrolysis	mg CO/g rock	CO_2 organic source
*TpS3*CO	–	°C	Temperature of peak *S3*CO maximum
*S3'*CO	IR/Pyrolysis	mg CO/g rock	CO organic and mineral source
*S4*CO_2	IR/Oxidation	mg CO_2/g rock	CO_2 organic source
S5	IR/Oxidation	mg CO_2/g rock	CO_2 mineral source
TpS5	–	°C	Temperature of peak *S5* maximum
*S4*CO	IR/Oxidation	mg CO/g rock	CO organic source

From the FID and IR measurements other two indexes, can be estimated (David Sebag et al. 2022; Disnar et al. 2003; Delahaie et al. 2023):

- The Hydrogen Index (HI) [mg HC/g soil], is the amount of HC released during pyrolysis relative to the TOC, and is associated to the origin of the organic matter and its thermal stability. An increase in the HI indicates a higher hydrogen content suggesting a less thermally stable organic matter.
- The Oxygen Index (OI) [mg CO_2/g soil], corresponds to the amount of CO_2 released during the pyrolysis phase, is related to the level of oxidation of the organic matter and its elemental stoichiometry. In this case an increase in the OI indicates a higher oxygen content, suggesting a more oxidized organic matter.

Usually those two indexes are represented in the same graph, as shown in the figure below:

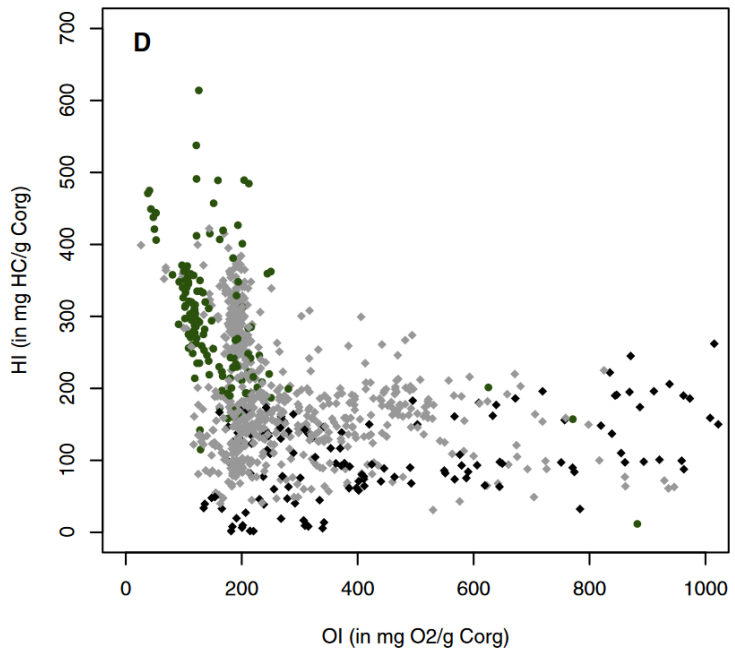

Figure 12: Example of HI/OI index representation (D. Sebag et al. 2016)

In 2006 D. Sebag et al. demonstrated how from the deconvolution of the S2 peak it was possible to obtain further insights into the soil organic matter composition and identify the so called I and R index. Starting from these considerations the I and R indexes have been reviewed, using an integration factor instead of deconvolution. These two indexes assess the contribution to the thermal stability of SOM, being:

- I-index (I for immature) related to the contribution of thermally labile immature SOM;
- R-index (R for refractory) related to the contribution of thermally stable OM (D. Sebag et al. 2016).

A very useful representation of those index is the I-R diagram, as shown in

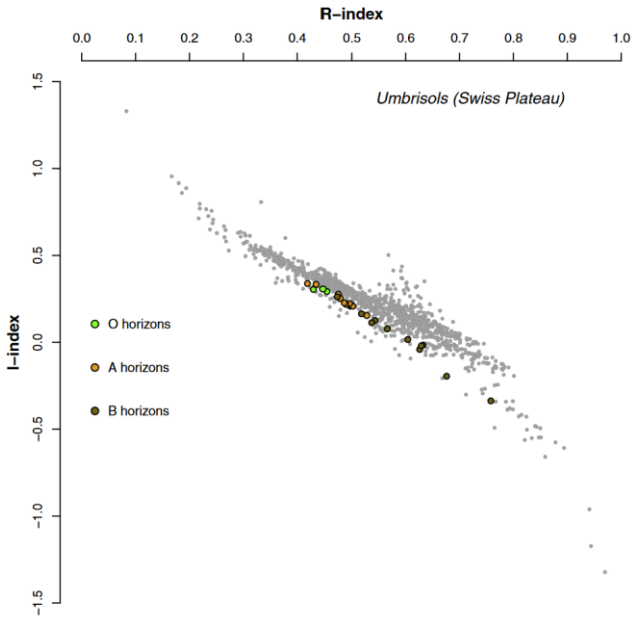

Figure 13: I-R index example of Umbrisols samples(D. Sebag et al. 2016)

When soils are characterized by homogeneous pedological processes, the I and R index present a distinct linear relationship, as shown in Figure 13. This linear relationship between the I and R indices can be attributed to the characteristic signature of soil organic matter stabilization processes: the decomposition of biopolymers increases the refractory SOM compounds and increased mineral protection of decomposing organic compounds.

2. Methods

2.1 General organization of the experiments

The objective of this experiment is to investigate, with the use of the Rock Eval method, the variations in soil organic carbon in soils with different soils textures in the presence of different microorganisms. To reduce the number of variables in this experiment, a simulated model soil was created in the laboratory using a mixture of sand and clay. Additionally, the model soil was inoculated with microorganisms, a source of easily decomposable organic matter (Tryptic Soy Broth), and a predetermined amount of water to achieve a specific moisture content.

To generate a range of soil textures in the model soils, kaolinite clay and pre-treated sand were combined on a dry weight basis. Kaolin clay was ordered by Sigma Aldrich, while Sand with particle size between 0.1 and 3 mm was ordered by the local company Carlo Bernasconi AG. The following soil textures were chosen based on the typical range of clay content present in Swiss soils ('SoilMaps' 2023) :

- 20% clay and 80% sand (20%)
- 10% clay and 90% sand (10%)
- 5% clay and 95% sand (5%)

For each soil texture, 3 different microorganism amendments were used, and for each combination triplicates were prepared:

- *Trichoderma asperellum* (received from company, colture on a potato agar plate) (T)
- *Streptomyces sp.* (DSM 687) (received from University of Zurich, frozen strains) (S)
- *Trichoderma asperellum* and *Streptomyces* together (TS)
- Control set without any microorganisms (C)

Table 2: Combinations used

Soil texture	Microorganisms	Replicate
20% clay	Trichoderma	3
	Streptomyces	3
	Trichoderma +Streptomyces	3
	Control	3
10% clay	Trichoderma	3
	Streptomyces	3
	Trichoderma + Streptomyces	3
	Control	3
5% clay	Trichoderma	3
	Streptomyces	3
	Trichoderma + Streptomyces	3
	Control	3
	Total jars	36

Counting the triplicates in total 36 jars with around 100g of soils were prepared, with the scheme in Figure 14:

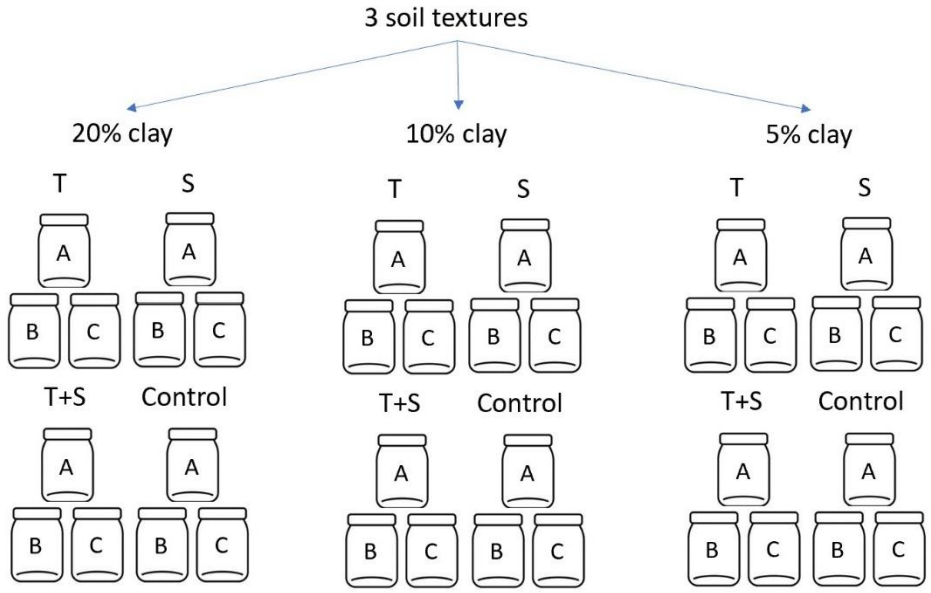

Figure 14: Experiment's scheme

To ensure that the correct amount of liquid was added to each jar, an experiment was conducted to determine the Water Holding Capacity (WHC) of each soil texture. The results of this experiment were then utilized to estimate the appropriate quantity of liquid broth needed to supply organic matter for the growth of microorganisms. To maintain osmotic balance between the two liquids, a mixture of deionized water and NaCl was added, having a salt concentration that corresponds to the one of the broth, equal to 10 g/l.

The jars were placed in a closed incubator, devoid of sunlight, and incubated for a period of 4 weeks at a constant temperature of 25°C. To facilitate the exchange of gases between the jar and the environment while maintaining consistent water content and contamination between the different treatments, the jars were covered with parafilm. This protective covering allows for gas exchange while preventing water from escaping, thereby ensuring the stability of soil moisture throughout the incubation period (Miller 2021).

The samples taken at the beginning of the incubation period were only tested using the Rock Eval method, without any specific pretreatments, while the samples at the end of the incubation were analysed for water extractable organic carbon, microbial biomass using the Chloroform direct extraction method, and Rock Eval on the total fraction, MAOM and POM.

The timeline of the analysis made is shown in Figure 15 and Figure 16.

Figure 15: Analysis made at the different timepoints

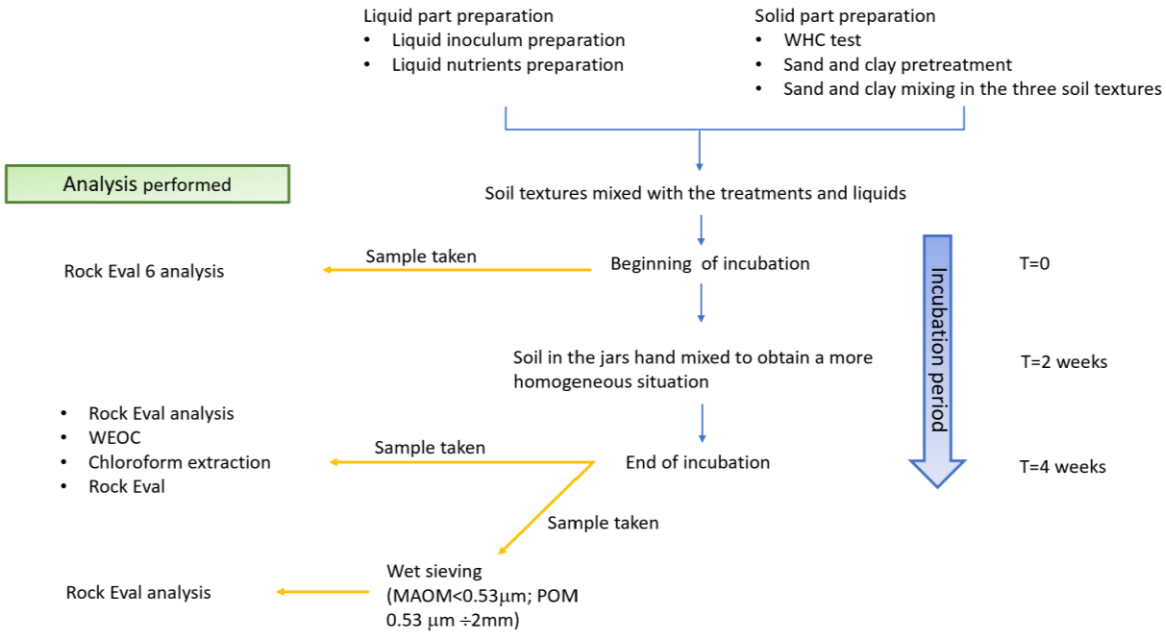

Figure 16: General scheme of the experiments

2.1.1 Microorganisms description

Trichoderma asperellum is frequently isolated from soil samples, especially from the rhizosphere, which is the narrow region of soil that is directly influenced by root secretions and associated microorganisms (Sehim et al. 2023). This microorganism is characterized by a high growth rate in culture medium, indicating its rapid proliferation under suitable conditions, and efficient sporulation (Gupta et al. 2014). The characteristics of this microorganism make it a valuable resource for sustainable and environmentally friendly approaches to crop protection and disease control (Matas-Baca et al. 2022; Wu et al. 2017; Yang et al. 2021).

Streptomyces sp. (DSM 687) is a versatile bacterium found in diverse environments, particularly in the rhizosphere of plants like tomatoes, suggesting its potential as a plant growth-promoting rhizobacterium. Other Streptomyces strains, such as Streptomyces sp. TOR3209, have demonstrated the ability to enhance the growth of various plants and exhibit antimicrobial properties against phytopathogens, positioning them as promising biocontrol agents for plant disease management (Hu et al. 2020). Streptomyces sp. (DSM 687) and related strains offer valuable attributes for sustainable agricultural practices, including plant growth promotion and biocontrol of plant diseases (Liu et al. 2013).

2.2 Model soil preparation

2.2.1 Sand pre-treatment

To ensure the removal of any carbonate present in the sand and facilitate accurate Total Organic Carbon (TOC) calculations, a process of acid washing was performed prior to use. Approximately 3.5 kg of sand was measured, and a 1% hydrochloric acid solution was prepared starting from concentrated acid. This involved mixing 10 ml of 37% hydrochloric acid in 1000 ml of solution.

To execute the acid washing, filter cones were placed on plastic bottles to collect the acid waste in a fume hood. A small quantity of sand was then washed at a time to allow the acid to evenly penetrate throughout the sand. The sand was initially saturated with the acid solution, followed by three subsequent rinses with deionized water to eliminate any remaining traces of acid.

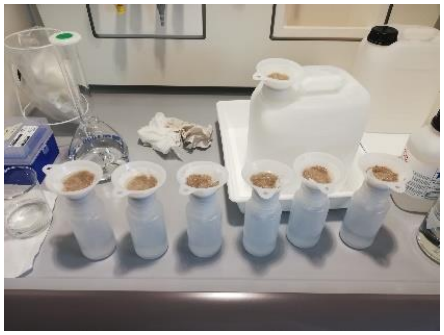

Figure 17: Acid washing of the sand

Approximately each time 300g of sand where treated with 250 ml of acid and the three washing with water required 1 l of deionized water. The wet sand was then let dry overnight at 85°C.

The clay utilized in this study is Kaolinite and was not subjected to any pre-treatment to achieve homoionic conditions (Domeignoz-Horta et al. 2020) . However, the composition of the clay was examined through X-ray Fluorescence (XRF) analysis. This analysis was conducted to determine the presence of various ions within the clay, as the composition of ions can potentially influence the stability of soil organic carbon (Rakhsh et al. 2017) .

2.2.2 Soil mixing

The amount of each soil texture needed for the experiment was first hand mixed in big glass jars on a dry weight basis and then autoclaved once at 125°C for 25 min with a wet cycle in order to sterilize the soil. These amount of soils were weighted with the jar before and after the autoclave cycle in order to estimate the amount of water absorbed by the soil during the sterilization process and to allow a more homogeneous distribution of soil between all the jars.

Figure 18: Soil after autoclave

The jars used for the experiments could not undergo to autoclaving and for this reason in order to ensure sterile condition the jars were washed with ethanol 70% and let dry open in the fume hood for around 30min/1 hours and then labelled.

In the end around 100g of autoclaved soil of each soil texture were added in each jar, ready for the liquid inoculum addition and water.

