Soil quality — Methods to estimate biogeochemical stability or residence time of organic carbon in soils

Qualité des sols — Méthodes pour estimer la stabilité biogéochimique ou le temps de résidence du carbone organique des sols

Contents

Introduction

Soils are the main terrestrial reservoir of organic carbon on Earth. They contain two to three times more carbon than the atmosphere, with which they exchange CO_2 through photosynthesis, plant respiration and the mineralization of soil organic matter, thus participating in the carbon cycle. By this process, it is estimated that around 30% of total global anthropogenic CO_2 emissions are captured by soils. Therefore, soils act globally as carbon sinks and contribute to mitigating climate change. Locally, depending on land usage and management, carbon emissions from soils can be higher than carbon sequestration (for example by deforestation) or vice-versa (for example by reforesting). It is therefore important to be able to trace the movement of soil carbon, in order to evaluate the impact of a given practice on the climate.

To this end, the ISO 23400:2021 standard provides recommendations for assessing organic carbon stocks in soils at the plot scale. This standard does not account for residence time of organic carbon in the soil, nor the fraction of it that returns to the atmosphere as CO_2. However, the soil organic matter is mineralized with CO_2 released at various rates, depending on its chemical structure, interactions with surrounding minerals and spatial distribution. In an attempt to quantify the various fractions or pools of soil organic carbon, which display different turnover rates, the approach of researchers is to describe these pools according to their biogeochemical stability or mean residence time in the soil.

The purpose of this document is to provide a list of methods to soil managers wanting to characterise the stability of their soil carbon stock, and its evolution over time or following a change in practice or usage.

This document presents standardized or published methods that currently allow:

(1) either quantifying the relative stability and level of decomposition of soil organic matter. This method follows two steps: 1. quantification of total organic carbon content and of carbon-hydrogen bonds thermal stability; 2. interpretation in terms of relative stability and level of decomposition of organic matter. It allows assessment of the stability of soil organic carbon over time,

(2) or quantifying the organic carbon content in soil according to its estimated biogeochemical stability or mean residence time. These methods follow two steps: 1. quantification of different pools of organic matter in soil, by physical, chemical, thermal or biological processes; 2. interpretation of these fractions in terms of biogeochemical stability or mean residence time.

This document describes the principle and operating mode of each method and refers to standards or scientific publications for detailed implementation. It also presents the outputs, their interpretation in terms of stability or residence time, and mentions the advantages and limitations. At least one example of application is provided per method. A figure representing the characterised carbon pools provides an indicative comparison of the ranges of stability and residence times according to the method. A comparative table of the methods allows one to get a synthesised view of the existing possibilities to assess organic carbon stability or residence time. It can also help choosing the most appropriate method according to the project, objectives and resources.

Scope

- The methods included in this document meet the following criteria:
    - they can be used to estimate the residence time of organic carbon in soil or its level of biogeochemical stability;
    - they are described in the scientific literature or in standards;
    - examples of applications have been published.
- Methods for quantifying total organic carbon content, without differentiating its stability or residence time, are not covered by this document (e.g. elemental analysis).
- Methods that have not been standardized or published are outside the scope of this document.
- To date, some methods that meet these criteria, spectroscopic methods, isotopic methods, do not appear in this document because they are still in the research stage and have not been routinely used in operational projects.
- The methods described in detail in this document are:
    - Physical method : the organic soil particulate fractionation
    - Thermal method : the Rock-Eval® analysis
    - Chemical method : the permanganate oxidisable carbon method
    - Biological methods
        - Soil basal respiration – field or laboratory tests
        - Enzymatic activities

- This document is applicable to a wide range of soils and biomass:
    - Agricultural soils
    - Forest soils
    - Pastures and meadows
    - Peatlands and wet grasslands
    - Permafrost
    - Urban soils
    - Technosols / constructed soils
    - Organic amendments (biochar, compost, digestate, etc…)

Organic carbon geochemical stability and residence time

Soil organic matter, which is mainly comprised of carbon, follows complex dynamics of mineralisation, dissolution, oxidation, adsorption, etc. (Basile-Doelsche et al., 2020). These processes lead to continuous modification of the organic carbon present in soil and of the size of carbon pools of various biogeochemical stabilities or residence times.

The biogeochemical stability and residence time are controlled at different scales by different processes:

- At the molecular scale, the intrinsic chemical properties of organic compounds impact the rate of their biotransformation. Recalcitrant organic compounds are not easily degraded due to their chemical structure. But the molecular structure of organic matter alone is not the only factor controlling its stability at this scale: both biological and environmental factors contribute (Schmidt et al., 2011; Lehmann & Kleber, 2015). Bacterial community composition can also drive the soil organic matter composition and stability (Domeignoz-Hurta et al., 2021).
- At the aggregate scale, organo-mineral associations impact the processes of adsorption, desorption and destruction of organic matter (Lehmann & Kleber, 2015; Lehmann et al., 2020). The physical and chemical interactions between organic compounds and mineral particles are influenced by their respective chemical composition, mineral particle size and crystallographic state, and soil conditions such as pH and water saturation. At this scale, the spatial dispersion of organic matter and living organisms limits the extent of mineralisation by decreasing the probability that microorganisms decomposers will encounter the organic substrate.
- At the ecosystem scale, environmental conditions (i.e. temperature, humidity, oxygen, etc.) as well as soil practices (tillage, amendments, etc.) can have a dominant influence on carbon stability (Schmidt et al., 2011; Basile-Doelsch et al., 2020; Lehmann et al., 2020).

The lability and stability of the organic matter can be explained by bioenergetic constraints, i.e. "the organic matter capacity to provide the energy necessary for growth of decomposing microorganisms. The more complex and the less accessible organic compounds are, the more energy decomposers have to invest. If the return-on energy investment is unfavourable, the soil carbon tends to persist" (Henneron et al., 2022).

Despite the increasing scientific knowledge, the soil organic matter dynamics are not yet well understood and the big picture understanding of carbon pools has not yet been fully developed. Different concepts or divisions of organic carbon pools in soils have been proposed in the scientific literature and accordingly different approaches and analytical methods are used to measure or estimate them. Two main types of description are proposed, the first one being based on residence time of organic carbon in soil and the second one being based on organic carbon biogeochemical stability in soil (Poeplau et al., 2018; Sanderman and Grandy, 2020; Derrien et al., 2023).

For the 'residence time' approach, for example Parton and co-workers described carbon pools as active pool (1–5 years), slow poll (20–100 years) and passive pool (400–2000 years) (Parton et al., 1987 in Nayak et al., 2019). Using the 'organic matter biogeochemical stability' approach, carbon pools can be categorised into labile, stable, refractory and inert pools (Nayak et al., 2019). In this document it was decided to describe three pools of organic carbon, based on the concepts and terms discussed in Parton et al. (2017), Balesdent et al. (2018), Nayak et al. (2019) and Basile-Doelsch et al. (2020): labile, intermediate and stable organic carbon pools. Figure 1 shows the generic vocabulary used in this document to describe the pools of organic carbon according to their level of stability in the soil, as well as the associated dynamics of organic matter decomposition and the related mean residence time of organic carbon in soil. This figure also attempts to link generic vocabulary to the vocabulary specific to each method described in this document. It also provides a comparison of the estimated stability ranges depending on the method chosen. These stability ranges are indicative, as they are based on current scientific knowledge, which remains incomplete.
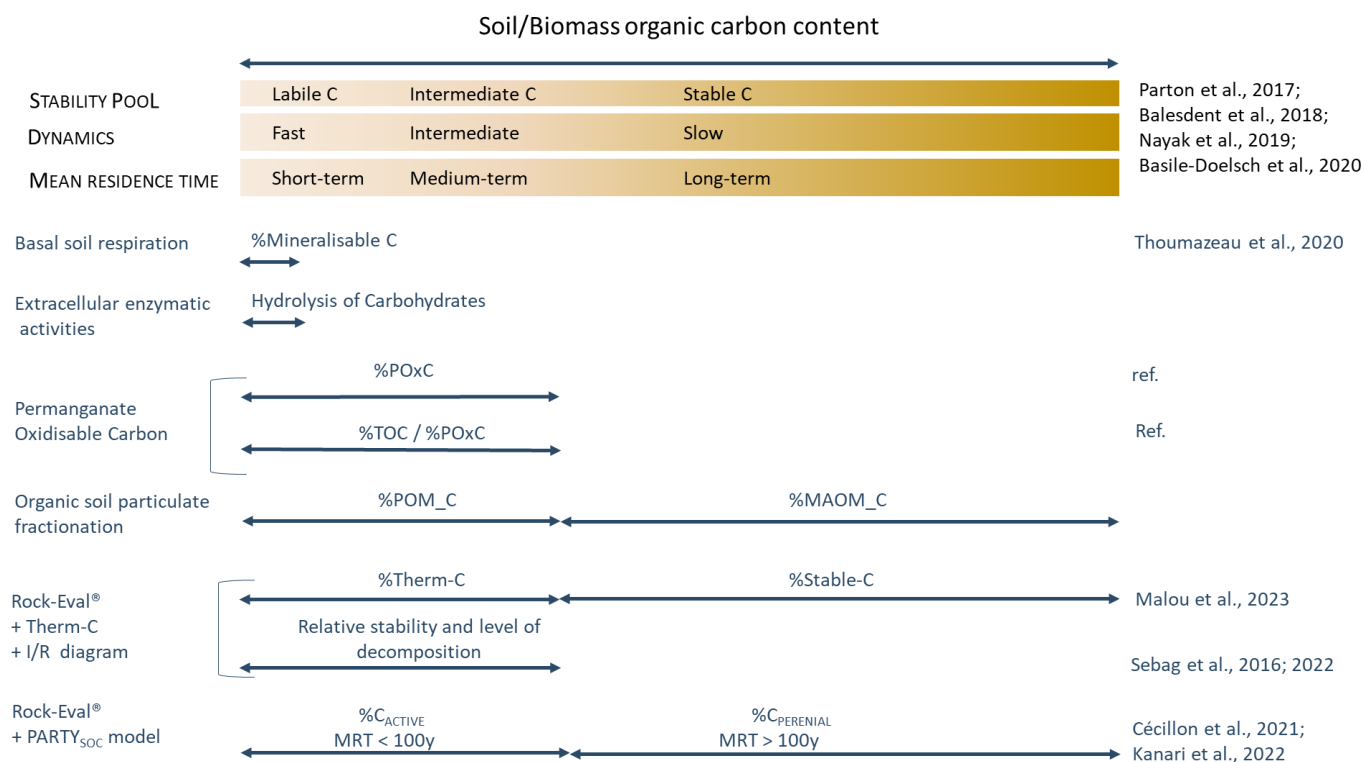
Figure 1 : Carbon pools and associated indicative stability ranges – generic and method–specific

Sample collection

It is recommended that sample collection, transport and storage adhere to the following standards:

- ◆ ISO 18400–101:2017 – Soil quality – Sampling – Part 101: Framework for the preparation and application of a sampling plan

- ◆ ISO 18400–102:2017 – Soil quality – Sampling – Selection and application of sampling techniques

- ◆ ISO 18400–105:2017 – Soil quality – Sampling – Packaging, transport, storage and preservation of samples

- ◆ ISO 18400–104:2018 – Soil quality – Sampling – Part 104: Strategies

- ◆ ISO/FDIS 18400–206:2017(E) – Soil quality — Sampling — Part 206: Guidance on the collection, handling and storage of soil for the assessment of biological functional and structural endpoints in the laboratory

- ◆ ISO 23400 –Guidelines for the determination of organic carbon and nitrogen stocks and their variations in mineral soils at field scale

Normative references

Vocabulary

- ISO 11074 – Soil quality – Vocabulary

Sampling

- ISO 18400-101:2017 – Soil quality – Sampling – Part 101: Framework for the preparation and application of a sampling plan
- ISO 18400-102:2017 – Soil quality – Sampling – Selection and application of sampling techniques
- ISO 18400-105:2017 – Soil quality – Sampling – Packaging, transport, storage and preservation of samples
- ISO 18400-104:2018 – Soil quality – Sampling – Part 104: Strategies
- ISO/FDIS 18400-206:2017(E) – Soil quality — Sampling — Part 206: Guidance on the collection, handling and storage of soil for the assessment of biological functional and structural endpoints in the laboratory

Analysis

- ISO-10694:1995– Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)
- ISO 14235:1998– Soil quality — Determination of organic carbon by sulfochromic oxidation
- ISO 16072:2002– Soil quality — Laboratory methods for determination of microbial soil respiration
- ISO 17505:2023 – Soil and waste characterization – Temperature dependent differentiation of total carbon (TOC400, ROC, TIC900)
- ISO 20130:2018– Soil quality — Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates
- ISO/TS 22939:2019– Soil quality — Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates

Organic carbon stocks determination

- ISO 23400:2021– Soil quality —Guidelines for the determination of organic carbon and nitrogen stocks and their variations in mineral soils at field scale

Terms and definitions

→ **Basal soil respiration**

Steady state CO2 emissions or O2 consumption linked to the microbial decomposition of organic matter content in soil under either field conditions or defined laboratory conditions.

→ **Soil organic carbon (SOC)**

Carbon related to the soil organic matter (SOM), including pyrogenic carbon (issued from fires). It excludes the organic carbon related to organic pollution that may come from petroleum products (*e.g.* crude oil, polycyclic aromatic hydrocarbons, organic volatile compounds, chloride hydrocarbons, distillation residues), pesticides or plastics.

→ **Soil inorganic carbon**

Carbon related to carbonates in soil

→ **Lability of soil organic matter**

Degree to which soil organic matter is readily decomposed by soil organisms and microbial activity. It reflects the ease with which organic compounds can be transformed into simpler forms, releasing CO2, nutrients, and energy in the process. The lability of soil organic matter is influenced by various factors: its chemical composition, its physical protection within soil aggregates, soil conditions such as pH, temperature and moisture, and the activity of soil organisms. Soils with high organic matter lability contribute to agronomic fertility and soil health.

→ **Stability of soil organic matter**

Ability of soil organic matter to persist in the soil environment without being rapidly degraded or transformed into simpler organic compounds and CO2. Soil organic matter stability is influenced by chemical composition, physical protection within soil aggregates, interactions with soil minerals, and microbial activity. Soils with high organic matter stability tend to have a greater capacity to retain nutrients, store carbon, improve soil structure, and support overall soil health and fertility over the long term. Stable organic matter decomposes more slowly and persists in the soil for longer periods than labile organic matter.

→ **Labile organic carbon**

Carbon constituting labile soil organic matter, which has the ability to be rapidly decomposed.

→ **Stable organic carbon**

Carbon constituting stable soil organic matter, which is decomposed very slowly.

→ **Mineral soil**

Soil composed largely or entirely of mineral (inorganic) constituents. [ISO 14688-1:2017]

→ **Organic soil**

A soil in which the organic component is dominant with respect to the mineral component.

Note: Please note that the definition of 'organic soils" varies between different soil classification systems. In the ISO 23400:2021 standard, organic soils are considered to contain more than 50% organic matter by volume or more than 30% organic matter by weight, i.e. 17% of organic carbon.

➜ **Organic soil layer**

A soil horizon which is dominated by organic material, consisting of undecomposed or partially decomposed litter, such as leaves, needles, twigs, moss, and lichens, which has accumulated on the surface; it may be on top of either mineral or organic soils. [ISO 23400:2021]

➜ **Permafrost**

Ground consists of mineral soil and sediment, rock, ice, peat and other organic materials that remain below 0°C for at least two consecutive years. [ISO 23400:2021]

➜ **Technosol**

Soil with strong human influence as it contains significant amounts of artefacts. Its pedogenesis is significantly modified. [World reference base for soil resources 2014, FAO]

➜ **Residence time :** duration of residence of organic matter in the soil, during which it is gradually biodegraded and transformed into CO_2 by mineralisation, until its complete disappearance. By extension, duration of residence of organic carbon in the soil until its complete transformation into CO_2.

➜ **Time scale sensitivity** : for a given soil practice, duration from which the method is sufficiently sensitive to detect and quantify a change in soil organic carbon content as well as a change in the quality of soil organic matter and/or in its interaction with organic matrix that impact its residence time.

Methods for assessing soil organic carbon pools

Four families of methods are presented: physical, thermal, chemical and biological.

I. Physical methods

Organic soil particulate fractionation methods

The soil is characterised in its solid phase by organic and inorganic particles. The organic compounds are vegetal and animal origin residues. The soil organic matter (SOM) presents a continuum of increasingly fragments that can be separated.

The organic particles, in particular those in size bigger than 50 μm are differentiated from finest organic material by their origin properties, chemical characteristics, reactivity and their mean time of residence in the soil. The organic soil particulate fractionation is an "old" procedure [1], it was standardized in France [2].

The quantification of organic particles in different particle-size classes, that is the granulometric distribution of carbon, provided by the separation of these organic particles from mineral forms, is an indicator of the organic status of the soil. The fractions separated can be used for others purposes, for example their chemical or biochemical properties, etc.

Thanks to the experimental procedure developed in water, without any other chemical agent, the organic fractions conserve their *in situ* properties.

    A. Analysis

        ● **Principle**

The objectives are to separate SOM particles by size from mineral particles, with no alteration of their biochemical nature, to characterise their chemical composition, to characterise the particle size distribution of soil carbon, and then express it for example in the SOM stocks.

The analytical sequence is based on a process of physical dispersion of the soil sample in the water, separation of SOM particles by sieve at different sizes, concentration by densimetry, finally the analysis of the mass and C & N concentration of the different fractions.

        ● **Sample storage, preparation and quantity**

Different ways are possible, depending on the objectives. By routine, it is recommended to use air-dried soil samples sieved to 2 mm (fine earth) [3].

In some cases, the use of fresh samples from field work were studied. In this case, the sample is manually and gently crushed to obtain aggregates less than 5 mm in diameter in order to obtain a good dispersion. When the coarse fraction > 2 mm contains an important proportion of gravels, and/or vegetal macro-debris from roots, a peculiar attention is needed, and in the case of evaluation of SOM stocks the mass these fractions have to be considered [4].

This document is considered for 2 mm sieved samples. Otherwise, add a 2 mm sieve after the dispersion-shaking (cf.Implementation paragraph).

Sample mass: from 30 to 50 g air dry soil, measure the residual water content (g.kg at 105°C).

An aliquot of the same sample is reserved and treated for C and N elemental determination [5].

- **Implementation**

A complete version of this method and variances is proposed for a new standard. A French standard is already available [2].

Tool : Most soil routine laboratories are equipped for the realisation of the particle fractionation, by analogy to the determination of the particle size analysis of soils samples [6] the material and tools are the same.

It is recommended to make the analysis of carbon and nitrogen concentrations of the organic fractions by elementary analysis [5].

Disaggregation and dispersion of soil sample in water : 50 g sample in a 250 mL plastic bottle with 180 mL of distilled water and ten glass balls 5 mm in diameter. Agitate the bottle on a rotary shaker at 40 rpm for 16 h.

For a better reproduction of the results, and in some cases if the organic level of the soil appears low, 3 repetitions by sample of this sequence are made.

Wet sieving: Sieve the suspension underwater on a sieve with a 200–µm square mesh, the residue is suspended in a beaker, then the organic fragments are separated during their transfer to the 200–µm sieve by decantation, the operation is repeated several times until the sands no longer contain any visible organic fragments. The result permits the separation of the OF200-2000 µm and MF200-2000 fraction (organic fraction and mineral fraction). The dry weight of the two fractions is determined at 60°C.

The same operation on the fraction of less than 200 µm with a 50 µm sieve to obtain OF50–200 and MF50–200. The dry masses are determined.

The residual suspension contains the < 50µm particles and the hydrosoluble.

 Treatment of the 0–50 µm fraction: Depending on the objectives different ways are possible.

- ➔ The analysis of the total 0–50 µm needs a flocculation-centrifugation of the suspension, the separation permitting the analysis of the solid and the water-soluble phases.
- ➔ Study the OM associated to the 0–2 µm fraction needs the separation by sedimentation (pipette method) or centrifugation of these particles after a sonication of the suspension (10 min at an applied energy of approximately 300 J mL–1) [7].
- ➔ Method with sampling of aliquots is faster than the preceding one for measurement of the silt and clay fractions   [8].

- **Parameters output**

Masses of the different fractions, compare the sum of fraction to the sample dry weight. The mass balance is from 99 % to 100 %.