Figure 19: Subdivision of the soil samples in the jars

2.3 Water Holding Capacity Test

In order to evaluate the correct amount of liquid to add to the model soils a water holding capacity test was done on all the three soil textures. Other than the previous described soil textures an additional one composed by 30% of clay and 70% of sand was tested. Between 40 and 43 grams of model soil of each texture were inserted in a tubes open on both sides, one closed with a Whatman #40 filter and a rubber band. Those tubes were immerged in water for 10 minutes until fully saturated and then positioned on a tube holder in order to allow the water to percolate out of the tubes. The tubes where weighted before the immersion in water, one hour after the extraction from the water and then 4 hours and 12 hours after.

Figure 20: Water holding capacity test

Since some of the clay left the tube despite the filters, the first attempt of the experiment was followed by a drying of the tubes overnight to evaluate the new dry weight. The difference in weight of the dry sample before and after the experiment was considered negligible and for this reason the experiment was repeated and then the values of the water holding capacity where evaluated starting from the average result of the two experiments.

Furthermore the soil texture corresponding to 20% of clay content, in the second replication of the experiment, visibly lost some clay in the first step of immersion in the water. In order to check the validity of the value obtained for that sample, a linear interpolation of the values of the other soil textures has been done. The value of WHC identified with the linear interpolation for a soil

containing 20% of clay was equal to the one obtained with the calculations, for this reason the value obtained has been considered valuable for the next steps.

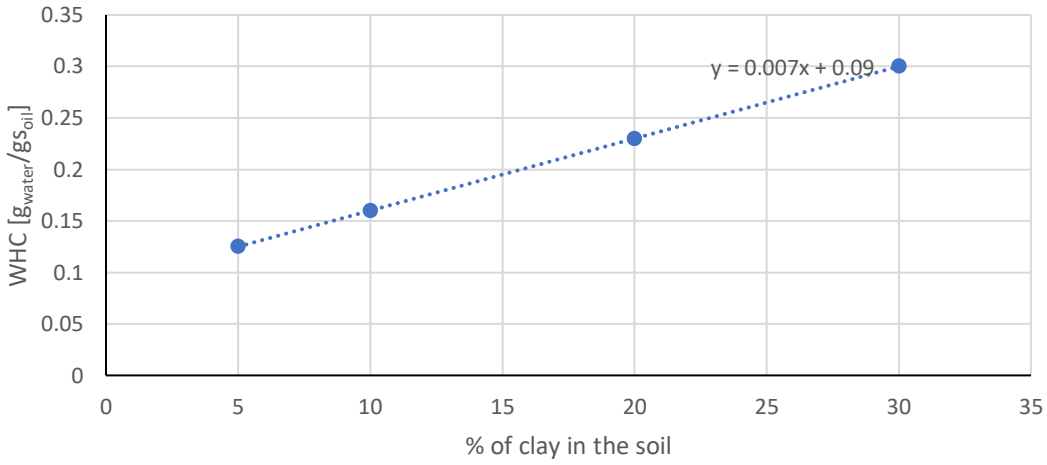

Figure 21: Water holding capacity test results

The water holding capacity was then calculated as:

$$WHC = \frac{wet\ weight_{10min}\ (g) - dry\ weight_{12h}(g)}{dry\ weight_{12h}(g)} = g_{water}/g_{soil}$$

Knowing the average weight of each soil sample equal to 100 g , the total amount of liquid to add was calculated as equal to the 60% of the total water holding capacity of each soil texture:

$$Liquids = 60\%\ WHC$$

2.4 Liquid inoculum preparation

2.4.1 Preparation of LB Liquid Medium

To prepare the liquid medium for microorganisms growth before the soil inoculation 50 g of LB broth powder for 2 litre of medium were mixed and autoclaved. To reach the amount of broth needed in each 1l bottle 25g of LB broth were added and then around 0.7 l of deionized water was added and the solution was mixed till complete dissolution of the powder in the liquid. Finally water till reaching 1l in each flask was added, then was divided into 3 flask to allow enough headspace in each bottle to autoclave at 125° for 25 min.

The pH of the broth was checked with a pH paper and resulted equal to 7.0, for this reason any adjustment with NaOH or HCl was needed.

2.4.2 Preparation of the liquid colture

The medium was let cool down to room temperature before proceeding to the inoculation step. Inoculation of Trichoderma from agar plates and Streptomyces from liquid colture tubes to LB medium.

The fume hood work area was disinfected with 70% ethanol and approximately 30 ml of sterile LB liquid medium were transferred in a sterile glass using a sterile pipette. For the Trichoderma inoculum approximately 2 cm^2 of the agar plate were removed from the plate and added to the

sterile broth, ensuring that the mycelium is transferred to the medium, and then mixed using the vortex for around 20 seconds. Finally the jar was filled with sterile broth and closed.

For the streptomyces the frozen sample were retrieved by mixing with the vortex to ensure an homogeneous suspension, than repeating the same procedure used for the Trichorderma, 2 ml of the streptomyces samples were added to the sterile broth.

In order to check the compatibility of the two microorganisms also a mixed liquid colture was prepared, adding around 1 cm^2 of Trichoderma sample and 1 ml of streptomyces colture in the same glass with sterile LB broth.

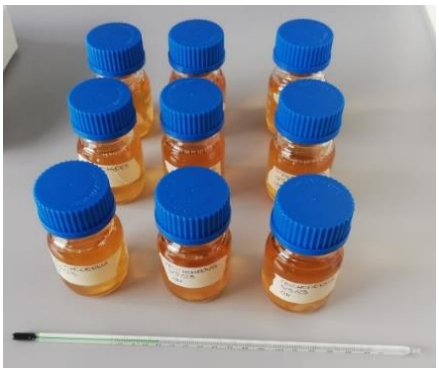

Figure 22: Liquid coltures

All the different liquid colture were than left first at 25°C in the incubator to start the growth of the microorganisms for 3 days, after moved at room temperature of 21°C for other 5 days. Each day the jars were open and shaken in a sterilized fume hood in order to ensure the presence of oxygen inside the liquid colture, since both organisms are aerobic.

2.4.3 Preparation of the Tryptic Soy Broth

As source of easy organic matter for the microorganisms a solution of concentrated Tryptic Soy Broth (TSB) was used, since its carbon content is higher than the LB broth. In order to reach approximately 0.12 g of carbon in each jar the TSB solution was prepared mixing 50g of TSB powder in 1l of deionized water.

Table 3: TSB component

Component	Final concentration
Pancreatic Digest of Casein	34 g/l
Papaic Digest of Soya Bean	6 g/l
Sodium Chloride	10 g/l
Dipotassium Hydrogen Phosphate	5 g/l
Glucose Monohidrate	5 g/l

After mixing the powder with water the solution was autoclaved at 125°C for 25 minutes.

2.5 Calculation of the amount of liquids needed

To ensure the same condition of soil saturation the amount of liquids added to each jar were calculated starting from the WHC values of each soil texture. To each jar a total of 2ml of liquid colture were added, corresponding to 2g. Furthermore, an equal addition of nutrients to each jar was important and, to ensure the addition of the maximum amount of nutrients, the amount of TSB was calculated starting from the minimum WHC value, as follows:

$$Liquids_{5\%} = 60\%\ WHC_{5\%}$$

$$TSB = Liquids_{5\%\ clay\ soil} - Liquid\ inoculum$$

In the end, the amount of water needed to reach in all the jars the wanted WHC value was calculated as:

$$Water = Liquids_{each\ soil\ texture} - TSB$$

Table 4: Calculation of liquids to add

Soil texture	WHC (g_w/g_{soil})	60% WHC	Liquids [$g_{liquids}$/jar]	Water to add after the inoculum [g]	TSB	water to add at the end [g]
5% clay	0.125	0.075	7.5	5.5	5.5	0
10% clay	0.16	0.096	9.6	7.6	5.5	2.1
20% clay	0.23	0.138	13.8	11.8	5.5	6.3

2.6 Inoculation of soil

In each jar filled with model soil were added firstly the liquid colture for each group of samples:

- Trichoderma only 2 ml of liquid colture
- Streptomyces only 2 ml of liquid colture
- Trichoderma and Streptomyces 1ml of each liquid colture was added.

After the correct amount of TSB was added, and later water mixed with salt was used to reach the desired water content. The deionized water was mixed with NaCl (10 g/l) in order to reach the same concentration of salt as the TSB and ensure the same osmotic circumstances in the whole jar. Finally all the soils were mixed and samples of the time zero of the incubation were taken, before that the jars were placed in the incubator at 25°C and 50% fan option.

After 2 weeks the soil mixtures in the jars were hand mixed to ensure a better distribution of the microorganism in the soils since they were visibly present only on the surface of the model soils.

2.7 Water Extractable Organic Carbon extraction

Water-extractable organic matter (WEOM) refers to the portion of organic matter that can be dissolved and extracted from the soil using water, employing different laboratory methods. It represents the highly dynamic and mobile component of soil organic matter (SOM) (Sun et al. 2017), therefore influences a lot of ecologically relevant processes even id is only a small part of SOM.

Different methods to obtain the water extractable organic matter exist:

- Pressurized hot-water-extractable organic carbon (PH-WEOC)
- Water-extractable organic carbon (WEOC)
- Leaching -extractable organic carbon (LEOC)

Among them the WEOC was used (Guigue et al. 2014).

To evaluate the water extractable organic carbon approximately 5g of the soils at the endpoint of incubation were collected in 50ml tubes and stored at 4°C until the procedure, in order to decrease the microbial activity.

20 ml of ultrapure water were added to each sample and shaken at 120 rpm for 60 minutes, afterwards the samples were centrifuged for 15 minutes at 4600 g in order to ensure a better separation of the liquid from the solid phase and enhance the successive filtration.

The supernatant was then collected with a syringe and filtered with 0.45 μm, the filters were previously washed with ultrapure water to release eventual DOC coming from the membrane. The collected liquid was then stored frozen before the TOC and TN analysis.

Figure 23: WEOC extraction procedure, after mixing

Figure 24: WEOC extraction procedure, after centrifuge

This procedure was performed only on the samples taken at the end of the incubation period, since at the starting point the organic source added was in a liquid form, hence all the carbon would have been water extractable.

2.8 POM & MAOM

To perform the Rock Eval analysis on the different fractions approximately 15g of soils were wet sieved with a 2mm and a 53 μm and deionized water, the fractions collected were than dried in order to be powdered with a ball mill and used for the analysis. Rock Eval analysis were performed on a sample without previous sieving both at starting and endpoint of the incubation period, additionally on MAOM and POM fraction at the conclusion of the incubation period.

2.9 Direct Chloroform extraction

Chloroform is as an effective agent to eliminate living organisms, but it does not dissolve non-microbial organic matter found in the soil. Using potassium sulphate it is possible to extract soluble organic compounds, with the consequence that extracting DOC on Chloroform treated samples the DOC resulting will be related only to non microbial derived carbon. Performing the extraction with potassium sulphate on both samples treated and not with Chloroform and evaluating the TOC in them, through the difference in the amount of carbon between the two samples it will be possible to evaluate the microbial biomass carbon as follows:

$$\begin{matrix} microbial \\ biomass\ C \end{matrix} = \begin{matrix} C\ extracted\ from \\ not\ treated\ sample \end{matrix} - \begin{matrix} C\ extracted\ from \\ Chloroform\ treated\ sample \end{matrix}$$

It is important to note that the efficiency of the potassium sulphate extraction procedure is estimated to be approximately 45% according to Vance et al. in 1987, hence the final estimates of microbial biomass should be adjusted accordingly.

The procedure used to extract the microbial biomass is the Chloroform direct extraction method instead of the fumigation (Setia, Verma, and Marschner 2012), and the protocol used is the one from (Domeignoz-Horta et al. 2020).

From each jar approximately 4 grams of soil were taken, then separated in 2 different tubes in order to allow the different treatment. From each jar 2g of soils were treated with 0.250 ml of ethanol free Chloroform, added directly to the soil and then gently shaken. Afterwards in all the samples 10ml of 0.05 M Potassium sulphate solution (4°C) were added and vortex for about 5 seconds.

The samples were then shaken for 30 minutes at 175 rpm at room temperature, and subsequently let sit for around 30 minutes at 4°C to let the supernatant clear. With a pipette approximately 5 ml of supernatant were taken and filtered through Whatman filters #40. The samples not treated with Chloroform were ready for storage, while the Chloroform treated one were injected with air in order to volatilize the residual Chloroform. Using some needles air was injected inside the tubes for approximately 30 minutes, and after the samples were left open for another hour to ensure the total volatilization of the Chloroform before storing frozen waiting for the TOC and TN analysis.

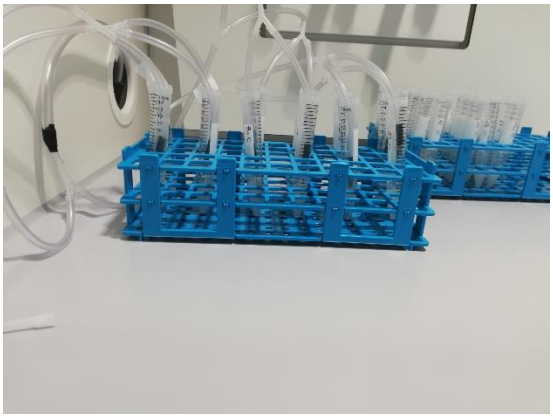

Figure 25: Injection of air to volatilize residual Chloroform

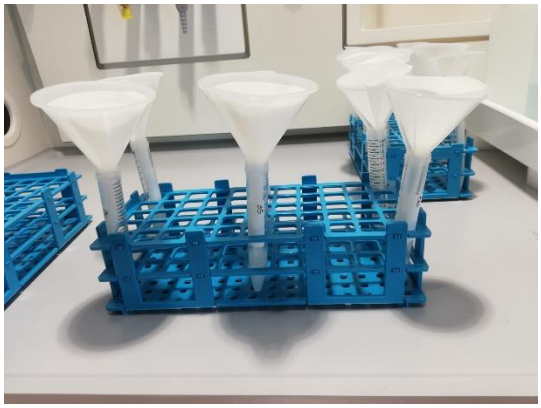

Figure 26: Filtration of the liquid extracted

The amount of liquid obtained for this procedure was too low to ensure a TOC analysis with TOC analyser, and for this reason a 2:10 dilution was made, mixing 2 ml of liquid extracted with the procedure with 8 ml of ultrapure water at 4°C.

2.10 TOC & TN

TOC and TN analysis were performed on liquid samples for the water extractable organic carbon and Microbial biomass carbon using and Elementar Vario TOC Cube. With this instrument the result obtained is in term of mgC/l (or mgN/l), converted in mg/g_{soil} with the following equation:

$$TOC\ \left[\frac{mg}{g_{soil}}\right] = \frac{TOC\left[\frac{mg}{l}\right]}{1000\ \left[\frac{ml}{l}\right]} * \frac{liquid\ extracted\ [ml]}{soil\ mass\ used\ for\ the\ extraction\ [g]}$$

TOC analysis on solid samples were avoided since the Rock Eval analysis already gives this parameter with good accuracy compared to the TOC analysis, with the advantage of not requiring preliminary treatment such as decarbonatation (Disnar et al. 2003).

2.11 Rock Eval

Prior to Rock-Eval analysis, all samples were subjected to a drying process overnight at approximately 65°C in an oven. Subsequently, the dried samples were pulverized using a ball mill for a duration of 15 seconds at a frequency of 12 Hz. Between 60 and 80 mg of samples were analysed with this method with the following parameters:

- Pyrolysis
  - Starting temperature 200 °C
  - Final temperature 650 °C
  - Heating rate 25 °C/min
- Oxidation
  - Starting temperature 300 °C
  - Final temperature 850 °C
  - Heating rate 20 °C/min

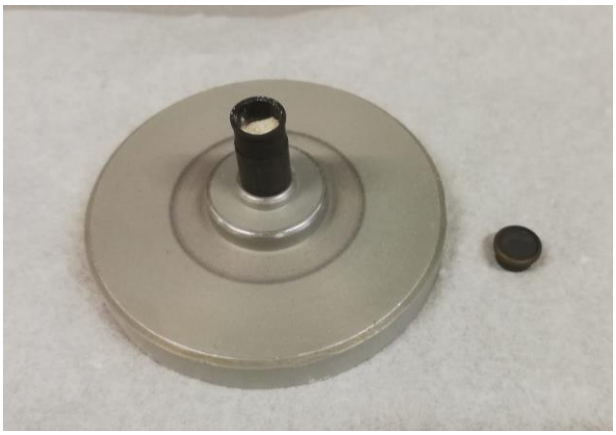

Figure 27: Sample for Rock Eval

Figure 28: Carousel used to insert multiple samples in the Rock Eval

The analysis of the sample was performed at Sediment geochemistry lab of UNIL, and the results obtained with this method are:

- For each sample one file containing all the parameters, including the baseline values for the FID and infra-red cell to correct the results
- The thermograms for HC, CO and CO_2 both during pyrolysis and combustion
- One final excel with alle the values of TpS2, TOC, MINC, HI and OI

2.12 Rock Eval results correction

The result obtained with the analysis will have to be corrected taking into account different factors.

Even if the instrument is able to give as result the TOC and MINC separately, it is important to check if in the sample there is actually some mineral carbon. The instrument itself usually separates between mineral and organic carbon taking into account the temperature range in which the C is released, but the real difference between the two types of carbon is related not only to the temperature but also to the chemistry. To verify if the carbon accounted as mineral is actually mineral, the graph representing the emissions of CO_2 over the temperature during the oxidation phase must be controlled. If in this graph there is a clear peak ok CO_2 released after 600°C, there is carbonate in the sample and the results obtained from the instrument about TOC and MINC are correct. Otherwise if in the graph there isn't a clear peak after 600°C, also the carbon accounted ad mineral will be organic, hence in this situation the TOC will be calculated as:

$$TOC_{real} = TOC + MINC$$

An example of the graph with (Behar, Valérie, and Penteado 2001b) and without (example of one sample) the peak is shown in

Figure 29 and Figure 30:

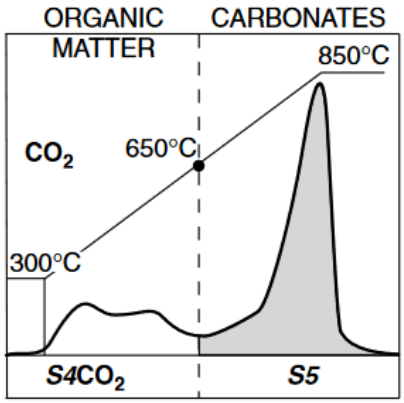

Figure 29: Example of CO2 curve with peak (Behar, Valérie, and Penteado 2001b)

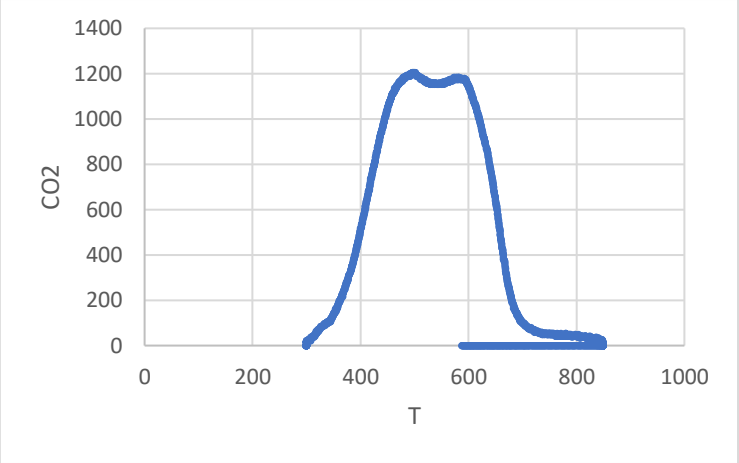

Figure 30: Example of typical CO_2 emission curve in the combustion phase, obtained from the experiments

This situation is present in all the samples analysed, situation expected after the acid washing of the sand that had the purpose of removing most of the carbonates. For this reason the TOC has been calculated as sum of the TOC and MINC for all the samples. As consequence of this, also the values of HI and OI has been corrected as follows:

$$HI_{real} = \frac{(S2 * 100)}{TOC_{real}}$$

$$OI_{real} = \frac{(S3 + S3CO_2) * 100}{TOC_{real}}$$

Furthermore to evaluate the areas needed for the calculation of the different indices it is necessary to subtract the baseline value of the detectors from the values obtained, as follows:

$$HC_{real} = HC - Baseline_{HC}$$

$$CO_{real} = CO - Baseline_{CO}$$

$$CO_{2_{real}} = CO_2 - Baseline$$

3. Results and discussion

To simplify the analysis of the samples and the representation, in the following results representation the different combinations of soil textures and treatment will be identified in some graphs with simple numbers. The correspondence between type of sample and number is shown in Table 5.

Table 5: Correspondence between sample and numeric values

%Clay	Treatment	Replicate	N°
20%	Trichoderm	T-a	1
		T-b	2
		T-c	3
	Streptomyces	S-a	4
		S-b	5
		S-c	6
	Trichoderm+Streptomyces	TS-a	7
		TS-b	8
		TS-c	9
	Control	C-a	10
		C-b	11
		C-c	12
10%	Trichoderm	T-a	13
		T-b	14
		T-c	15
	Streptomyces	S-a	16
		S-b	17
		S-c	18
	Trichoderm+Streptomyces	TS-a	19
		TS-b	20
		TS-c	21
	Control	C-a	22
		C-b	23
		C-c	24
5%	Trichoderm	T-a	25
		T-b	26
		T-c	27
	Streptomyces	S-a	28
		S-b	29
		S-c	30
	Trichoderm+Streptomyces	TS-a	31
		TS-b	32
		TS-c	33
	Control	C-a	34
		C-b	35
		C-c	36

3.1 Water Extractable organic Carbon and Nitrogen at end of incubation

The results obtained with the TOC analyser of the water extractable organic carbon in the samples taken at the end of the incubation are shown in Figure 31 and Figure 32.

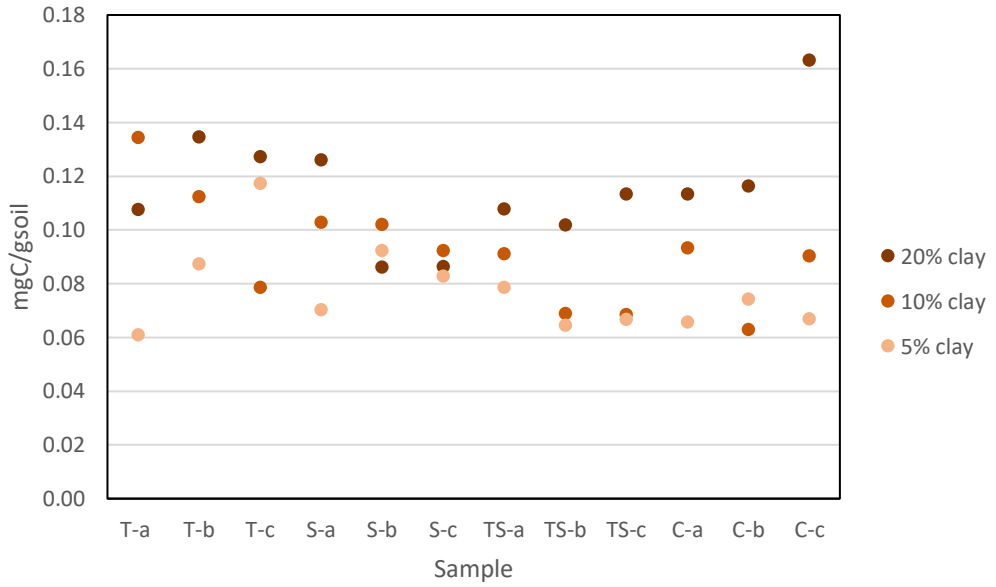

Figure 31: WEOC results, represented for the different soil textures

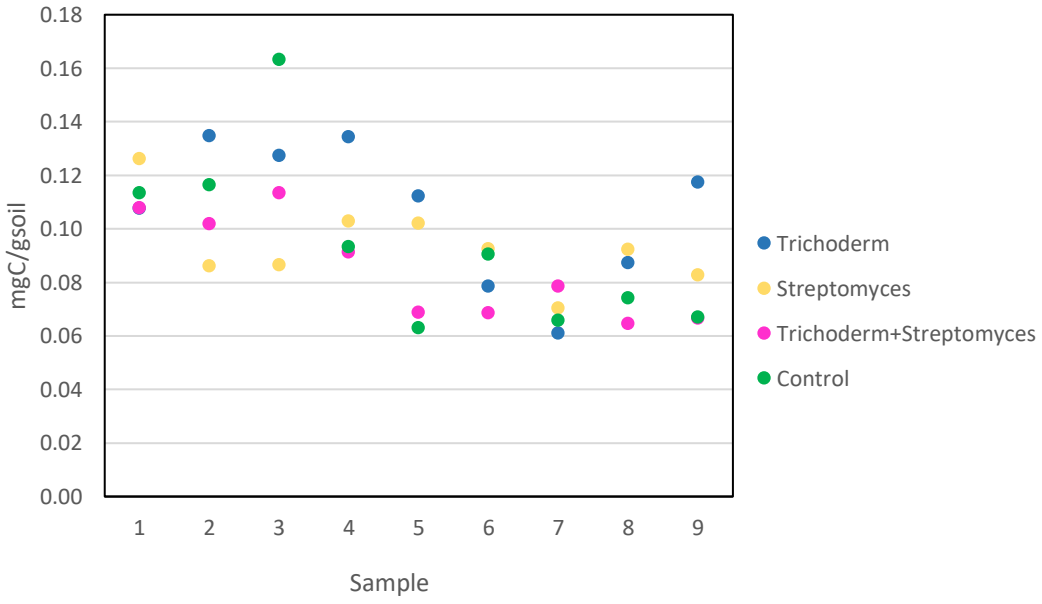

Figure 32: WEOC results, represented for the different soil treatments

Those results can be also displayed in term of soil texture or type of treatment with box plot graphs in order to highlight mean values and variance:

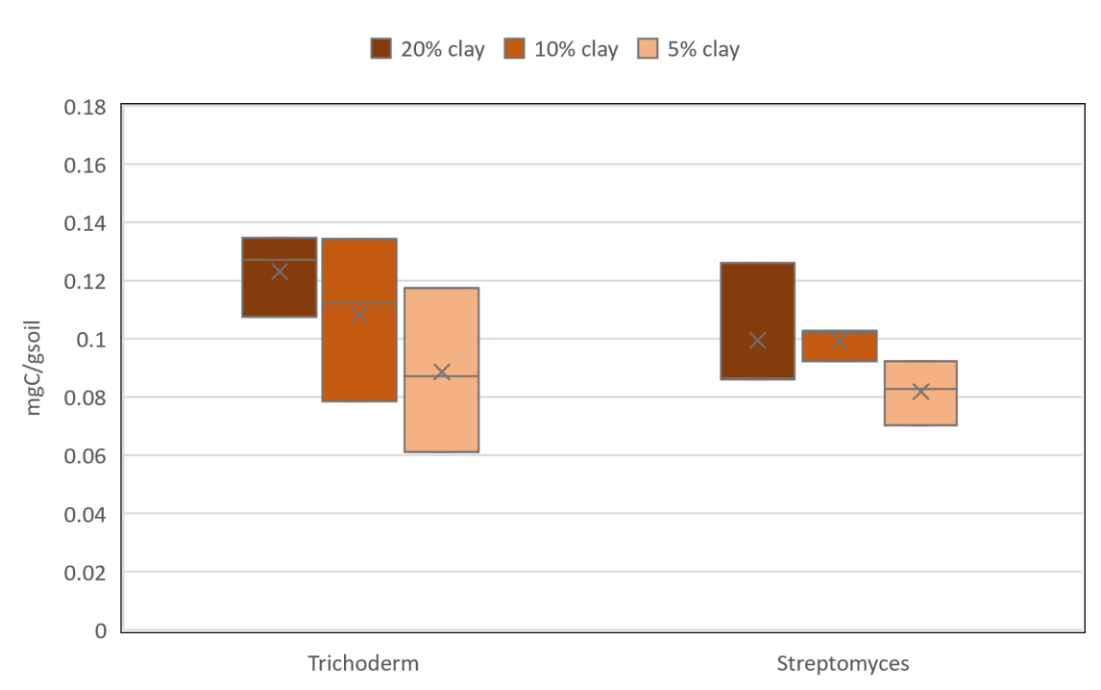

Figure 33: WEOC results, box plot representation

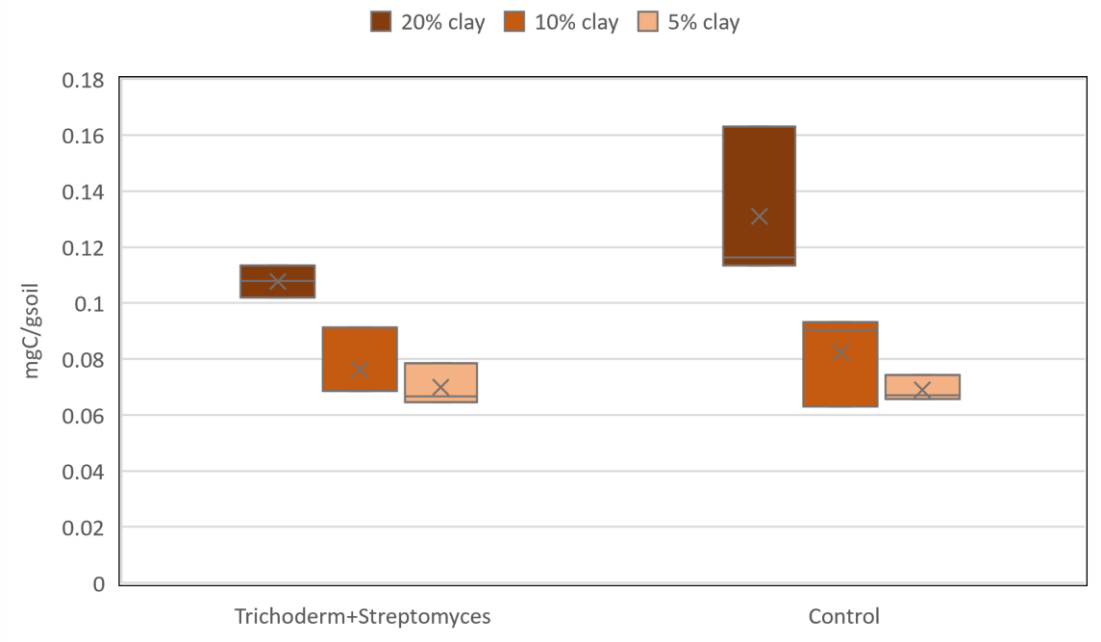

Figure 34: WEOC results, box plot representation

In Figure 33 and Figure 34 it is possible to identify a low positive correlation between the percentage of clay in soil and the water extractable organic carbon; sample with higher amount of clay resulted in a slightly higher water extractable organic carbon measured.

The same representation can be applied to investigate a relation between the WEOC and the type of microorganisms added to the soil:

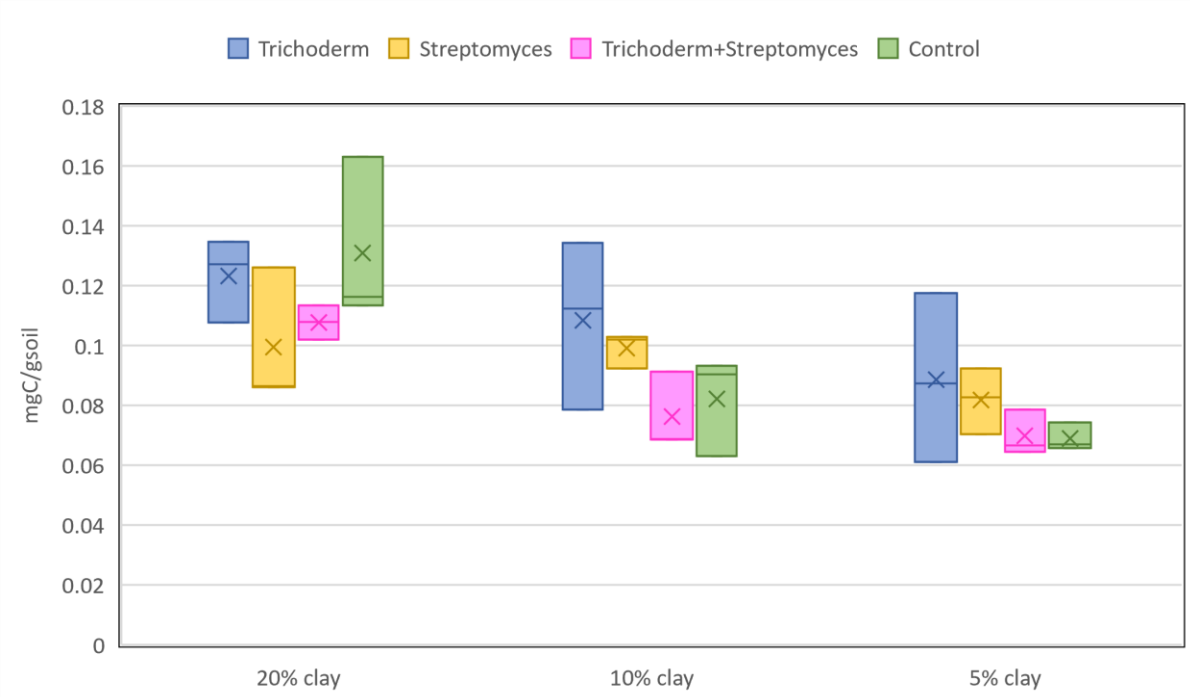

Figure 35: WEOC results, box plot representation

From this figure it is not possible to identify any relationships between the WEOC and the type of soil treatment used.