Determination of the C and N fractions concentrations. Calculate the carbon content of the different soil fractions. The results illustrate the carbon (and N) granulometric distribution. The C/N of the fraction is also provided.

- **Advantages**

Ultrasonic treatment is not applied on particulate organic matter > 50 µm (pure organic fractions size).

The carbon content of the mineral fraction is negligible in most cases (< 0.1% of soil C).

Light and coarse particulate SOM which has predominantly a vegetal signature, is assumed to have high turnover rate and is relatively undecomposed and vulnerable to land use change and the finer mineral-associated SOM, generally with slow turnover due to chemical bonding to minerals and physical protection in fine aggregates (depending the dispersion method) requires no expensive equipment, and is already used worldwide.

This approach is suitable before other techniques, it permits a simple approach of the distribution of the carbon into the soil. A perfect separation of each component of the soil organic matter is impossible. With the particle size separate a simple approach for a qualification of the organic and organo-mineral compartments of the soil is possible [9].

Precision and reproducibility were summarised by [7]: "In general, the smaller the quantity of the fraction, the greater the variability. Repeatability increases with the particle size of the fraction. Relative error resulting from fractionation varies in the same way for percentages by weight or the percentage of the carbon of the fraction compared to total carbon."

Comparison with the particle-size distribution obtained from the reference method [6] demonstrated complete dispersion (from 98-102 % MW);

Carbon balance of the separation, including solubilized organic carbon, was 99.5 ± 1.0 %

- **Limits**

The disadvantage could be associated to the duration of the experimental procedure.

- **Operator and delivery time**

Dispersion corresponds to a night agitation; 1 to 2 h for the fractionation; determination of the mass fraction 1-2 h. Add the time for drying, crushing and homogenising the fractions and their elemental analysis. In general, a complete sequence is done and the results obtained in less than 4 four days (by a qualified technician/engineer).

- **On going R&I**

B. **Interpretation and associated tools**

The scientific background is large and provides an important state of methodological performances, adaptations, conceptual framework and results for the study of organic matter stabilization and evolution in the soil by this method. An important methodological discussion and adaptation are proposed by [7].

- **Time scale sensitivity**

The Carbon mean age of the fractions can be resumed [10]:

➜ Size > 2000 µm: less than 1 year
➜ 200 µm – 2000 µm: from 2 to 5 y
➜ 50 µm – 200 µm: from 10 to 20 y
➜ 0 – 50 µm: > 50 y
➜ water soluble: 5 to 10 y

This way, it is possible to analyse the fluctuations of carbon soil contents and stocks by the analysis of fluctuations of the carbon distribution in the coarser fractions (in general > 50 µm).

- **On going R&I**

- The ∂^{13}C tool was associated to this approach for diachronic approaches and modification of the carbon nature inputs [11].
- This particulate analysis can also be an approach to study other elements, like the nature of soil phosphorus and the relation to the soil phosphate dynamics [12], [13], [14].
- Recently the particulate fractionation was associated with the Rock–Eval® thermal analysis [15].

C. Examples   of   application

➔ **Dynamics of soil organic carbon pools comparing different land use**

[10], [16], [17], [18],

➔ **Stability of organic carbon in soil**

[19], [20], [21], [22].

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II. Thermal methods

Thermal methods involve progressively degrading a sample under increasing thermal stress in a specified atmosphere and recording a physical or chemical property of the sample over time or temperature, while the sample temperature is controlled. Different thermal methods allow to quantify and characterise soil organic carbon, thanks to adapted temperature programs:

➔ Thermogravimetric methods, where the sample property that is recorded is the weight loss from soil organic matter during heating (Tokarski et al., 2019, 2020)

➔ Calorimetric methods, where the sample property that is recorded is energy released during soil organic matter heating (Plante et al., 2011)

➔ Evolved gas analysis methods, such as the Rock-Eval® method (Disnar et al., 2003; Sebag et al., 2006) and the TOC400 method, where the property that is recorded during soil heating is the composition of the released gases by soil organic matter, especially carbon.

From a pure thermal point of view, labile organic matter degrades at lower temperatures than stable organic matter. From a bioenergetic point of view, labile organic matter is decomposed primarily by microorganisms because the return on energy investment is more favourable to them, than with stable organic matter (Plante et al., 2011; Pallasser et al., 2013; Barré et al., 2016; Henneron et al., 2022). Correlations were found between the thermal lability/stability and biochemical lability/stability of soil organic matter (Plante et al., 2011; Domeignoz-Horta et al., 2021).

Furthermore, soil organic matter decomposition has been shown to involve the degradation of hydrogen-rich compounds at an early stage (Sebag et al., 2006; Gregorich et al., 2015; Albrecht et al., 2015). The Rock-Eval® thermal method, allowing the quantification of Carbon-Hydrogen bonds released by organic compounds during its thermal degradation, is thus particularly suited to follow the stabilization of soil organic matter (Sebag et al., 2016; Soucémarianadin et al., 2018; Sebag et al., 2022).

The TOC400 method is not described here as it is not focused on carbon stability. Although it was found to be meaningful to get some information about carbon stability over time, data are still missing to build up solid proof that it is reliable for characterization of stable carbon. The data interpretation is a subject of debate, mainly regarding the programmed temperatures used as they have a significant impact on the results.

Thermogravimetric methods

A. Analysis

• Principle

Thermogravimetric analysis (TGA) measures the absolute amount and rate of weight changes of a sample as a function of time or temperature in a controlled environment. Differences in the molecular structure of the sample compounds will generate weight loss peaks at different temperatures. The TGA analysis of soil samples in general consists of heating the sample first under synthetic air atmosphere (e.g. O2 and N2, 10-50 mL min-1) with a protective gas (e.g. N2, 10-50 mL min-1) from room temperature up to the desired final temperature (e.g. around 550°C - 700°C). Some analytical protocols in the literature propose temperature bearing during the heating. The amount of the different carbon pools according to their thermal stability is estimated from the derivative of TGA curves.

The analytical protocols found in the literature are quite variable in terms of gas carrier (O2/N2 ratios), gas fluxes, the initial and final temperatures as well as the heating ramp and steps.

• Sample storage, preparation and quantity

It is recommended to store the sample after drying at 105°C at room temperature if the analyses are carried out in the following weeks or at 4°C for a long term survey (years), and grounding. The amount of sample required to carry out the analysis may vary from 5-1500mg, depending on the apparatus and the quantity of organic carbon.

• Implementation

Thermal analyser most often coupled with DSC or FTIR.

• Parameters output

Peaks area integration. Some authors propose to divide the thermogram original peak into three new peaks that represent contrasting levels of resistance to thermal oxidation/combustion (Demyan et al., 2013; Kurganova et al., 2019; Merino et al., 2014):

(i) 200–350 °C (labile organic matter);

(ii) 350–400 °C (recalcitrant organic matter); and

(iii) 400–550 °C (highly recalcitrant organic matter).

Nevertheless, as there is no standard protocol, the temperature ranges for peak integration are variable.

- **Advantages**

More time-effective than chemical and fractionation methods (1 to 3 hour/analysis and continuous 24/7 unattended operation; no need for preliminary treatment)

No need for chemical solvents and related laboratory facilities

- **Limits**

No standard protocol and data treatment which makes it difficult to compare the results from different publications.

- **Operator and delivery time**

From 1 hour up to 3 hours/sample depending on the temperature ramp used.

- **On going R&I**

Continuous R&I on soil analytical methods by coupling TGA with other techniques such as FTIR, DSC, or Gas Chromatography (GC).

B. **Interpretation and associated tools**

- **Time scale sensitivity**

- **On going R&I**

Linking thermogravimetric data with SOC fractions (Tokarski et al., 2019, 2020)

Attention should be paid to the fact that thermogravimetric information were validated on proxies

Continuous R&I on soil analytical methods by coupling TGA with other techniques such as FTIR, DSC, or Gas Chromatography (GC).

C. **Examples of application**

➔ Thermal stability of soil organic carbon after long-term manure (Barreto et al., 2021)

➔ Thermal stability of soil organic matter within an erosional landscape under agriculture (Zhang et al., 2021)

D. **References**

Differential Scanning Calorimetric methods

A. Analysis

- **Principle**

Differential scanning calorimetry (DSC) is a technique that records the energy balance of a substance during temperature changes by measuring the temperature differences between the sample and a reference. A material that consists of various substances with different molecular structures exhibits several energy flux peaks at different temperatures.

- **Sample storage, preparation and quantity**

The same TGA: it is recommended to store the sample after drying at 105°C at room temperature if the analyses are carried out in the following weeks or at 4°C for a long term survey (years), and grounding. The amount of sample required to carry out the analysis may vary from 5- 1500mg, depending on the apparatus and the quantity of organic carbon.

- **Implementation**

Differential scanning calorimeter most often coupled with TGA

- **Parameters output**

- **Advantages**

More time-effective than chemical and fractionation methods (1 to 3 hour/analysis and continuous 24/7 unattended operation; no need for preliminary treatment)

No need for chemical solvents and related laboratory facilities

- **Limits**

No standard protocol and data treatment which makes it difficult to compare the results from different publications.

- **Operator and delivery time**

From 1 hour up to 3 hours/sample depending on the temperature ramp used.

- **On going R&I**

Continuous R&I on soil analytical methods by coupling DSC with other techniques such as TGA, FTIR, or Gas Chromatography (GC).

B. Interpretation and associated tools

- **Time scale sensitivity**

- **On going R&I**

Continuous R&I on soil analytical methods by coupling TGA with other techniques such as FTIR, DSC, or Gas Chromatography (GC).

C. **Examples of application**

D. **References**

Rock-Eval® methods

A. **Analysis**

- **Principle**

Continuous measurement through heating ramp programs : Pyrolysis [from 200°C to 650°C – 30°C/min] + Oxidation [300°C to 850°C – 20°C/min]

HC, CO, CO2 and SO2 signals continuously measured by Flame Ionisation Detector (HC), IR (CO, CO2) and UV (SO2) spectrometers

- **Sample storage, preparation and quantity**

Storage :

➢ preferably after drying
➢ at room temperature if the analyses are carried out in the following weeks
➢ at 4°C for a long term survey (years), to avoid the lost of the most labile organic compounds and consequently the alteration of the S2 and HI parameters

Drying for 12 to 24h at 40°C

Grounding at less than 200μm

Quantity : 5-200 mg, depending on the content of organic carbon and of mineral carbon

- **Implementation**

Rock-Eval® device

- **Parameters output**

HC, CO, CO2 and SO2 thermograms in pyrolysis and oxidation

Total carbon content (TOC + MinC)

Total organic carbon content (TOC)

Inorganic carbon content (MinC)

Indexes (TPeak S2, R-index, I-index, HI, OI, TOC, PC, RC, PseudoS1, $T70_{HC-PYR}$, $T90_{HC-PYR}$ and more)

- **Advantages**

Total organic carbon content and inorganic carbon carbon contents obtained from one single analysis on a single soil sample have better accuracy, saving time and cost compared to chemical methods.