3.2 Water Extractable Nitrogen results

The results of water extractable nitrogen at the end of the incubation period are shown in Figure 36 and Figure 37.

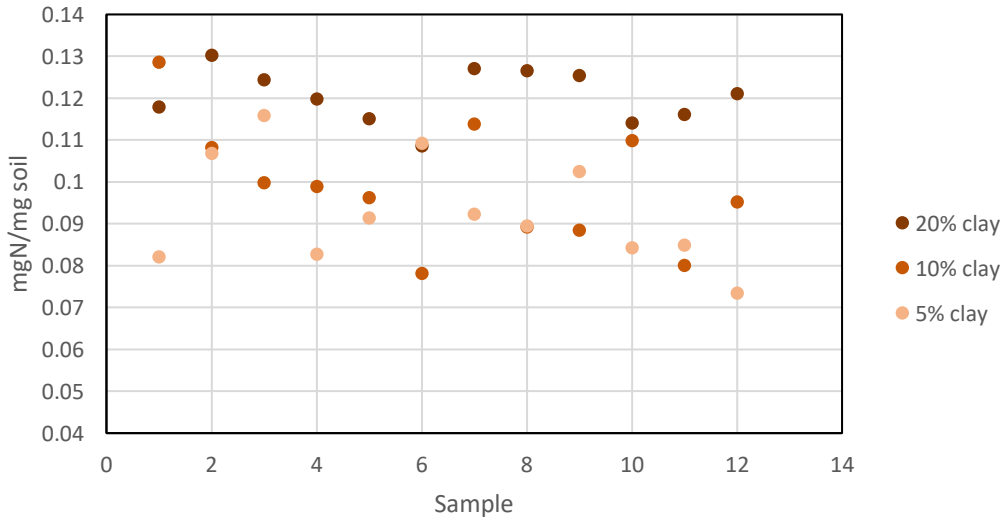

Figure 36: WEN results, represented for the different soil textures

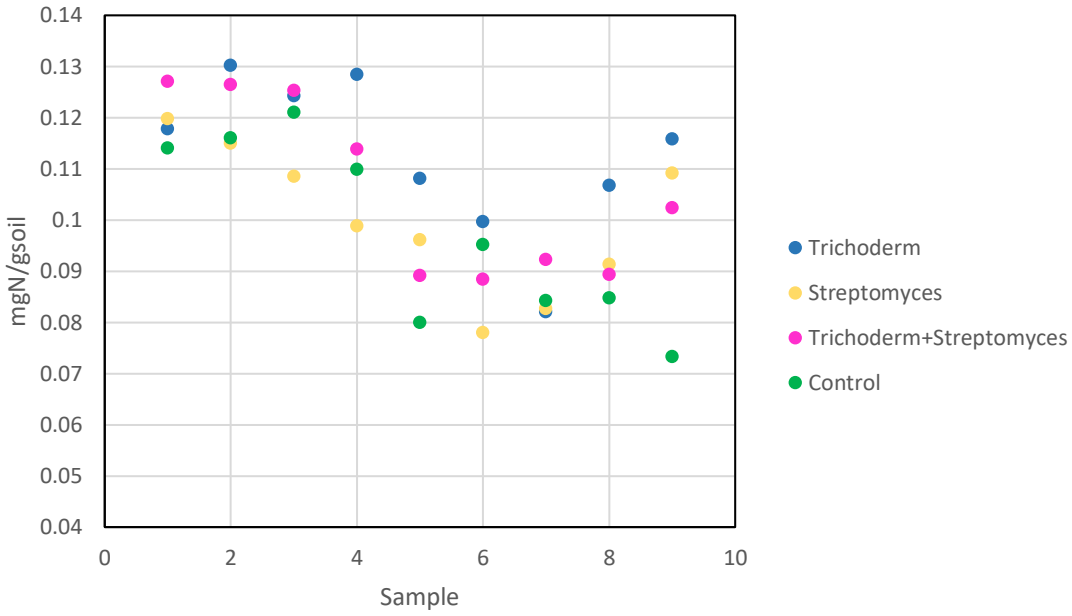

Figure 37: WEN results, represented for the different soil treatments

From those results and the WEOC ones it is possible to immediately evaluate the C:N ratio, which is important to the growth of microorganisms in soil. This ratio has been calculated and is shown in Figure 38:

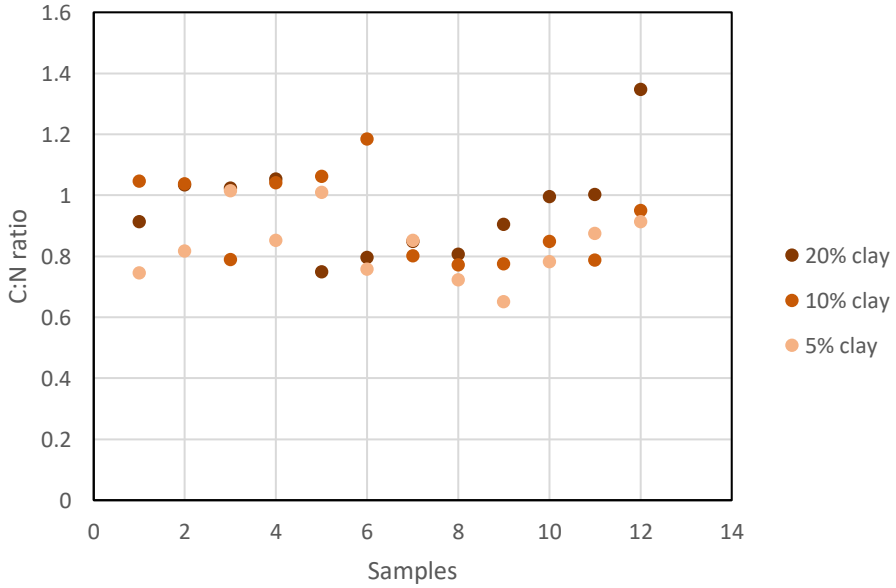

Figure 38: C:N ratio, represented for the different soil textures

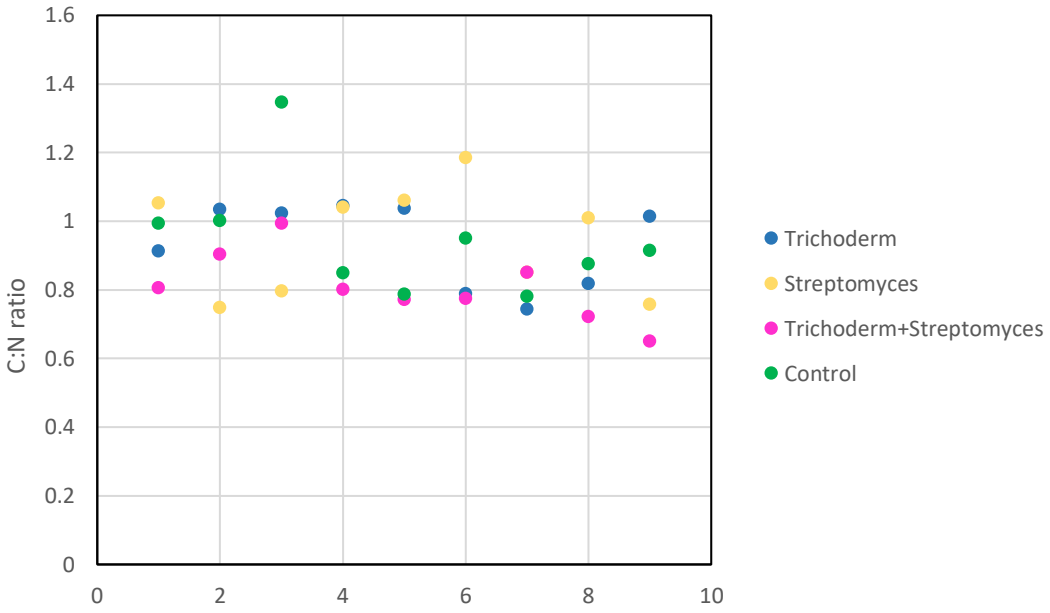

Figure 39: C:N ratio, represented for different soil treatment

The values obtained for the C:N ratio are very low if compared to the values registered in soils, around 8, or to the optimal value of 24 (Brust 2019, 9). This may be due to excessively high content of Nitrogen in the broth used as nutrient, or to problem in the measurements. The nutrient used is specific for microorganisms growth but it is possible that the source added was not enough to provide the necessary amount of organic matter for bacteria growth.

Again the results can also be displayed with box plot graphs that highlight the mean value and variance of the values, both for the different soil textures and treatment to highlight eventual pattern:

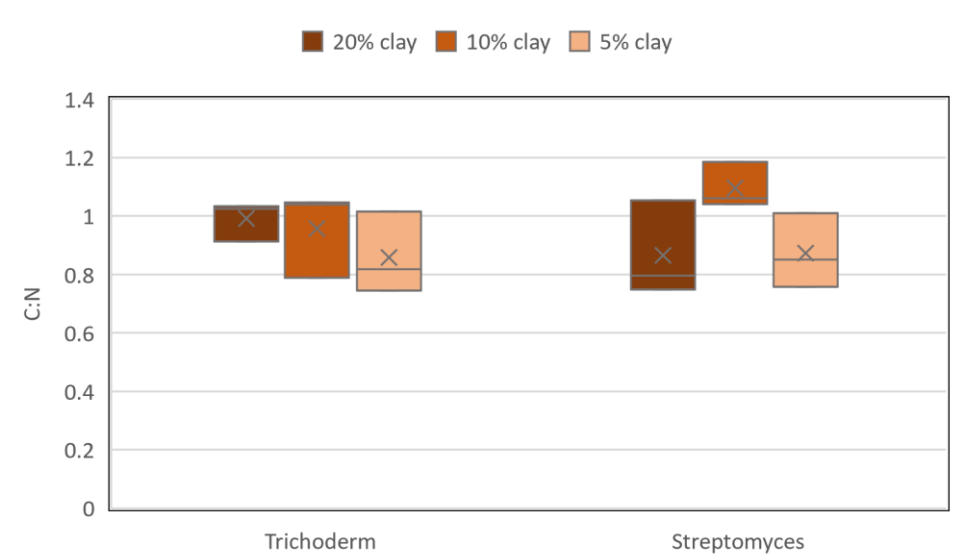

Figure 40: C:N ratio, box plot representation

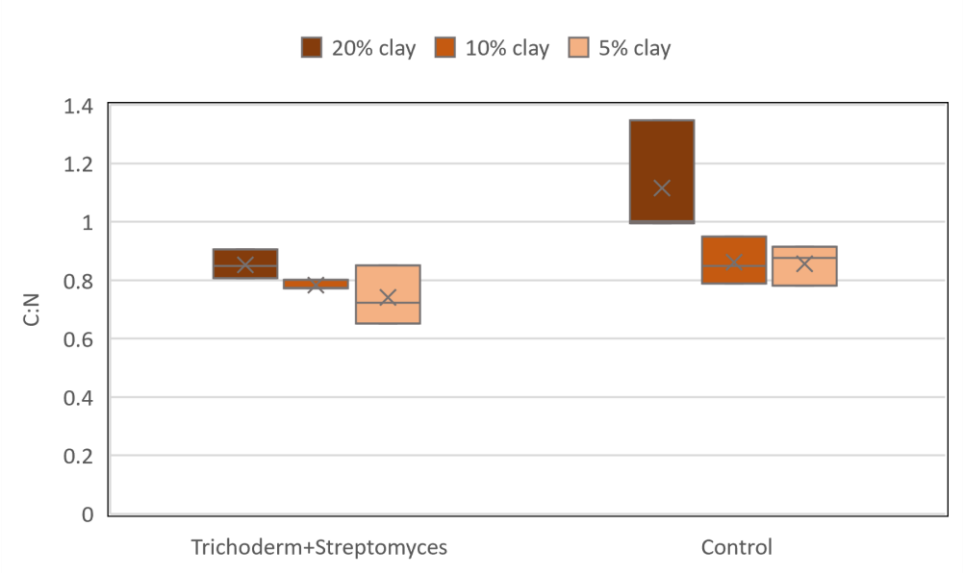

Figure 41: C:N ratio, box plot representation

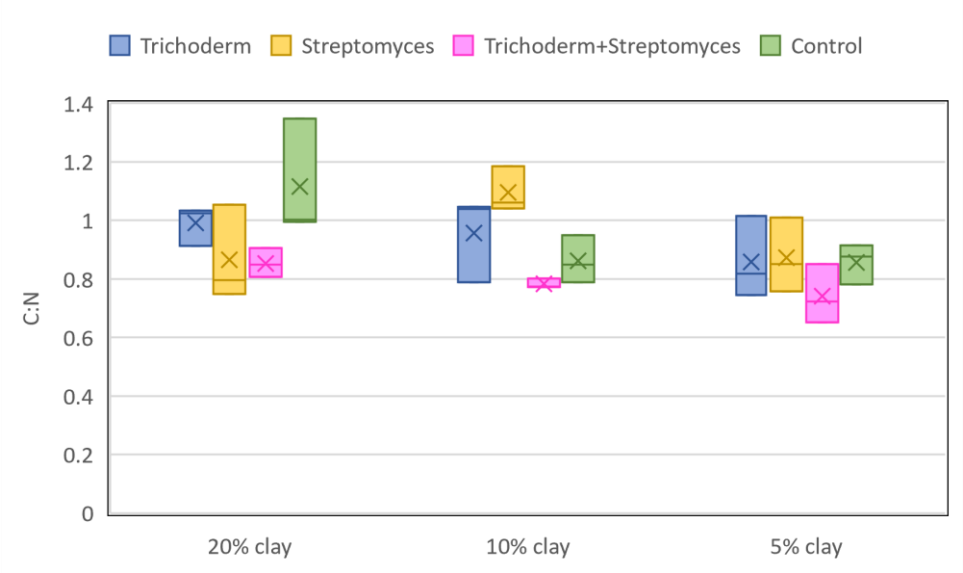

Figure 42: C:N ratio, box plot representation

In both cases there is no evidence of a correlation between C:N ratio and soil texture or type of treatment.

3.3 Chloroform extraction results

As described in the method chapter 2.9 an attempt to evaluate the microbial biomass at the end of the incubation period was performed. The results of the TOC analysis on the liquids for the two type of samples, Chloroform treated and not, and the results of the microbial biomass calculation are shown in Table 6.