Normalised analytical methods : good reproducibility (Pacini et al., 2023) and interlaboratory comparability (Pacini et al., 2023)

More time-effective than chemical and fractionation methods (1 to 1:30 hour/analysis and continuous 24/7 unattended operation; no need for preliminary treatment)

No need for chemical solvents and related laboratory facilities

TIC-TOC and SOTHIS method : provides correction to convert TOC and MinC into Corg and Cmin, which are more accurate (Sebag et al., 2022; Stojanova et al, 2024, in press).

- **Limits**

Requires expensive equipment, but academic and private laboratories provide Rock-Eval analysis and interpretation

- **Operator and delivery time**

About 1:30 hour / analysis for a batch of samples (48 samples), half a day of qualified technician/engineer work and results obtained in less than 4 days (including calibration checks)

- **On going R&I**

Continuous R&I on soil analytical methods

Continuous innovation and development on Rock-Eval device, detection and interpretation

Continuous innovation on models for soil organic carbon stability and dynamics

B. **Interpretation and associated tools**

Three methods of interpretation in terms of carbon stability or residence time have been developed independently and provide complementary results: PartySOC model, I/R Diagram, Therm-C.

PartySOC model

The PartySOC model is one of the two main approaches for soil characterization using Rock-Eval® thermal analysis. This model allows estimation of SOC kinetic pool sizes (Cécillon et al., 2018, 2021).

Calculation of active (degradable within a few decades) and stable (centennially persistent carbon- PartySoc - Cécillon et al., 2021) soil organic carbon contents obtained from a machine-learning random forest regression model for topsoil samples based on the archives of a dozen of European long-term agricultural sites including a long term bare fallow treatment (LTEs) (Cécillon et al., 2021). The model was verified to be valid on agricultural sites in specific conditions (Kanari et al., 2022).

➔ Active organic carbon content (degradable within a few decades)
➔ Stable organic carbon content (centennially persistent carbon- PartySoc - Cécillon et al., 2021; Kanari et al., 2022)

PARTYSOC incorporates recent key elements of the new understanding of SOC dynamics (Dignac et al., 2017), showing that the centennially stable- SOC fraction has specific chemical and energetic characteristics that are measurable quickly (ca. 1 h per sample) and at a reasonable cost (less than USD60)

using Rock-Eval®; it is thermally stable (i.e. high activation energy), and it is depleted in hydrogen (Barré et al., 2016; Hemingway et al., 2019; Gregorich et al., 2015; Poeplau et al., 2019; Chassé et al., 2021)

Since PartySoc has been calibrated using absolute values of TOC, on soils sampled over several decades on LTEs, the results of the analyses performed with unknown soil samples can be considered as absolute results, and not as relative information and quantification, provided the analysed samples are coming from comparable pedoclimatic conditions with which PartySoc has been calibrated.

I/R diagram

The I-index and R-index parameters, provided by the Rock-Eval analysis, allow the construction of the I/R diagram. These parameters both account for the fraction of organic matter whose carbon-hydrogen bonds are cracked into smaller organic compounds during open-pyrolysis: between 200°C and 400°C for the I-index and between 400°C and 650°C for the R-index. So the I-index gives information on organic matter which is easily decomposed by temperature and refers to a "labile" carbon pool, while the R-index gives information on organic matter which is more difficult to decompose by temperature and refers to an "intermediate" organic carbon pool. Diagram I/R quantifies the progressive stabilization of the most reactive fraction of soil organic matter (hydrocarbon compounds), linked to the preferential decomposition of the most thermally labile organic compounds and the consequent enrichment of soil organic matter by more thermally stable compounds (Sebag et al. 2016).

Application of I/R diagram to a compost of increasing degree of decomposition (9 levels) showed that the I-index decreases and the R-index increases with the degree of biological decomposition (Figure 2) (Albrecht et al., 2015; Sebag et al., 2016).

Application of I/R diagram to 1300 samples representing various soil types and horizons gathered in different bioclimatic environments, pure compounds (cellulose, lignin, humic substances, lignite) and organic materials (coal, charcoal) showed that I-index and R-index can be used to study soil organic matter dynamics during pedogenesis. I-index emphasises the degree of transformation of the immature organic pool, while the R-index highlights the contribution of a more stable pool (Figure 3). A humic trend describing organic matter decomposition and the level of biodegradation during pedogenesis could be drawn in the I/R diagram (Figure 4) (Sebag et al., 2016) and proved to be consistent over various environments.

Application of I/R diagram to 46 pedologic profiles in the Swiss Alps confirmed that it allows describing a decomposition and stabilisation trend along the soil profile (Figure5) (Matteodo et al., 2018).

In a study on an incubated soil, the negative relationship between R-index and cumulative respiration observed by Domeignoz-Horta et al. (2021) made them suggest that more thermally stable carbon (higher R-index) was less biodegradable (Figure 6). Good evidence for a link between the biological and thermal stability of soil organic matter was also reported by Gregorich et al. (2015).

All these studies suggest that the thermal indicators I-index and R-index can be interpreted as proxies of biogeochemical decomposition and stabilization, respectively.

A positive difference between the measured I-index and the I-index referring to the undisturbed soil profiles (delta-I) was calculated on four fields (n = 169 points) in various tropical land-use and management contexts based on rubber, soybean and oil palm cropping systems. This delta-I was negatively linked to the POXC-SituResp® indicator, which has proven to be a relevant, sensitive and robust indicator of soil organic matter dynamics according to Thoumazeau and coworkers (2020). This corroborates that the I-

index refers to the labile carbon pool and that a positive delta-I indicates an excess of labile carbon that is potentially mineralizable.
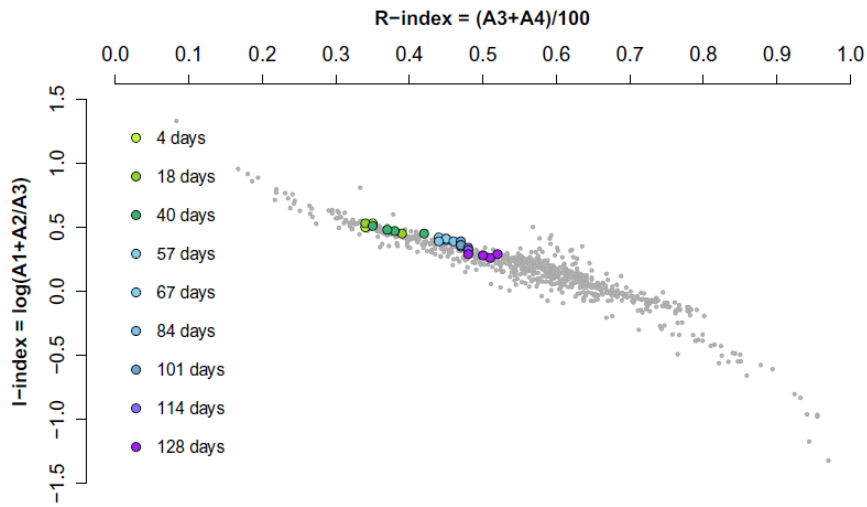

Figure 2: Evolution of compost with its level of decomposition (Sebag et al., 2016)
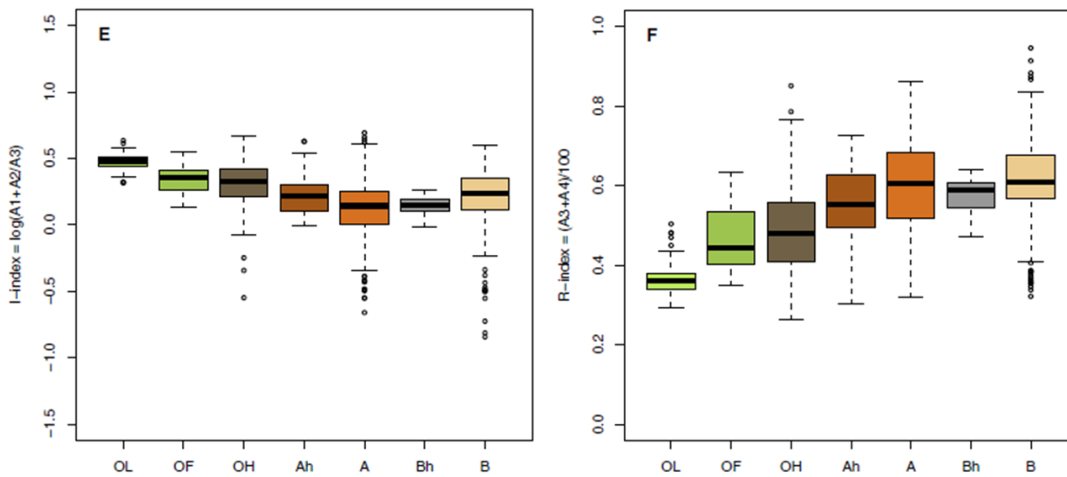

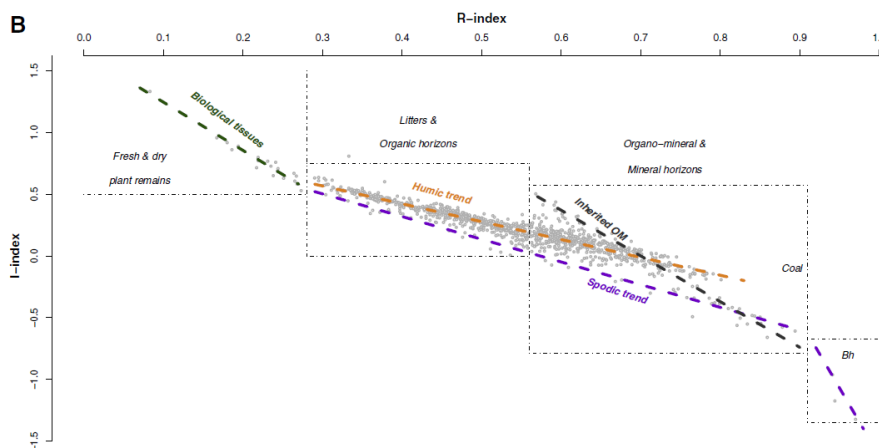Figure 3: I-index and R-index along the pedologic profile (Sebag et al., 2016)