Table 6: Chloroform Extraction results

%Clay	Treatment	Replicate	TOC not treated samples [mg/l]	TOC Chloroform treated samples [mg/l]	Microbial biomass [mg/gsoil]
20%	Trichoderm	T-a	55.5	47.9	-84.3
		T-b	50.2	57.3	78.3
		T-c	78.3	53.7	-273.2
	Streptomyces	S-a	53.9	58.1	46.8
		S-b	19.1	34.1	165.7
		S-c	40.7	43.2	27.3
	Trichoderm + Streptomyces	TS-a	67.6	42.5	-278.9
		TS-b	45.8	45.7	-1.8
		TS-c	40.9	48.5	85.1
	Control	C-a	46.3	53.2	76.2
		C-b	59.3	48.8	-116.3
		C-c	96.6	76.9	-218.5
10%	Trichoderm	T-a	76.2	69.8	-70.8
		T-b	39.6	51.4	131.4
		T-c	30.9	37.9	77.9
	Streptomyces	S-a	44.9	42.7	-24.6
		S-b	39.9	50.2	114.1
		S-c	41.8	50.6	97.7
	Trichoderm + Streptomyces	TS-a	37.3	33.5	-42.2
		TS-b	24.5	58.6	378.8
		TS-c	33.5	27.8	-62.6
	Control	C-a	38.7	55.1	181.1
		C-b	32.1	45.1	144.3
		C-c	34.8	85.4	562.4
5%	Trichoderm	T-a	34.0	43.5	105.8
		T-b	38.2	59.9	241.4
		T-c	40.7	56.2	173.2
	Streptomyces	S-a	49.6	31.3	-202.6
		S-b	39.3	45.5	68.4
		S-c	29.6	39.6	110.7
	Trichoderm + Streptomyces	TS-a	33.2	30.4	-31.1
		TS-b	31.9	31.1	-10.1
		TS-c	29.7	36.1	70.4
	Control	C-a	36.4	63.3	298.7
		C-b	29.1	35.9	75.7
		C-c	27.8	32.6	53.1

From the results it is clear that some mistake has been done in the procedure, the results of the final microbial biomass are inconsistent since values are very variable in a range that goes from -278.9 and 562.4 [mg/g_{soil}]. In particular it is clear that negative values of biomass present in the sample are not realistic.

3.4 Rock Eval results

3.4.1 TOC

The values obtained from the Rock Eval method about the TOC are in term of percentage of weight [%w] and the following results will be showed in this unit of measurement, which corresponds also to mg/g, hence mg_C/g_{soil}.

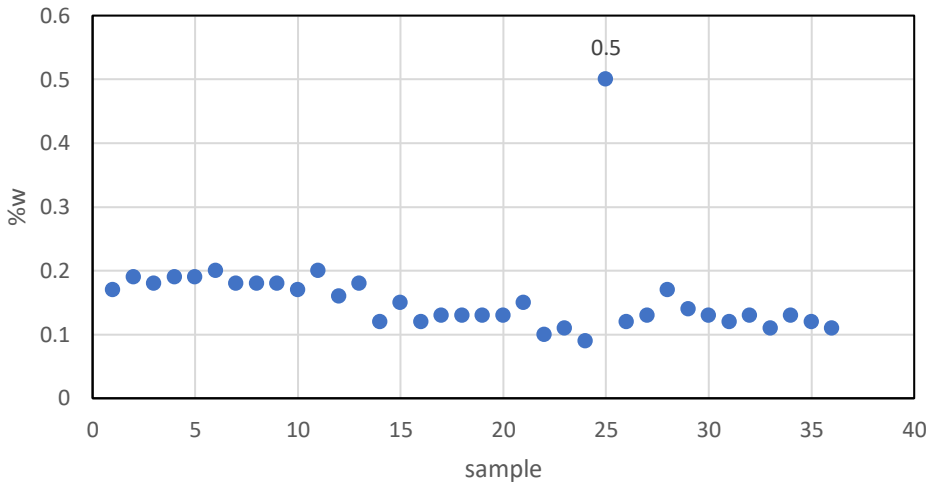

Figure 43: TOC results at the beginning of the incubation

Looking at this series of data is clearly visible an outlier, characterized by a TOC equal to 0.5 mg/g, much higher if compared to the average values of the other sample, all below 0.25 mg/g. For this reason this specific sample, corresponding to the Trichoderma treatment, duplicate A, in a soil with 5% of clay, won't be taken in consideration for further evaluations.

The results of the TOC measurements on the total sample at the end of the incubation are shown in Figure 44.

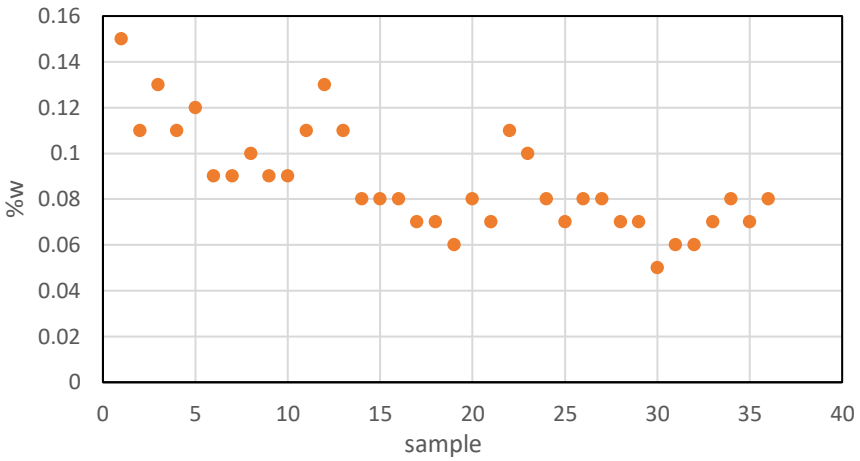

Figure 44: TOC results at the end of incubation

At end of the incubation some samples has also been taken to perform the size separation to identify the composition of TOC in terms of POC, MAOM and WEOC (results in table 1 annex). Calculating the percentage of each component over the total the following mean values are obtained:

- WEOC = 110.83 % of the TOC
- MAOM = 1124.2 % of the TOC
- POM = 10.69 % of the TOC

It is clear that those results does not reflect the theorical/real distribution of the TOC in the different classes. The use of a model soil prepared in the lab, without any complex form of organic matter pre-existent or soil aggregates as we could find in real soils, helped with the interpretation of the final results from one side, but on the other is not properly representing the reality, hence those results cannot be used as base to formulate conclusions about what would happen in real situations.

Firstly avoiding the use of other sources of organic matter the only action happening during the incubation for the short period is the growth of microorganisms using the nutriments present in the broth added to the soil. Furthermore, the technique used to separate particulate and mineralogical associated organic matter involved a liquid separation. One clear consequence of this separation method is that all the aggregates present in the sample are destroyed, hence the particulate part will be very low since the totally disaggregated soil present at the beginning.

The excessive value correspondent to the MAOM could be explained with a concentration process that might occur as consequence of the method used. Collecting all the water deriving from the wet sieving and drying the sample leaded to a very low amount of dry soil to use for the Rock Eval. All the carbon present in the leachate, that if we compare to the WEOC seems be the majority of the TOC, will be concentrated in the small amount of solid remaining. Furthermore, being the sample smaller and well homogenised, the part that will be analysed for the TOC in the Rock Eval instrument will be very rich in C, almost all the carbon in the sample (starting from 5/10g of soil) ended up in around 1g of dried sample (all the carbon of 10 grams, concentrated in 1).

Finally it is useful to compare the TOC values before and after the incubation period, as shown in Figure 45.

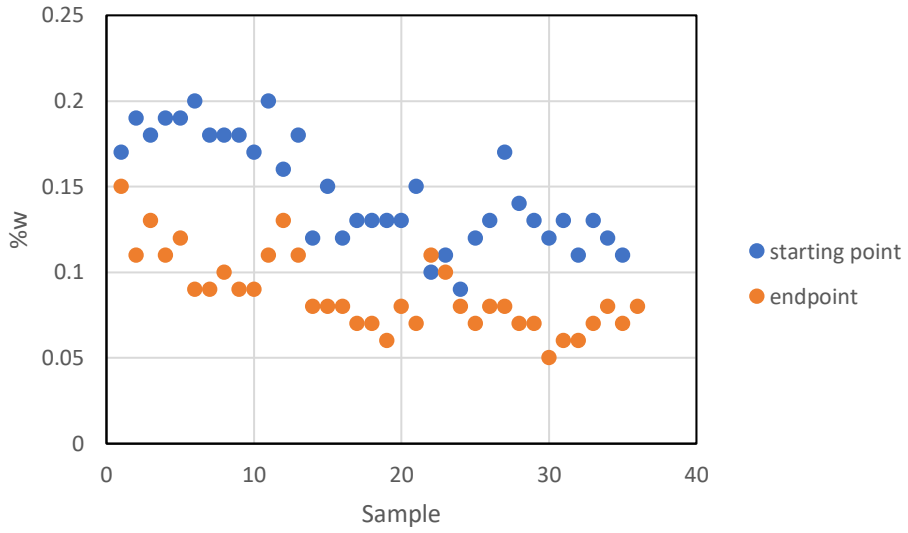

Figure 45: TOC values before and after the incubation, comparison of results

The box plot representation of those values are shown in Figure 46 and Figure 47:

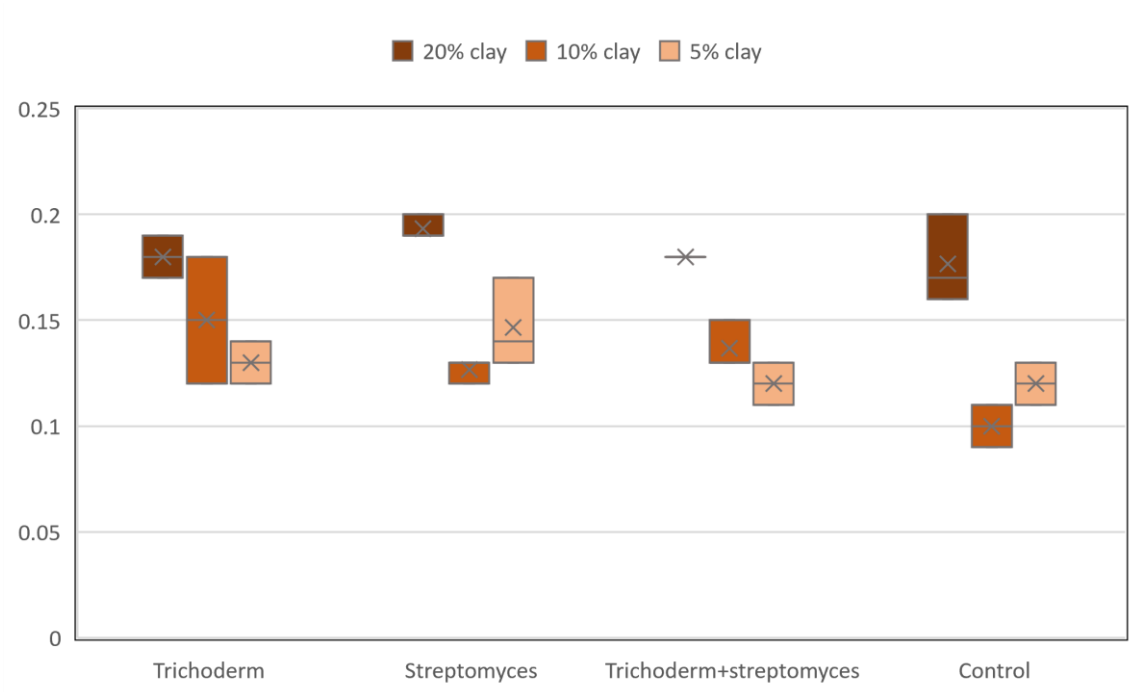

Figure 46: TOC at the beginning of the incubation, box plot representation

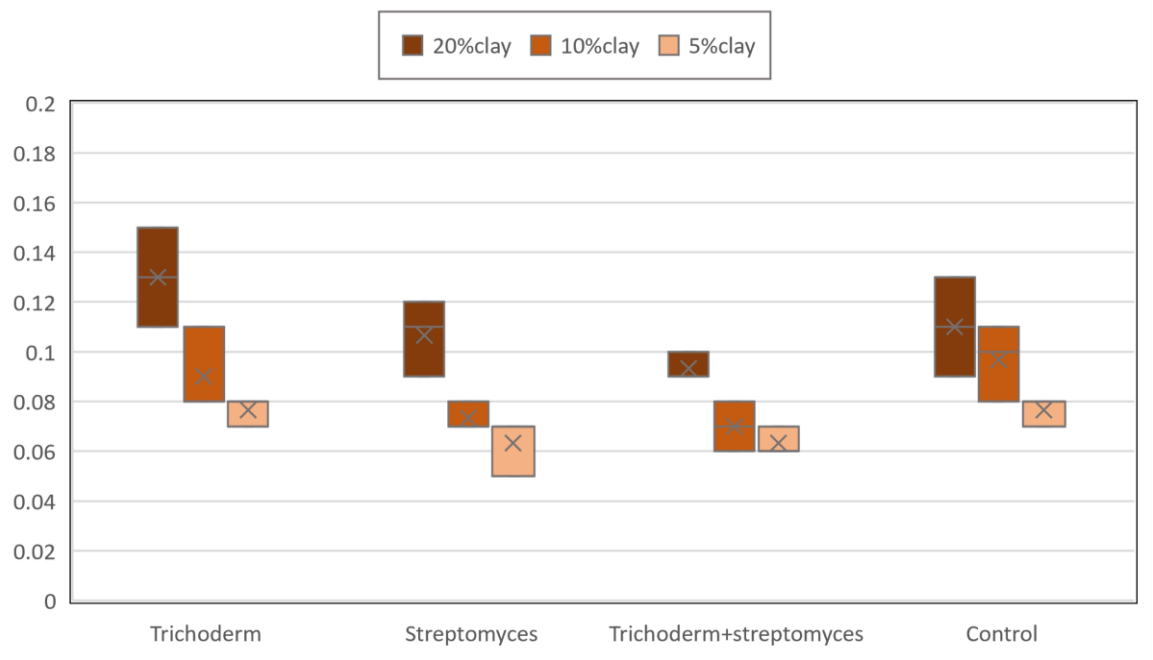

Figure 47: TOC at the end of the incubation, box plot representation

As expected the TOC in the sample slightly decreased due to microorganisms respiration, which is higher than microorganisms accumulating it for growth. It is still useful to investigate weather some treatment leaded to an higher decrease in TOC as in Figure 48 and Figure 49.

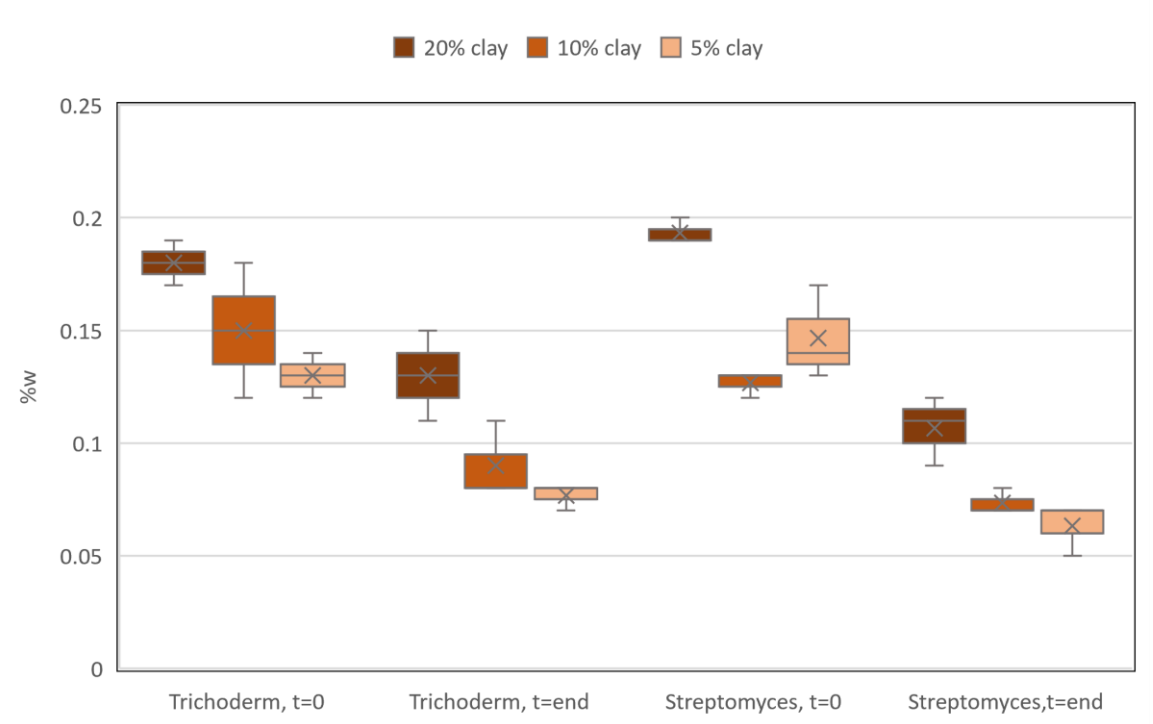

Figure 48: TOC results, box plot representation comparing beginning and end of incubation

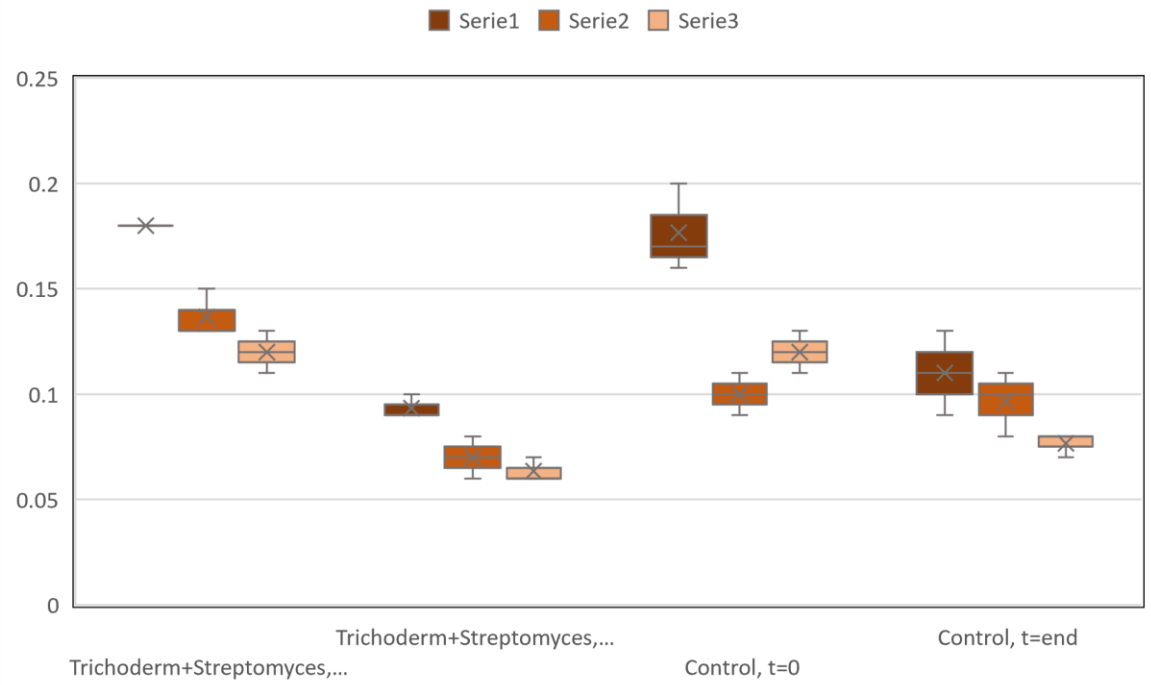

Figure 49: TOC results, box plot representation comparing beginning and end of incubation

With the values of TOC at beginning and end of the incubation period it has been evaluated the variation of it for each sample and this decrease has been reported in terms of percentage of the initial value to allow the comparation between the different samples, taking into account the different amount of samples analysed. Those results are shown in the following graph:

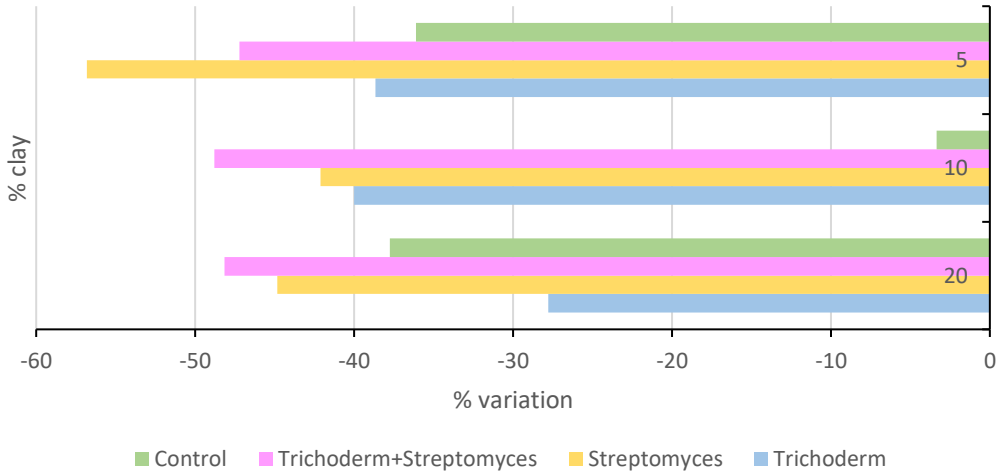

Figure 50: Percentage variation of TOC during the incubation

This representation highlights an higher decrease of TOC in the samples treated with Streptomyces or Trichoderm+Streptomyces (yellow and pink), while any clear difference between the different soil textures is visible.

Figure 50 shows also how in the jar with the control samples (green in the figure) the variation of TOC are comparable to the ones in the jars with microorganisms treatment, except for the samples

with a 10% of clay as soil texture. Since in the "Control" there should be absence of microorganisms at the beginning of the incubation, and that during the incubation period the jars were closed with parafilm to avoid exchange of solids or liquid with the environment, the value of TOC decrease in the samples with 10% of clay seems more reasonable (less than 5%). The higher decrease in TOC in the other Control samples could be explained by a contamination between the different samples that might be occurred during the midpoint collection of samples from the jars.

3.4.2 TpkS2

Another important parameter obtained from the Rock Eval method is the so called TpkS2, corresponding to the temperature at which the peak of the S2 curve occurs. This value is related to the thermal stability of the organic matter present in the sample. The higher is the TpkS2, the higher will be the thermal stability of the organic matter. The values of TpkS2 at the two timepoints are represented in Figure 51.

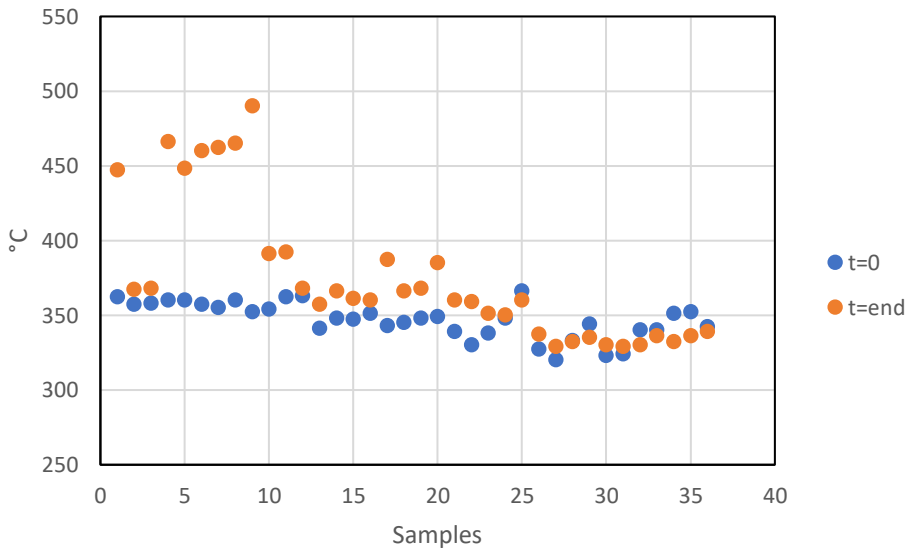

Figure 51: TpkS2 results, comparison between starting and ending point

In this representation it is possible to see how for most of the samples the TpkS2 stayed almost unchanged during the experiment, with an exception for some samples.

As done for the TOC values, also the percentage variation of Tpsk2 has been calculated, the results are shown in Figure 52:

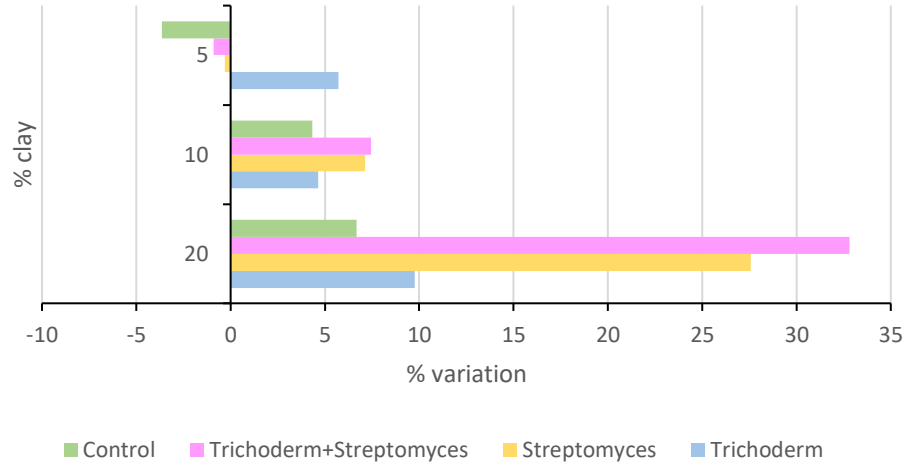

Figure 52: Percentage variation of Tpks2

From Figure 52 is possible to notice that soils with higher amount of clay, resulted in an higher increase in the value of Tpks2, hence in a more thermally stable organic matter at the end of the incubation period. This could be due to the protective effect of clay on organic matter (Chen et al. 2018) and the higher possibility of organo-mineral associations with clay particles.

An important representation that can be done with this data is particularly useful to plot the TpkS2 values with the TOC values, in order to highlight together the variation in the amount of TOC and its stability. To highlight any possible correlation between soil texture or soil treatment the graphs in Figure 53 and Figure 54 has been done.

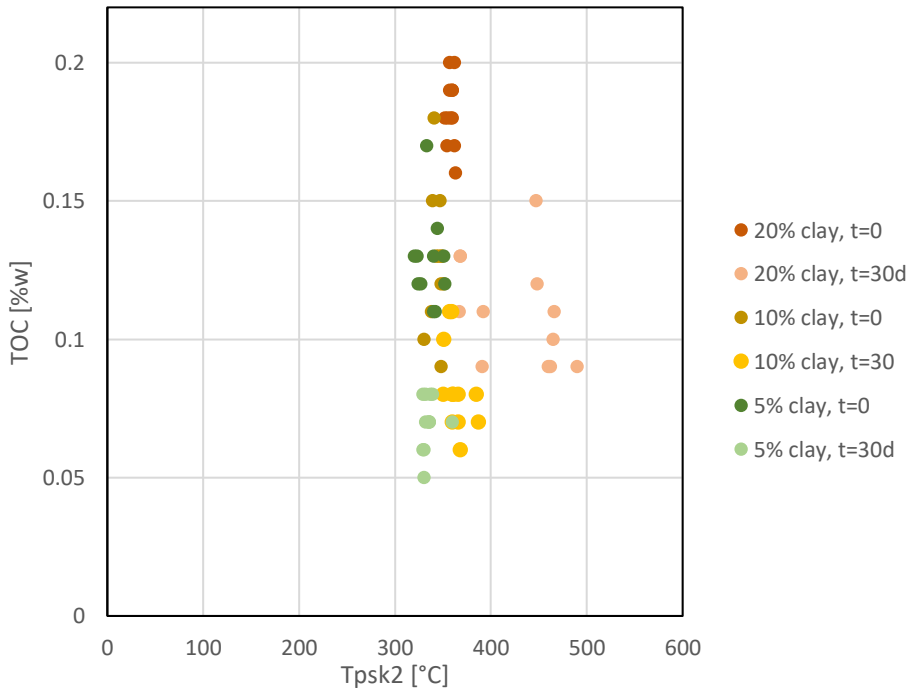

Figure 53: TOC-TpkS2 graph, representation for soil textures

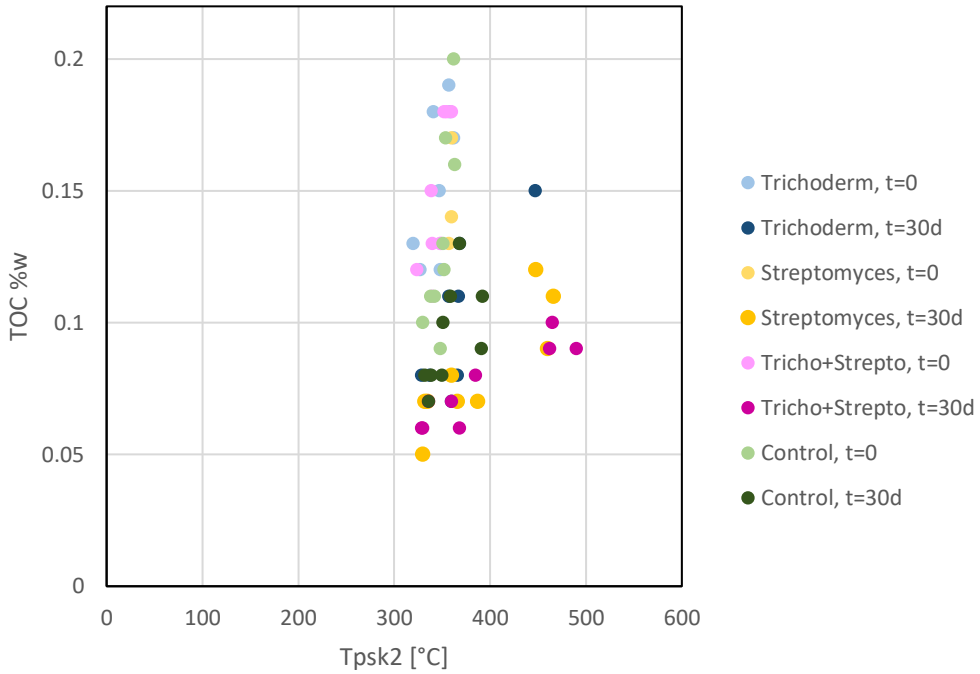

Figure 54: TOC-TpkS2 graph, representation for soil treatment

A group of samples clearly increase the value of TpkS2, and all of them were taken from a 20% clay soil texture samples at the end of the incubation period.

With the colour representation in Figure 54 it is possible to identify the same samples of the previous representation, which were treated with Streptomyces and Trichoderma + Streptomyces. Again the presence of Streptomyces seems to be connected to higher effects on the soil organic matter.

3.4.3 HI and OI

The results of the HI and OI index are shown in Figure 55:

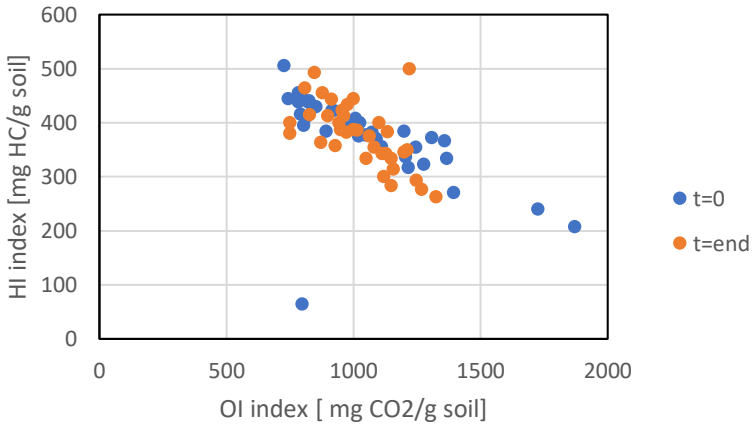

Figure 55: HI/OI graph results

From this representation it is clear that no visible changes occurred during the incubation period. Also for HI and OI index the percentage variation in time was calculated and shown in Figure 56 and Figure 57.