Figure 4: The Rock-Eval I/R diagram and its interpretation
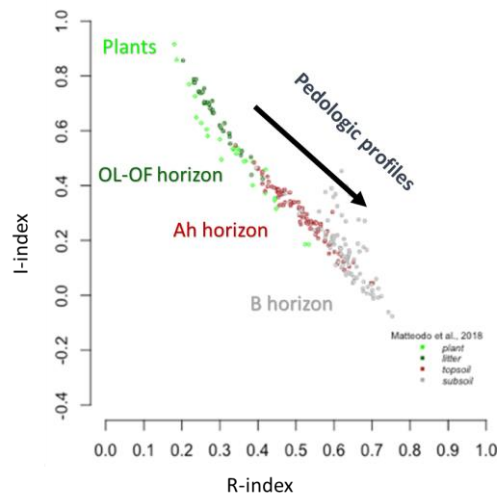(Sebag et al., 2016)

Figure 5: I/R diagram of 46 pedologic profiles in Swiss Alps (derived from Matteodo et al., 2018)
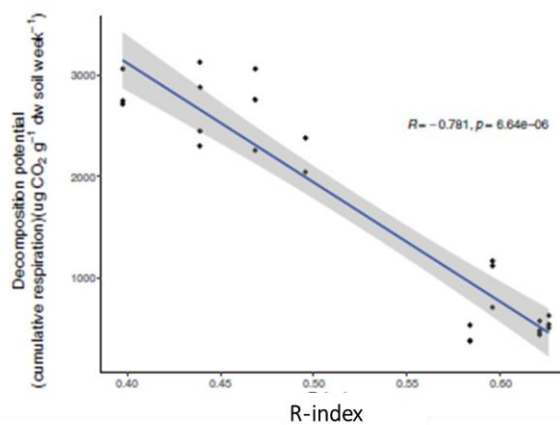

Figure 6 : Cumulative respiration vs Rock-Eval R-index on incubated soil (Domeignoz-Horta et al., 2021)

ThermC

Malou and coworkers developed an indicator of labile soil organic carbon content, based on Rock-Eval parameters : Therm-C (Malou et al., 2023). Therm-C results from organic matter cracking during its Rock-Eval pyrolysis between 200 and 520 °C. It corresponds to the carbon of hydrocarbons, CO and CO2 that are released by organic matter on this range of temperature. Malou et al. (2020) showed that thermally labile (200–400 °C) and even more resistant (400–520 °C) pyrolizable hydrocarbon pools are sensitive to mineralization" in the condition of Senegalese sandy soils.

- **Time scale sensitivity**

- **On going R&I**

C. Examples of application

PartySOC model

➜ **Impact on of SOC dynamics simulations accuracy**

In the study from Kanari et al., 2022, it is shown that the PartySOC machine-learning model built on a totally independent dataset from northwestern Europe, could be used to initialise the distribution of SOC pools of the AMG model (Clivot et al., 2019) and improve the accuracy of its simulations. The default version of AMG is currently one of the most accurate model for reproducing the observed SOC stock dynamics in diverse agricultural LTEs at the pluri-decadal scale (Martin et al., 2019). Here SOC is simply divided into two pools, the "stable SOC (CS)" that is considered inert at the timescale of the simulation and the "active SOC (CA)" that has a mean turnover time of a few decades. It has been recently highlighted (Clivot et al., 2019) that the model's default value (65% of stable SOC in croplands) can deviate and Kanari et al., 2022 study shows that initialization of the AMG model with values calculated by PartySOC based on Rock-Eval data would significantly improve AMG simulations of SOC dynamics. This last point is all the more important as it is especially true for areas with historical change in land use and soil management which represent a large yet poorly known part of arable land in France and Europe.
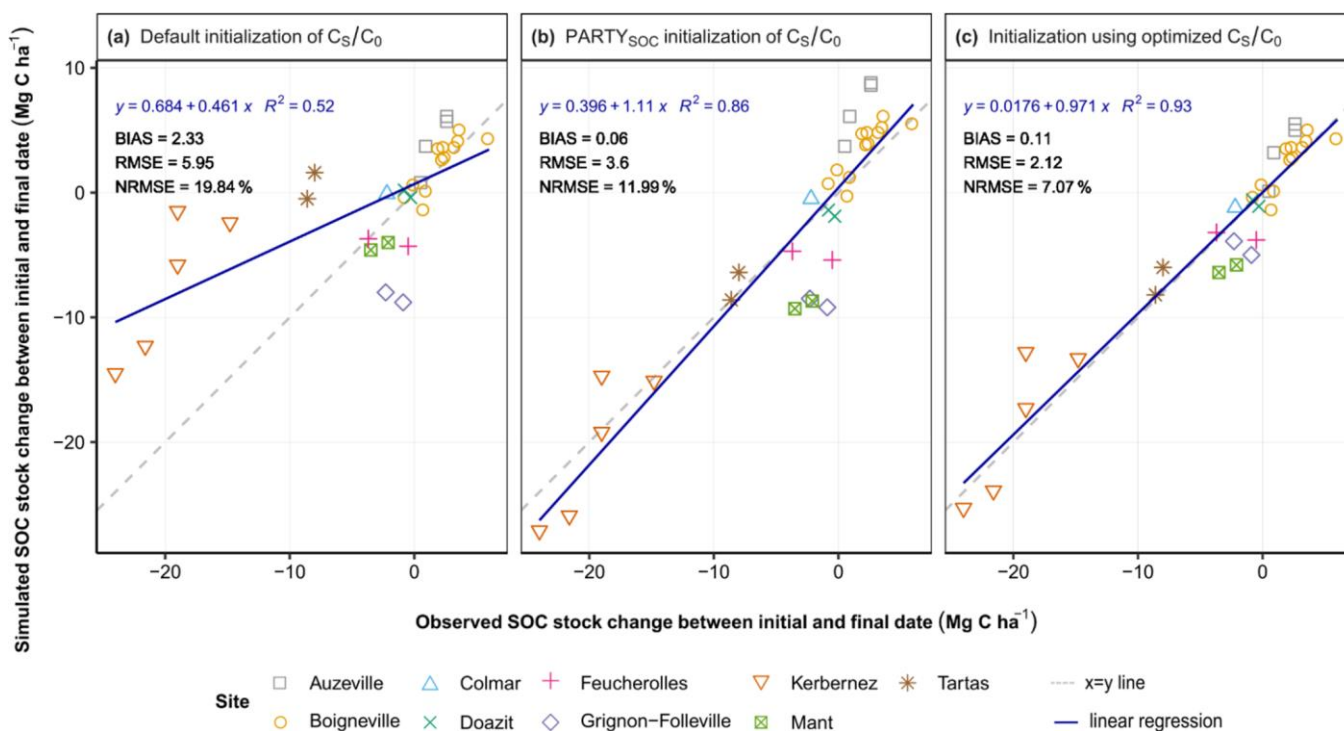

Figure 11 : Observed vs. simulated change in SOC stocks between the initial and final date of 32 treatments from nine French long-term experiments. The three panels show the performance of the AMG model for three different initialization approaches. Initial SOC kinetic pool sizes were defined using (a) the default value for cropland (C_S/C_0 = 0.65), (b) the centennially stable-SOC proportion predicted by the PARTYSOC model, and (c) the ex post AMG-optimized C_S/C_0 proportion. Statistics refer to the linear regression between x and y values (blue solid line). Points represent the values for the 32 treatments for which AMG simulations were run. (Kanari et al., 2022).

The PartySOC machine learning model has already been expanded to new soil types and climates (tropical; Cecillon et al., 2021) and is actually being validated on a wider range of pedoclimates. The good agreement between AMG-optimal stable-SOC proportion values and PARTYSOC predictions reported here suggests that most agricultural LTEs with accurate AMG simulations could be used as reference sites for the PARTYSOC model, lifting an important technical limitation to its geographical expansion (Cécillon et al., 2021) and allowing its use for other multi-compartmental SOC dynamics models such as RothC (Coleman et al., 1997).

➜ **Impact on of SOC dynamics simulations accuracy**

In their last study from 2024, delahaie et al., compare physical fractionation and its particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) with Rock-Eval thermal analysis coupled with PartySOC machine-learning model which is also able to fractionate SOC into active carbon (C_a) and stable carbon (C_s). About 2,000 topsoil samples were recovered all over France, presenting contrasting land covers and 25 pedoclimatic characteristics, and analysed.

The influence of different environmental variables on the C_s, C_a, MAOC and POC were tested. The considered environmental drivers were related to soil characteristics (particle size distribution, pH, inorganic carbon content, exchangeable cations contents (calcium, magnesium, potassium), amorphous and crystalline iron oxyhydroxides contents), climate (mean annual precipitation, mean annual temperature), and land cover. The relative importance of each of these features as estimated by the Random Forest model allowed the authors to evaluate the main drivers of the quantity of each fraction.
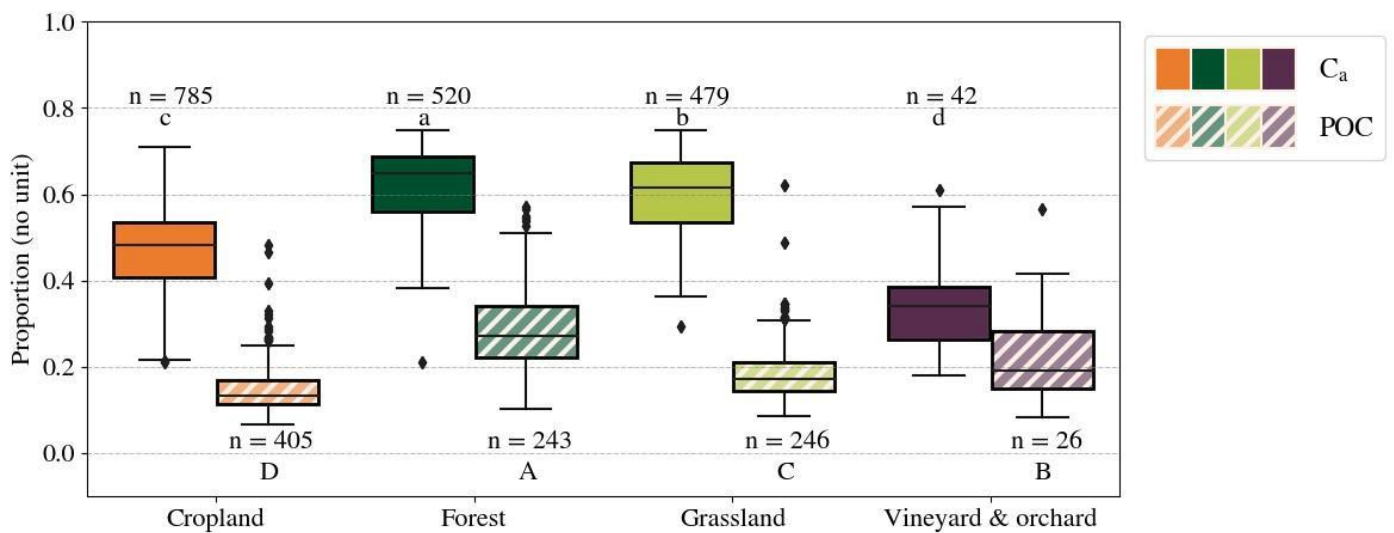

Figure 12 : Proportion of the Ca and POC fractions depending on the land cover. The black line in each box is the median, the lower and upper edges of the black rectangle are the respective first (Q1) and third (Q3) quartiles, and the lower and upper whiskers are the maximum between the minimum value or the first quartile minus 1.5 times the interquartile range (max [min; Q1-1,5×(Q3-Q1)]) and the minimum between the maximum or the third quartile plus 1.5 times the interquartile range (min [max; Q3+1,5×(Q3-Q1)]), respectively. Different letters indicate significant differences in the distribution of the values for the

land covers according to a Kruskal–Wallis test ($p < 0.05$) and a pairwise Wilcoxon rank sum test ($p < 0.05$); lowercase letters are used for Ca and uppercase for POC.
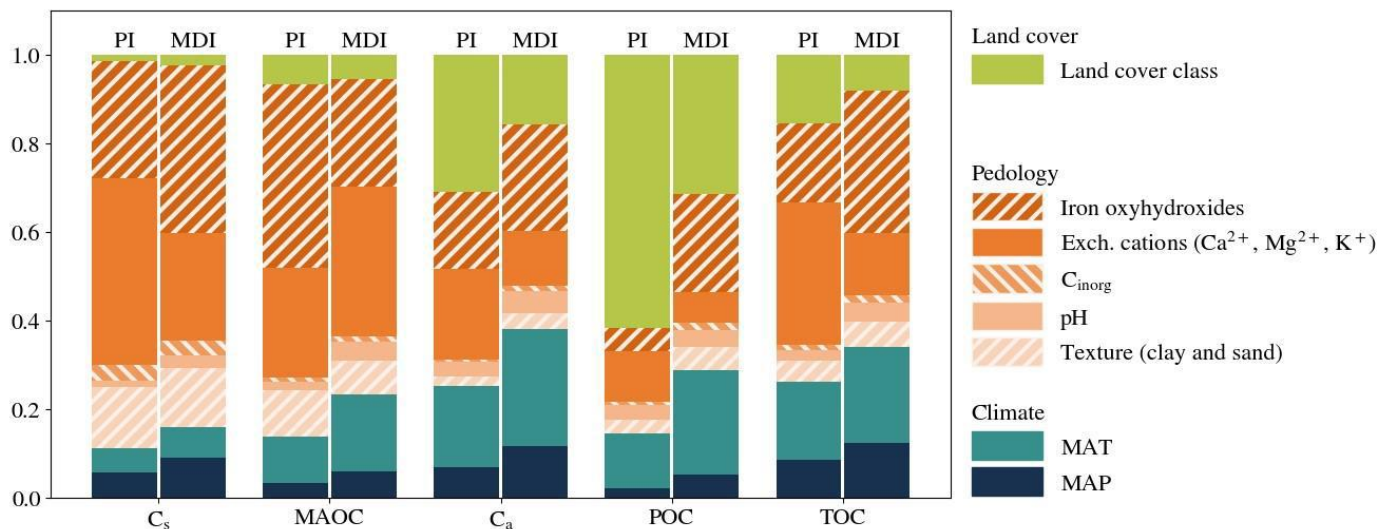

Figure XX : Importance of the different categories of soil and environmental variables (climate, pedology, and land cover) for the four fractions Cs, MAOC, Ca, and POC, and TOCea as a comparison (in g C kg-1 sample), assessed using MDI (mean decrease in impurity) and PI (permutation importance).

This study allowed the comparison of the POC/MAOC physical fractionation and thermal fractionation on an unprecedented amount of samples with an interesting diversity with respect to pedological characteristics, climatic characteristics and land covers. Results show that both the stable (Cs and MAOC) and labile (Ca and POC) fractions strongly differ in quantities. While the environmental drivers were close for the two stable fractions (respectively the two labile fractions) with a predominance of the soil characteristics (respectively the climate and land cover), they still presented differences suggesting that Cs and MAOC (respectively Ca and POC) correspond to different fractions with different biogeochemical stability. This means that both fractionation techniques display different thus complementary information.

➔ **Thermal stability of organic matter in French topsoils**

This study is an unprecedented effort to carry out widespread thermal analysis measurements on a national soil quality monitoring network. It demonstrated that Rock–Eval® may be used as a rapid and cost-effective method to assess the thermal stability and elemental stoichiometry of SOM on national soil monitoring networks. The very satisfying organic and inorganic carbon yields could make Rock–Eval® thermal analysis a very suitable tool for research work in carbonate soils or even for routine soil analysis if commercial laboratories take advantage of the method.

Some Rock–Eval® – PartySOC temperature parameters describing SOC thermal stability (T90_HC_PYR, T50_CO2_PYR, and T50_ CO2_OX) could be used as reliable proxies for SOC biogeochemical stability, whereas other parameters could not. These Rock–Eval® results on French topsoils can be used as input to the PartySOC machine learning model (Cécillon et al., 2021) to infer the size of the centennially stable SOC fraction. They can also be compared to other proxies for SOC biogeochemical stability, such as SOM physical fractionation results. In the medium term, it will be interesting to test whether this analytical information can be used to improve the accuracy of SOC stock evolution simulations at the scale of a

national soil monitoring network, as was observed for the Andriulo–Mary–Guérif (AMG) model of SOC dynamics in several French long-term agronomic experiments (Kanari et al., 2022).

(a) Rock-Eval® Hydrogen Index (SOM stoichiometry indicator; g HC kg⁻¹ SOC)

| 67 | 150 | 174 | 206 | 247 | 287 | 515 | missing |

(b) Rock-Eval® T50_CO2_PYR (SOM thermal stability indicator; °C)

| 347 | 370 | 376 | 383 | 389 | 397 | 497 | missing |

(c) Map of the land covers

- Gardens [3]
- Croplands [785]
- Forests [526]
- Grasslands [481]
- Vineyards and orchards [42]
- Environments with little human disturbance [40]
- Wastelands [14]
- Not sampled

(d) French regions for interpretation

Normandy · Paris Basin · Brittany · Massif Central · Alps · Landes · Pyrenees · Corsica
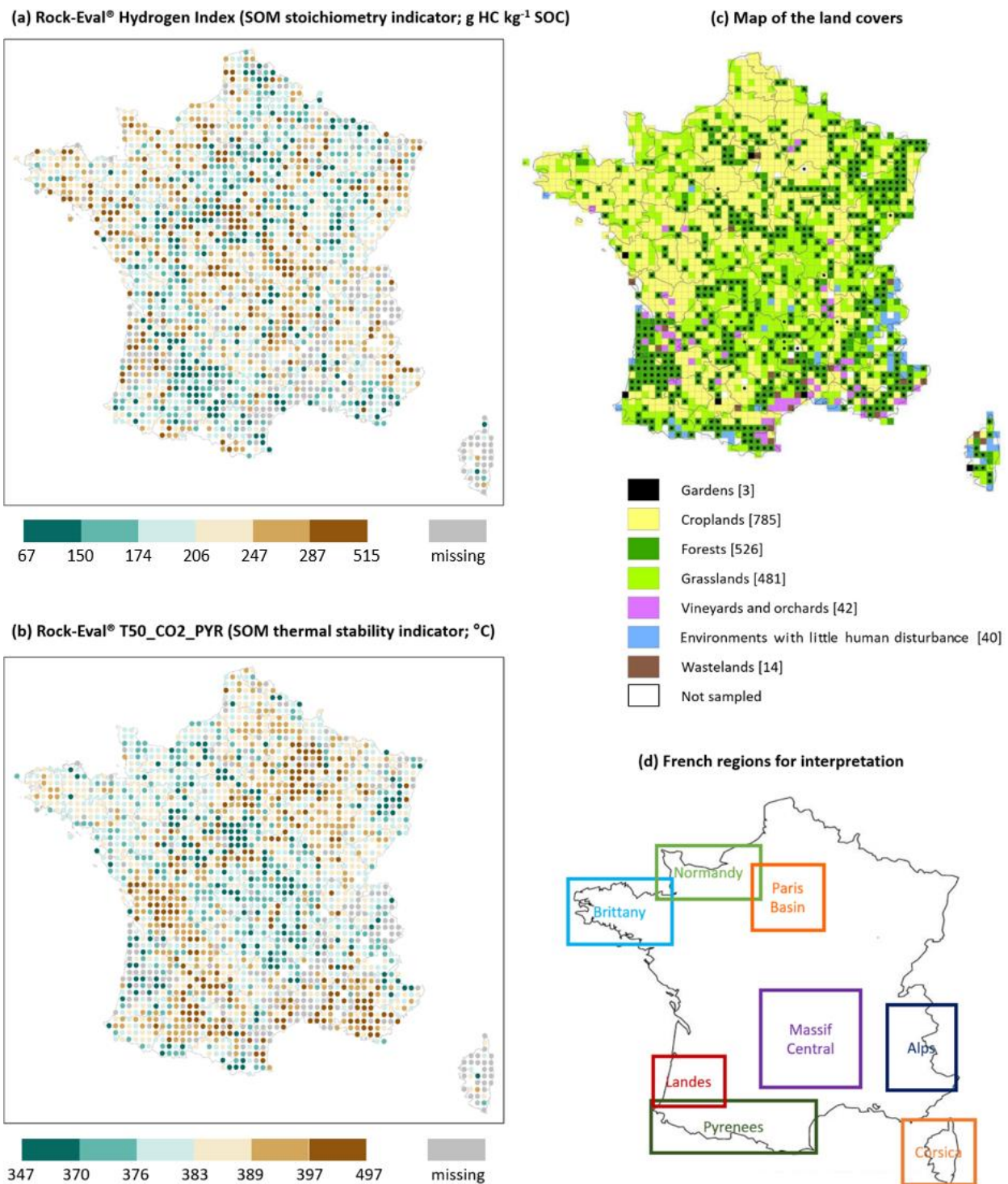
Figure XX : Point maps of two Rock-Eval® parameters – (a) hydrogen index values and (b) T50_CO2_PYR values – on the French mainland territory for the RMQS topsoil (0–30 cm) samples; (c) a map of the land cover at each sampling site (the numbers in square brackets correspond to the number

of sites for each land cover in our final dataset; n D 1891), modified from Jolivet (2011); and (d) a map of the main regions used for the interpretation.