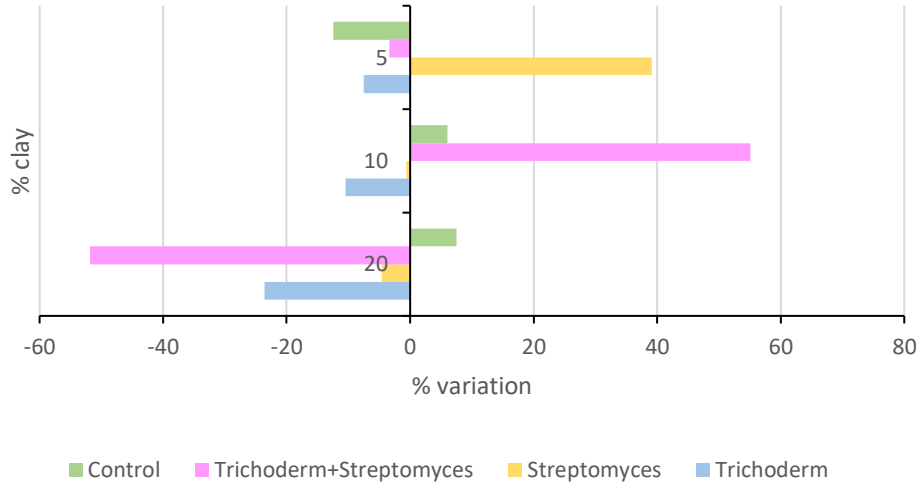

Figure 56: Percentage variation of HI index

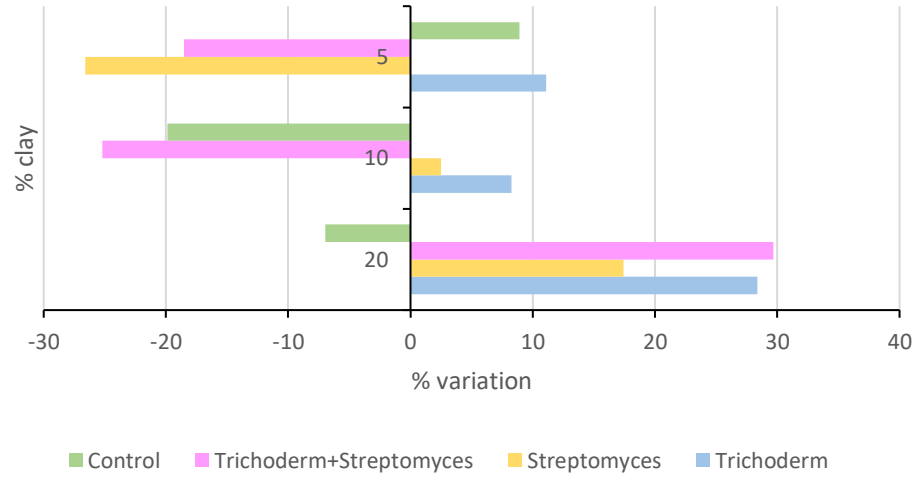

Figure 57: Percentage variation of OI index

The variation of HI index in Figure 56 did not show the same changes in all the combination of soil texture and treatment. The majority of combination showed a decrease in the HI index, suggesting an increase in the thermal stability of the organic matter, while the others showed the opposite behaviour, some with very high increase such as Streptomyces treatment in the 5% clay soil textures and the Trichoderm+Streptomyces in the soil containing 10% of clay.

Also in the case of the OI index the changes happened during the incubation period are various for different combinations, as showed in Figure 57. The samples presenting an increase in the OI index are characterized by an increase in the oxidation state of the organic matter.

It is also possible to note that no clear pattern between index variation and soil texture e/o treatment can be seen. These two indices are more commonly utilized in the petroleum field to identify crucial characteristics for oil utilization and extraction. For this reason further discussion on these findings will be omitted.

3.4.4 I and R index

The values of the I index are shown with a box plot representation, separated in Figure 58 by soil treatment and in Figure 59 by soil texture.

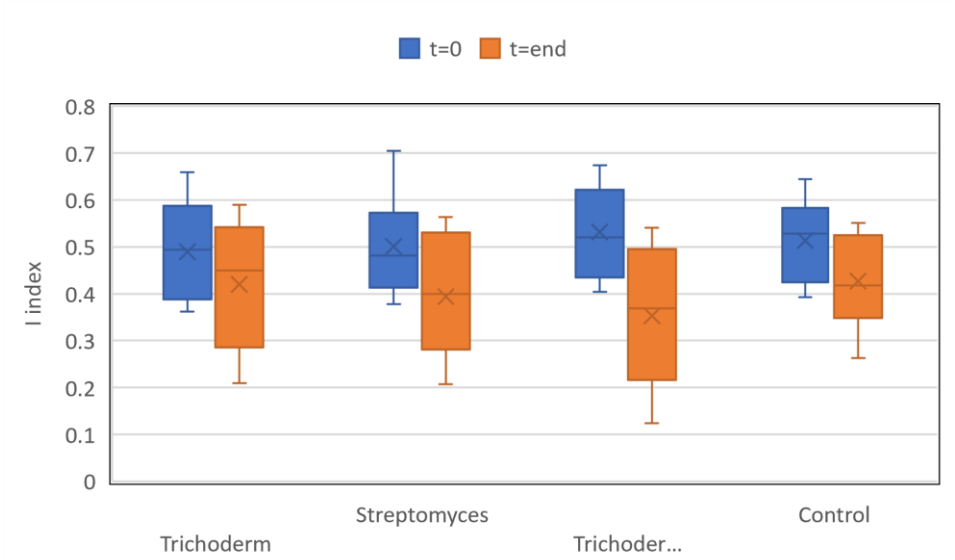

Figure 58: I index box plot representation for soil treatment

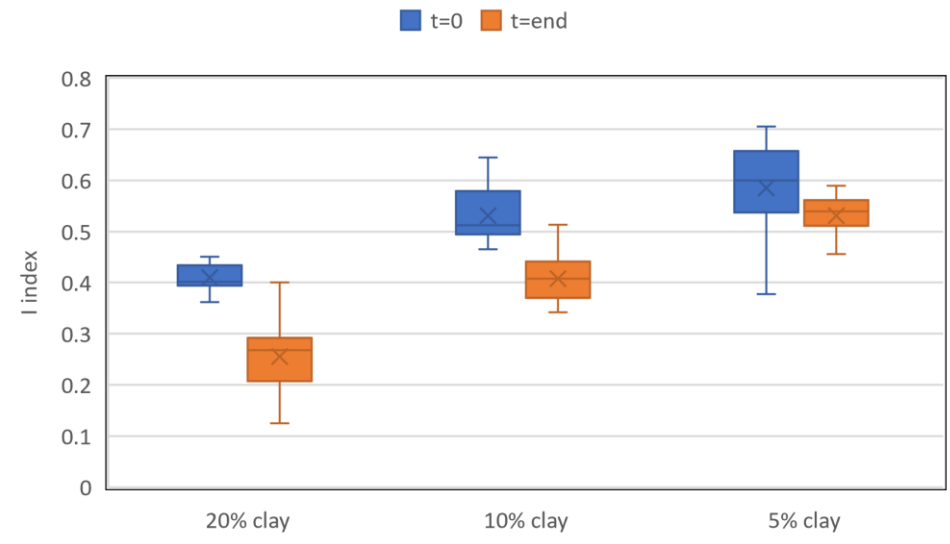

Figure 59: I index box plot representation for soil textures

The same representation was made for the R index in Figure 60 and Figure 61.

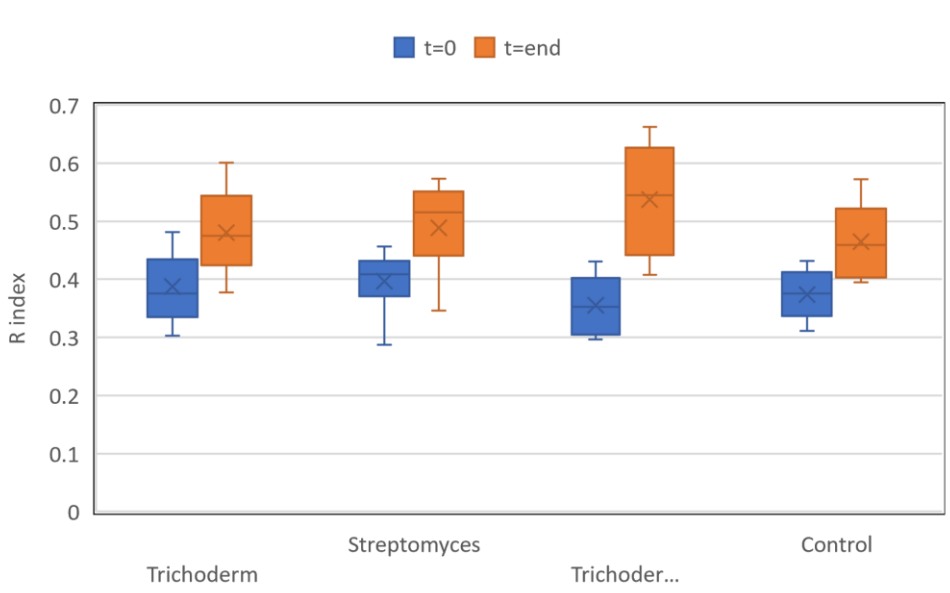

Figure 60: R index box plot representation for soil treatment

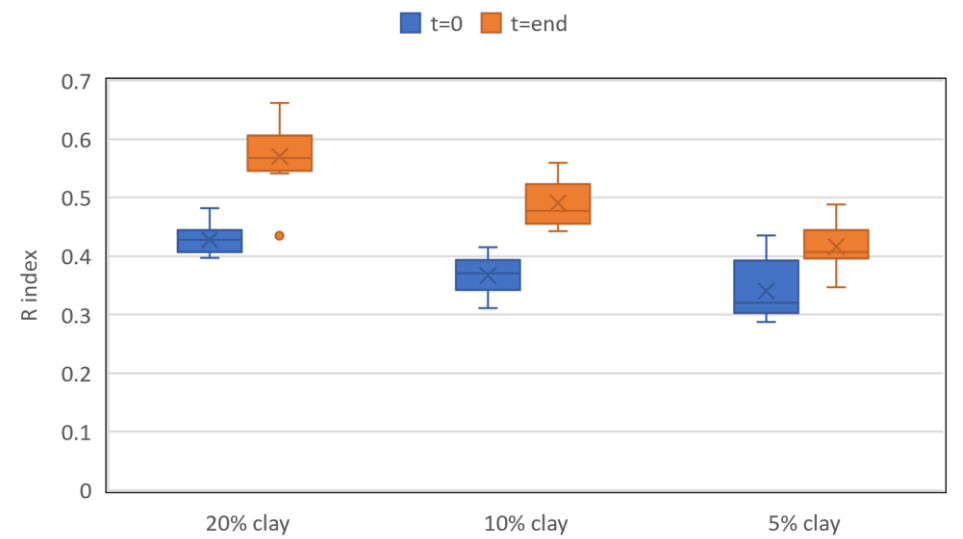

Figure 61: R index box plot representation for soil textures

For all the soil the I index of the samples taken, Figure 58 and Figure 59, at the end of the incubation is lower than in the ones taken at the start. Meanwhile the R index increased in all the samples during the incubation period, Figure 60 and Figure 61.

To obtain more information about the factors influencing the changes in those index, their percentage variation has been calculated and plotted in Figure 62 and Figure 63:

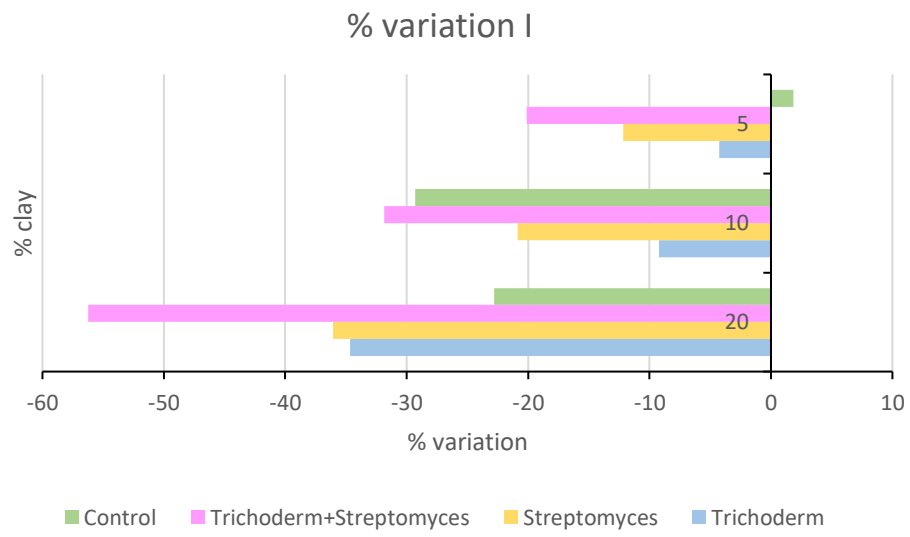

Figure 62: Percentage variation of the I index

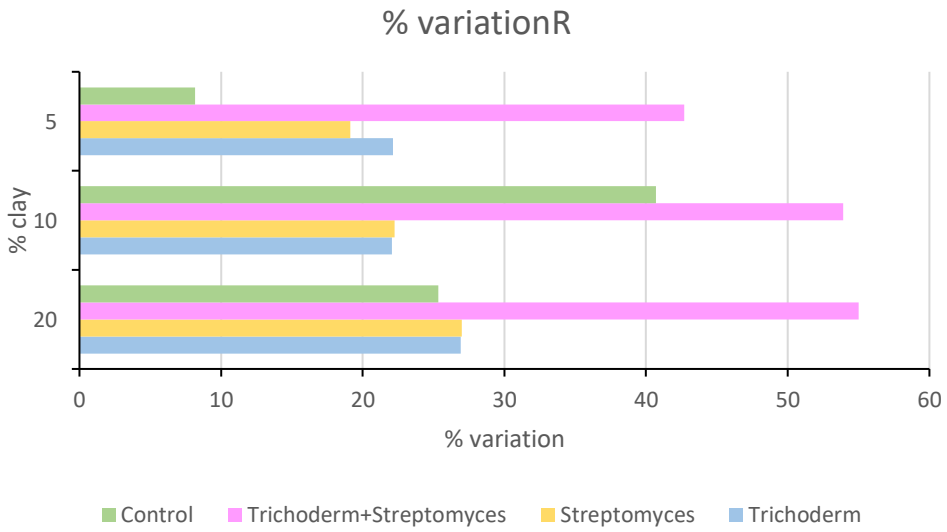

Figure 63: Percentage variation of the R index

It is possible to notice that for both the indexes, a higher effect was obtained with the soil treatment containing both Trichoderma and Streptomyces coltures. The parallel decrease in the I index and increase of R index shows how the thermal stability of the samples in the end has a higher contribution from the recalcitrant part of organic matter than the one from the labile one.

As explained in chapter 1.5 it is useful to represent the I and R index in the same graph to identify any relationship between those values.