This study ultimately highlights that the thermal stability defined according to different Rock–Eval® parameters varies in French topsoils. SOC biogeochemical stability is on average higher in croplands and vineyards than in forest or grassland soils (Poeplau and Don, 2013). Indeed, fresh organic carbon inputs to soil are usually higher in forest and grassland compared with croplands, where human exportation of biomass is higher (Murty et al., 2002). Thermal stability, as assessed using T90_HC_PYR, T50_ CO2_ PYR and T50_CO2_OX, was the highest in vineyards and orchards and in croplands compared with forest and grassland soils (Fig. 2).

These results suggest that, over-all, SOC thermal stability, as assessed using these Rock– Eval® parameters, is related to SOC biogeochemical stability.

I/R diagram

➜ **Stability and decomposability of organic amendments : composts, vermicomposts, digestates**
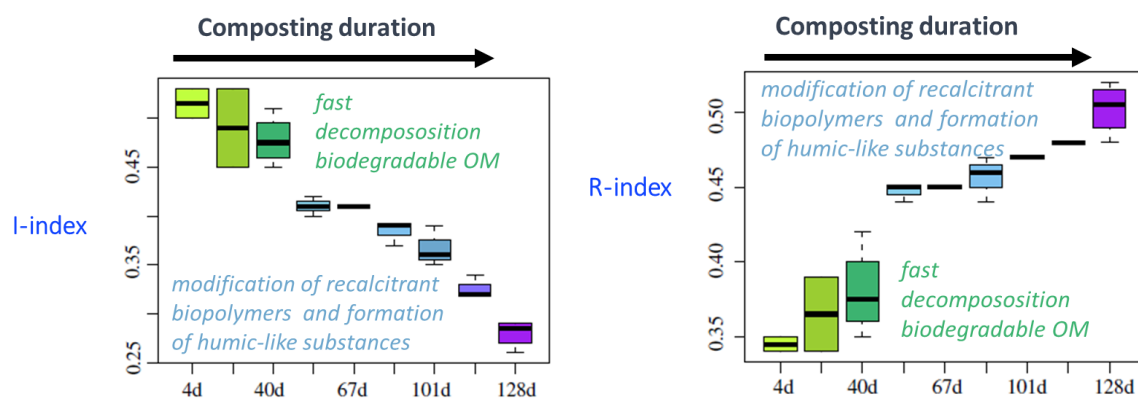

Figure 7: Impact of composting duration on compost stability and decomposability (Albrecht et al., 2015)
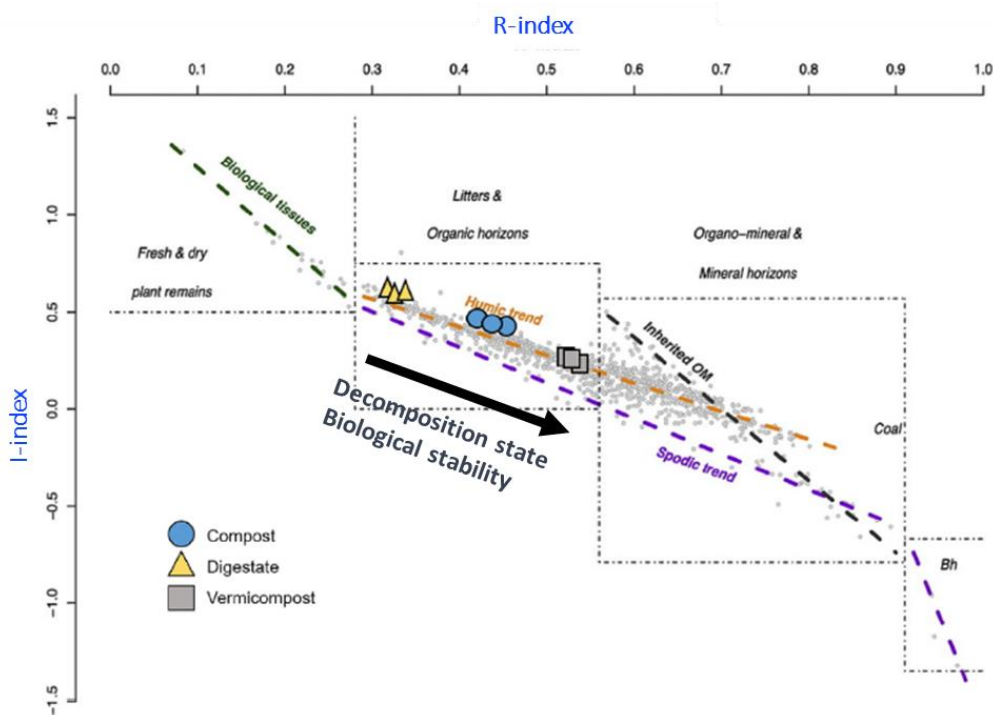
Figure 8: Stability and decomposition state of vermicompost, compost and digestate (Ducasse et al., 2023)

➔ **Impact of amendments and cropping on soil organic matter**

The Rock–Eval® method was proved to be sufficiently accurate and sensitive to discriminate in the short term the effects of amendments and cultivation on soil organic matter properties and organic carbon stability. After a 7-week supply of organic amendment (digestate, or biochar, or microalgae, etc.) and cultivation (sorgho or rapeseed), it was shown that the variations of the I–index and the R–index as well as the soil organic content were quantifiable (Lamoureux-Var et al., 2022). This study found that digestate and microalgae treatments increased organic carbon content in soil and reduced soil organic matter stability (Figure 9).
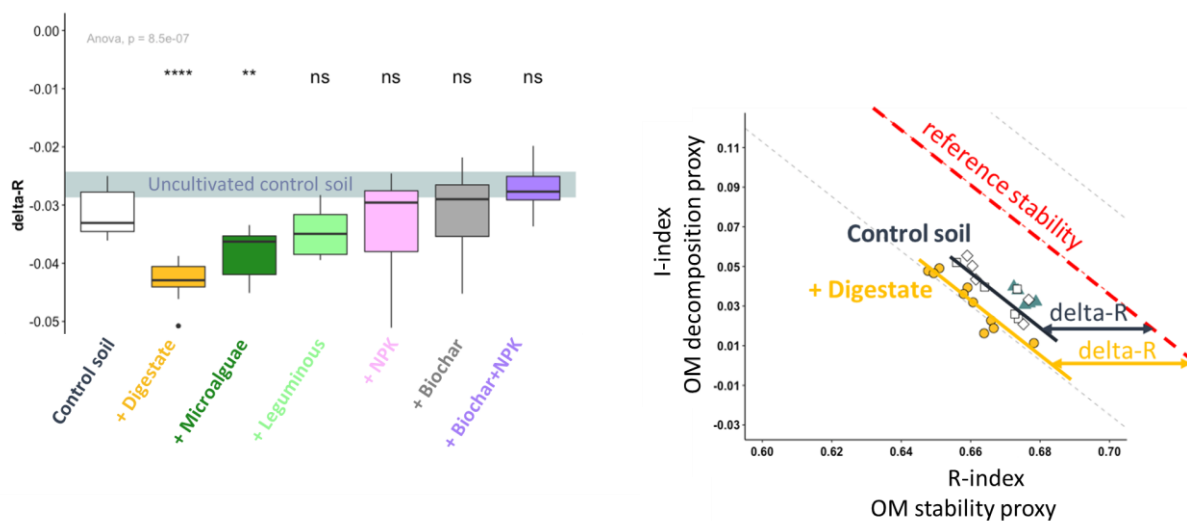
Figure 9 : Evolution of soil organic matter stability after amendment and 7-week cultivation of sorgho or rapeseed (Lamoureux-Var et al., 2022).