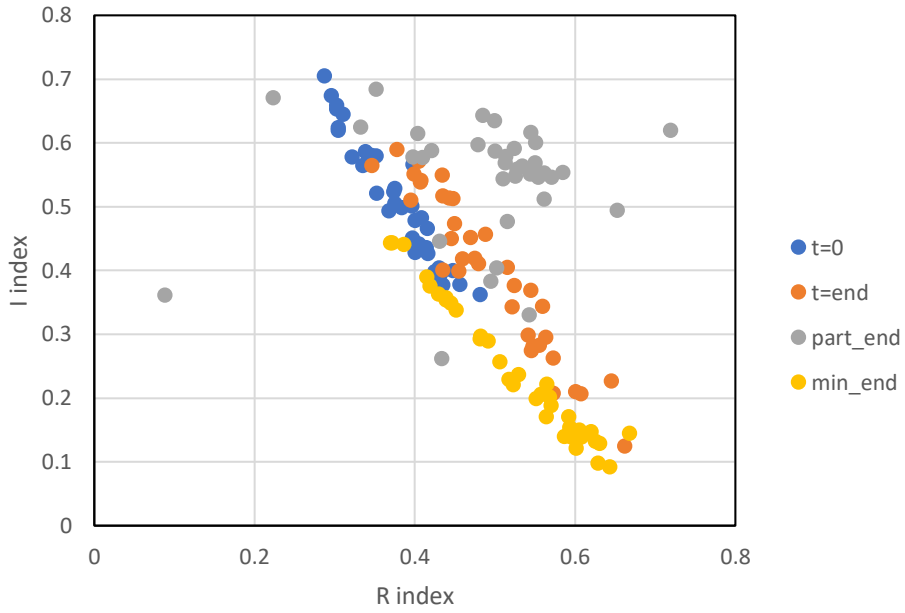

Figure 64: I-R index representation of all the samples

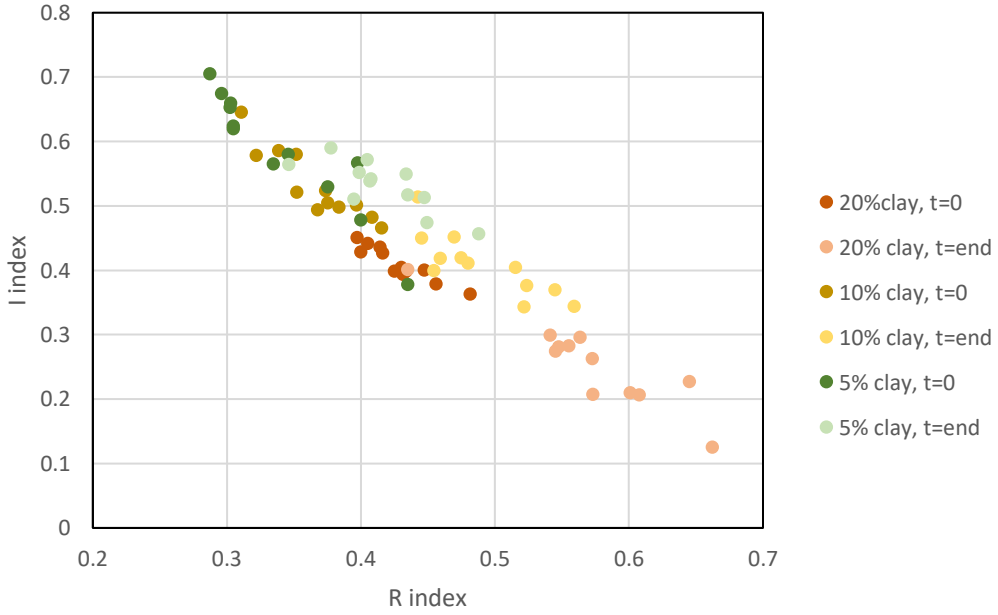

Figure 65: I-R index representation of the relevant samples, represented for soil texture

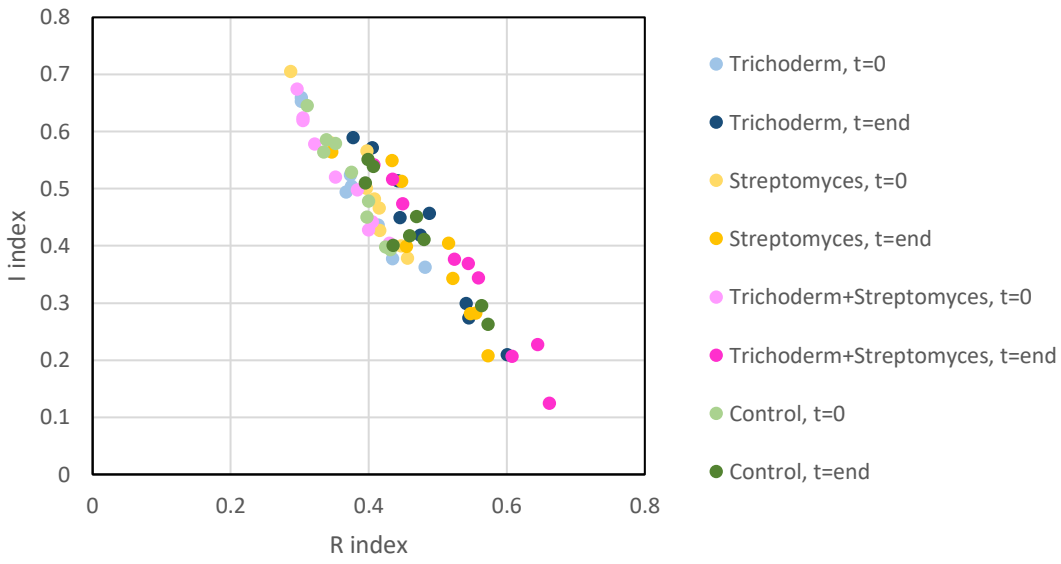

Figure 66: I-R index representation of the relevant samples, represented for soil treatment

Figure 64 and shows the I-R index representation for all the samples analysed. While most of the samples show a linear relationship between the two index, the group of samples representing the particulate organic matter samples taken at the end of the incubation, and obtained through the wet sieving, are outside the expected pattern. As explained in chapter 3.4.1 those samples are not representative of the real POM and for this reason in the next representations only the samples taken at the beginning and the end of the incubation that did not undergo to any sieving are represented.

The I-R index graph show as expected a linear relationship, and is possible to conclude that thermal stability is mainly explained by the preferential decomposition of the most labile compounds, used by the microorganism for their growth.

3.5 Comprehensive analysis of results

Providing a comprehensive overview of the results concerning the TOC values, and the separation of the fractions of MAOM and POM in particular, it is clear that under the experimental conditions employed this separation was worthless. Utilizing pure clay and sand as initial components, and introducing only a readily biodegradable organic matter and microorganism, resulted, within one month of incubation, in the exclusive proliferation of bacteria. This process did not induce the formation of substantial aggregates in the formulated model soils, and the application of wet sieving further destroyed any potential aggregate present in the jars. It is advisable to avoid wet sieving in tests similar to those presented in this work, as it disrupts soil aggregates, and to start with a more representative soil environment.

The primary objective of these experiments was to identify a methodology and investigate the feasibility of various analysis to comprehend the effects of different microorganism consortia on soils. In this context, while chloroform extraction returned inconclusive results regarding the quantity of microorganisms grown in the jars, the Rock Eval method provided valuable insights into the effects of diverse soil. Particularly this method is very useful for the quantification of soil organic carbon without necessitating extensive pretreatment. Despite each sample analysis requires approximately one hour to be analysed, the entire process and analysis are integrated into a single instrument, with the operator only required to prepare dry and homogeneous samples, weighing them in designated capsules for analysis.

Once the Rock Eval is performed it is possible to obtain numerous indices useful to acquire information about the organic matter within in the sample. Even with the simple composition of the soil and uncertainties surrounding the quantity of microorganisms introduced to each jar and their subsequent growth, the Rock Eval method revealed variations in TOC quantity and thermal stability. Significantly, the I and R indices variations revealed that the contribution to the thermal stability of the samples from the recalcitrant fraction of organic matter increased, while the one from the labile fraction decreased.

This method further enabled the identification of differences in changes resulting from different combinations of soil treatment or soil texture, rendering it an interesting and useful tool for the development of more complex microorganism consortia capable of enhancing the carbon stock potential of specific soils.

4. Conclusions

In conclusion, this research has examined the intricate dynamics of soil organic carbon and the efficiency of the Rock Eval method in understanding the impact of microorganisms on soil organic matter. The study gave valuable insights to the broader understanding of carbon cycling in soils, highlighting the significance of comprehending fluxes and factors influencing these processes.

The paradigm shift from traditional soil organic matter classification to the emerging soil continuum model required innovative techniques to investigate soil organic matter, such as the Rock Eval method. This project used the Rock Eval method, originally used to investigate the petroleum potential of rocks, and showed the applicability of this method in quantifying soil organic carbon and assessing its stability, thereby expanding its traditional use to a more environmentally focused application.

The Rock Eval method proved instrumental in showing the impacts of different soil treatments. It provided a robust means of quantifying soil organic carbon without demanding extensive pretreatment. However, it is crucial to note that, given the small sample size used by the Rock Eval method, a higher number of samples for each soil analysed should be considered to ensure a better representativity of the whole system studied.

Furthermore, the method's ability to highlight the presence of hydrocarbons opens the door to an exciting prospect. The Rock Eval method could potentially be employed to develop microbial consortia that are not only helpful to soil remediation but also at increasing soil carbon stock. This dual functionality makes it a promising avenue for future research in the realm of sustainable soil management practices.

The method's efficacy in discerning variations in total organic carbon quantity and thermal stability, as evidenced by changes in the I and R indices, underscores its utility in understanding the contributions of recalcitrant and labile organic matter fractions to the SOM stability.

Despite the simplicity of the soil composition and uncertainties surrounding microbial quantities, the Rock Eval method emerged as a powerful tool for identifying differences resulting from diverse soil treatments or textures. This suggests its potential for further exploration in developing intricate microorganism consortia aimed at enhancing carbon sequestration in soils.

As next steps for future research, it is possible to explore the use of more complex microorganisms consortia and transition to real soils containing authentic organic matter. This progression will enhance the ecological relevance of the findings, providing a more accurate representation of soil dynamics and microbial interactions.

Annex A

Results of TOC, POM, MAOM and WEOC

Table 7: TOC, POM, MAOM and WEOC results

	Starting point	Endpoint			
Sample	TOC [mgC/gsoil]	TOC[mgC/gsoil]	POM [mgC/gsoil]	MAOM [mgC/gsoil]	WEOC [mgC/gsoil]
20TA1	0.17	0.15	0.03	0.46	0.107614
20TB1	0.19	0.11	0.02	0.6	0.134631
20TC1	0.18	0.13	0.02	0.43	0.127242
20SA1	0.19	0.11	0.04	0.51	0.126144
20SB1	0.19	0.12	0	0.57	0.086112
20SC1	0.2	0.09	0	0.56	0.086401
20TSA1	0.18	0.09	0	0.63	0.107848
20TSB1	0.18	0.1	0.02	0.52	0.101902
20TSC1	0.18	0.09	0	0.46	0.11334
20CA1	0.17	0.09	0	0.73	0.113404
20CB1	0.2	0.11	0	0.5	0.116357
20CC1	0.16	0.13	0.01	0.72	0.163102
10TA1	0.18	0.11	0.02	1	0.134341
10TB1	0.12	0.08	0.02	0.64	0.112275
10TC1	0.15	0.08	0.02	0.75	0.078594
10SA1	0.12	0.08	0.01	0.57	0.102865
10SB1	0.13	0.07	0.01	0.66	0.102035
10SC1	0.13	0.07	0.01	0.82	0.092406
10TSA1	0.13	0.06	0.01	0.57	0.09123
10TSB1	0.13	0.08	0	0.61	0.068838
10TSC1	0.15	0.07	0	0.71	0.068494
10CA1	0.1	0.11	0	0.75	0.093259
10CB1	0.11	0.1	0.01	0.79	0.063003
10CC1	0.09	0.08	0	0.78	0.090373
5TA1	0.5	0.07	0.01	1.02	0.061046
5TB1	0.12	0.08	0.02	1.39	0.087268
5TC1	0.13	0.08	0	1.71	0.117433
5SA1	0.17	0.07	0.02	1.28	0.070378
5SB1	0.14	0.07	0.01	1.4	0.09226
5SC1	0.13	0.05	0.01	1.36	0.082733
5TSA1	0.12	0.06	0	1.32	0.078559
5TSB1	0.13	0.06	0.01	0.98	0.064519
5TSC1	0.11	0.07	0	1.27	0.066643
5CA1	0.13	0.08	0.01	1.72	0.06577
5CB1	0.12	0.07	0	1.62	0.074223
5CC1	0.11	0.08	0	1.23	0.066993

Annex B

X-ray fluorescence (XRF) analysis results for the clay.

SPECTRO AMETEK

7/6/2023 2:29:13

Sample Name	**GM Kaolinite**	Dilution Material	-
Method	**Geochemistry Traces Powders**	Sample Mass	**3.0000**
Sample Folder	**July 2023**	Dilution Mass	-
Sample Type	**Powder**	Dilution Factor	-
Sample Status	**A A A A X X X X**	Measurement Date	**7/6/2023 2:10 PM**
Operator	**Admin User**	Evaluation Date	**7/6/2023 2:11 PM**

Results - GM Kaolinite

The error is the statistical error with 1 sigma confidence interval

	Element		Concentration		Abs. Error
11	Na_2O	Sodium oxide	< 0.012	%	-
12	MgO	Magnesium oxide	< 0.0017	%	-
13	Al_2O_3	Aluminium oxide	43.51	%	0.03
14	SiO_2	Silicon dioxide	56.96	%	0.03
15	P_2O_5	Phosphorus pentoxide	0.06890	%	0.00088
16	SO_3	Sulfur trioxide	0.1469	%	0.0007
17	Cl	Chlorine	0.00096	%	0.00006
19	K_2O	Potassium oxide	0.1701	%	0.0003
20	CaO	Calcium oxide	0.01637	%	0.00011
21	Sc	Scandium	-		-
22	Ti	Titanium	8199	µg/g	3
23	V	Vanadium	142.1	µg/g	1.0
24	Cr	Chromium	116.3	µg/g	0.3
25	MnO	Manganese(II) oxide	6.0	µg/g	0.2
26	Fe_2O_3	Iron(III) oxide	0.6545	%	0.0007
27	Co	Cobalt	< 2.5	µg/g	-
28	Ni	Nickel	34.1	µg/g	0.4
29	Cu	Copper	12.6	µg/g	0.2
30	Zn	Zinc	43.8	µg/g	0.3
31	Ga	Gallium	53.6	µg/g	0.2
32	Ge	Germanium	2.1	µg/g	0.1
33	As	Arsenic	0.8	µg/g	0.1
34	Se	Selenium	0.24	µg/g	0.04
35	Br	Bromine	0.07	µg/g	0.03
37	Rb	Rubidium	4.49	µg/g	0.05
38	Sr	Strontium	45.0	µg/g	0.1
39	Y	Yttrium	6.0	µg/g	0.1
40	Zr	Zirconium	44.6	µg/g	0.1
41	Nb	Niobium	17.4	µg/g	0.1
42	Mo	Molybdenum	2.3	µg/g	0.1
44	Ru	Ruthenium	< 0.2	µg/g	-
45	Rh	Rhodium	< 0.2	µg/g	-
46	Pd	Palladium	< 0.2	µg/g	-
47	Ag	Silver	< 0.2	µg/g	-
48	Cd	Cadmium	0.7	µg/g	0.2
49	In	Indium	0.9	µg/g	0.3
50	Sn	Tin	2.8	µg/g	0.2
51	Sb	Antimony	1.0	µg/g	0.2
52	Te	Tellurium	1.7	µg/g	0.3
53	I	Iodine	2.9	µg/g	0.6
55	Cs	Cesium	5.2	µg/g	1.3

1 / 2

56	Ba	Barium	44.6	µg/g	1.7
57	La	Lanthanium	38.8	µg/g	2.4
58	Ce	Cerium	46.6	µg/g	2.8
59	Pr	Praseodymium	30.4	µg/g	2.0
60	Nd	Neodymium	29.7	µg/g	0.7
62	Sm	Samarium	8.5	µg/g	0.5
70	Yb	Ytterbium	< 0.2	µg/g	-
72	Hf	Hafnium	1.2	µg/g	0.2
73	Ta	Tantalum	< 0.8	µg/g	-
74	W	Tungsten	1.0	µg/g	0.4
79	Au	Gold	< 0.1	µg/g	-
80	Hg	Mercury	< 0.1	µg/g	-
81	Tl	Thallium	< 0.2	µg/g	-
82	Pb	Lead	29.0	µg/g	0.2
83	Bi	Bismuth	0.8	µg/g	0.1
90	Th	Thorium	18.6	µg/g	0.1
92	U	Uranium	3.1	µg/g	0.1

Annex C

Data sheet of the sand used for the experiments.

Carlo Bernasconi AG

Quarzsandmischung 0.10 - 3.20 mm

Produktbeschreibung

In unserem Werk werden natürliche Kristallquarzsande und Kiese nach selektiver Gewinnung mehrfach gewaschen und auf nassmechanischem Wege klassiert. Durch weitere Aufbereitungsprozesse in modernen Trocknungs- und Siebanlagen wird der feuergetrocknete Quarzsand in die einzelnen Fraktionen getrennt und in Silos gelagert.

Zusatzinformation

Preis pro	Sack
Körnung in mm	0.10 - 3.20
Kornform	Natürlich gerundet
Farbe	Grau
Verpackung	Plastiksack à 25 kg
Lagerort	Bärschwil, Bern
Zustand	Feuergetrocknet

Technische Daten

Härte nach Mohs	7
Schüttgewicht in kg/m³	1'500
Spezifisches Gewicht in kg/m³	2'650

Chemische Richtwerte

SiO_2	96.30%
Al_2O_3	1.90%
K_2O Na_2	1.41%
Glühverlust	0.30%
CaO MgO	0.05%
Fe_2O_3	0.04%
TiO_2	0..08%

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