➔ **Soil organic matter stability of constructed soils**

Constructed soils were formulated by mixing aggregate washing sludge (AWS) obtained from a quarry with agricultural soil. The Rock Rock-Eval® results suggest that biogeochemical stability of constructed soil organic matter increases with addition of AWS (Figure 10) (Coussy et al., 2024).
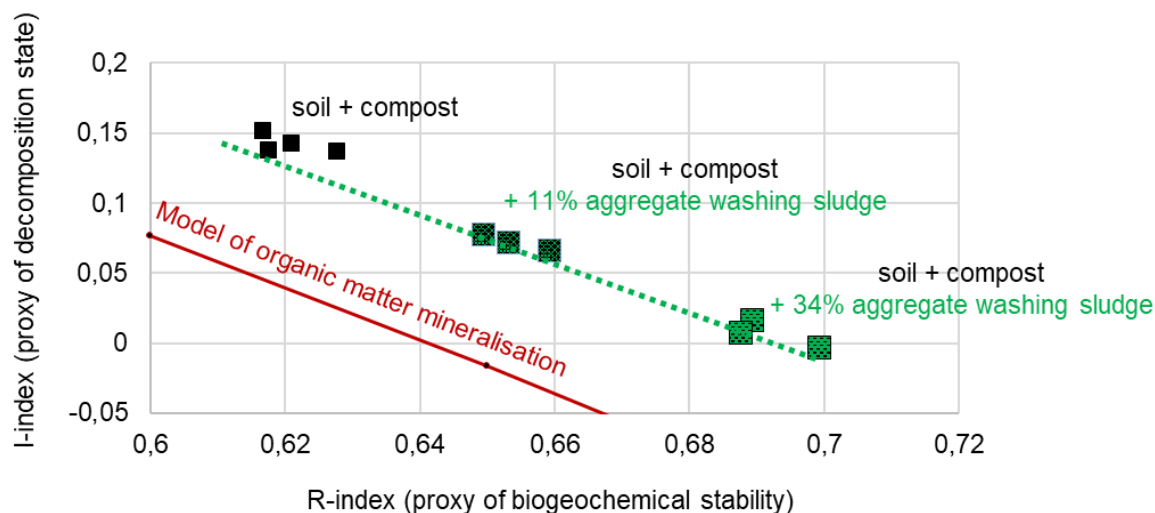

Figure 10: Stability of organic matter within a constructed soil after 9 months of lettuce cultivation (Coussy et al., 2024)

➔ **Impact of land-use and management on soil organic matter**

Agricultural practices : Thoumazeau et al., 2020; Malou et al., 2020, 2023

Forests : Sebag et al., 2022; Sun et al., 2023

➔ **Impact of earthworms on soil organic matter stability**

Schomburg et al., 2018, Le Mer et al., 2020

Therm-C

➔ **Impact of land-use and management on soil organic matter**

Agricultural practices : Malou et al., 2023

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Tokarski D., 2020

III. Chemical methods

Chemical methods are based on the reaction of a chemical agent (normally an oxidant) with soil organic matter (and in particular soil organic carbon), which breaks down the organic carbon into smaller, simpler molecules. If the chemical agent breaks down organic carbon completely, carbon dioxide is produced, which can be quantified. Alternatively, a drop in the concentration of the chemical agent can be measured, or the increase in concentration of the chemical product the original agent is converted to.

Various chemical methods exist for measuring soil organic carbon (SOC) that are based on oxidation with acidic dichromate solutions (e.g. Walkley and Black 1934; Heanes, 1984) however these methods are unable to fractionate SOC and cannot be used to estimate biogeochemical stability of SOC. Furthermore, since hazardous chemicals have to be used applying these methods, they have been replaced by combustion methods (e.g. EN 15936).

Other chemical methods exist that measure what has been termed 'labile' soil carbon. Various fractions of labile soil carbon have been conceived , and are named according to the procedure used to determine them. A sub-set of these methods determine soluble forms of soil organic carbon. Dissolved organic carbon (DOC) is determined by extracting field-moist soils with deionised water, or salt solutions such as 2 M KCl or 0.5 M K2SO4. Hot water extractable carbon (HWEC) as the name suggests determines the fraction of labile carbon soluble in hot or boiling water. However, none of these labile methods are useful for estimating the biogeochemical stability of soil organic carbon.

The chemical method most widely use in determining labile C, and for which results can be interpreted in terms of soil organic carbon stability, is based on the oxidation of soil using dilute solutions of potassium permanganate.

Permanganate oxidisable carbon method

The permanganate oxidisable carbon method (POxC) had its genesis with the work of Loginow et al. (1987). Since then, this method has evolved into a number of variants, many of which are in use world-wide today. Currently, the variant in widest use is the field-friendly version developed by Weil et al. (2003), and its laboratory equivalent as described by Culman (2017) and Tatzber et al. (2015). It is this latter version that is described   in   this   standard.

A. **Analysis**

- **Principle**

Duplicate 2.5 g samples of air-dried soil are weighed into 50 mL reagent tubes and extracted on a reciprocal shaker for 2 min with a dilute (20 mM) standardised solution of potassium permanganate ($KMnO_4$) in $CaCl_2$ solution, then allowed to stand for 10 min in the dark.

An aliquot of the supernatant is withdrawn from the tube and diluted, 20x to 100x according to the protocol, with de-ionised water and the concentration of permanganate is measured at 550 nm on a UV/vis spectrophotometer.

The difference in permanganate concentration between the original 'blank' solution and the solution that has reacted with the soil is used to determine the amount of permanganate consumed in oxidising the soil. The stoichiometry of the following equation is then used to calculate amount of permanganate oxidizable carbon (POxC):

$$4MnO_4^- + 3CH_2O \rightarrow 4MnO_2 + 3CO_2 + H_2O + 4OH^-$$

- **Sample storage, preparation and quantity**

Duplicate 2.5 g of air-dried soils

- **Implementation**

(Portable) colorimeter with 550 nm filter, or UV/vis spectrophotometer (double-beam preferable)

- **Parameters output**

The absorbance reading output by the UV/vis spectrometer is used to calculate a permanganate concentration from a calibration curve. The amount of permanganate consumed is used to calculate the permanganate oxidizable carbon (POxC) in units of mg C/kg soil.

- **Advantages**

Requires relatively inexpensive and readily available equipment (e.g. centrifuge tubes, balances, pipettes, portable colorimeters or small UV/vis spectrophotometers)

Chemical used are dilute and relatively safe

Procedure is rapid and low cost

- **Limits**

Short extraction time means that only small batch sizes can be run to maintain consistency and accuracy in extraction time

Lack of standardisation of methodology internationally. Researchers run variants of the method with different; extraction times, reagent concentrations, sample mass and sample preparation. Results are materially affected by such differences, meaning that international data comparison and interpretation is difficult. [BA1]

- **Operator and delivery time**

- **On going R&I**

Continuing research on effects of modifying/varying method conditions/parameters:

→ Sample mass (Gruver *et al*. 2015, Pullemann *et al*. 2020, Wade *et al*. 2021)

→ Sample preparation, e.g. hand sieving vs grinding (Hurisso *et al*. 2018), air–drying vs oven drying at 45°C or 65°C (Gasch *et al*. 2020)

→ Permanganate concentration (Dell 2009, Jones *et al*. 2023)

B. **Interpretation and associated tools**

Carbon management index (CMI) (developed by Blair *et al*. 1995) which is used to monitor changes in soil C pool size and turnover rate changes in total and labile C as a result of agricultural management practices. *Based on 333 mM permanganate concentration version of the method*.

SOC/C_{ox} ratio to characterize the part of "labile" C in the SOC pool (Tatzber et al.. 2015)

Used widely as a metric for Soil Health

- Time   scale   sensitivity

- On   going   R&I

C. **Examples of application**

D. **References**

Blair *et al*. 1995

Culman (2017)

Dell 2009

Gasch *et al*. 2020

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Wade *et al.* 2021

Weil et al. 2003

IV. Biological methods

These methods are based on the transformation of soil organic carbon by living organisms and more particularly microorganisms or by extracellular enzymatic reactions. Therefore, they can only access the rapidly degradable carbon and considering the short incubation times (generally 1h to 24h), only the most labile carbon sources are assessed. Therefore these methods should be considered as complementary to those assessing total organic carbon (ISO 10694) and the biogeochemically stable fraction. However, carbon mineralization was shown to be correlated to other organic carbon measurement methods (Hurisso et al., 2016).

Basal soil respiration (BSR)

CO_2 emission or oxygen consumption resulting from heterotrophic respiration, i.e. the mineralization of soil organic matter is assessed. It can be measured either directly on the field (in situ) or in the laboratory. Both methods have their advantages and disadvantages and will be described.

❖ **In situ BSR measurement using SituResp**

A. **Analysis**

- **Principle**

Estimation of the CO_2 emissions released during a 24 h incubation period (Thoumazeau et al., 2017). The principle is the same as for the MicroResp method (4.1.2.)

- **Sample storage, preparation and quantity** :

Microbial and enzymatic activity should be carried out as soon as possible after sample collection. If longer storage (> 7 days) is necessary, samples can be stored at -20°C according to ISO/FDIS 18400-206. Depending on the soil type and origin, air-drying can also be an adapted method for basal respiration measurements (Fromin et al., 2024).

40 g of fresh, coarsely sieved (5 mm) soil is needed for the incubation experiment.

- **Implementation**

40 g soil are incubated for 24h in airtight 267 ml jars, together with a 4.5 ml macro-cuvette containing a pH-sensitive colour gel. The gel is prepared according to Campbell et al., (2003). It contains 2.5 mM

NaHCO3, 150 mM KCl, 12.5 ppm (wt/wt) Cresol Red and 1 % Noble agar. Before and after the incubation, the absorbance of the macro-cuvette is measured at 570 nm using a portable spectrophotometer.

- **Parameters output**

The absorbance difference is converted to % CO2 using a calibration curve displaying an exponential relationship between the ΔAbs and % CO_2 :

$$y \, (\% \, CO_2) = \alpha.exp^{(\beta. \, \Delta Abs)}$$

α, β : constants of the calibration curve

- **Advantages**

Assesses in-field mineralization of accessible organic carbon sources

Avoids biases by transport and sieving and subsequently impacts on microbiological activity due to temperature or humidity changes of the soil samples

Robust, time- and cost-effective method

- **Limits**

Seasonal changes can impact the result, as the experiment is conducted under the weather conditions on site.

The estimation of the amount of available and readily degradable organic matter can be biased by edaphic factors (pH, soil structure, substrate accessibility, water content…). The presence of pollutants inhibiting microbial respiration could lead to underestimations.

Extrapolation of the BSR data to soil emissions and consequently amounts of labile soil organic carbon depends on the assumptions, such as soil density and horizon thickness

- **Operator and delivery time**

Results can be obtained in 24h. The cost for necessary devices is low.

- **On going R&I**

Studies of integrating SituResp with other methods are have been conducted (Thoumazeau et al., 2020; Braumann and Thoumazeau, 2020)

B. Interpretation and associated tools

If total carbon has been assessed, the results can be expressed as the mineralizable fraction of total carbon.

- **Time scale sensitivity**

BSR rapidly responds to changes in the availability of easily degradable organic carbon.

- **On going R&I**

C. Examples of application

➜ Study of soil conservation practice on soil carbon dynamics : Koun et al., 2023

➜ Establishment of a soil-indicator based on POXC and BSR : Thoumazeau et al., 2020

➜ Biofunctool, a tool to assess soil health : Brauman and Thoumazeau, 2020

D. References

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❖ Laboratory respiration test using Microresp

A. Analysis

- **Principle**

Estimation of the CO2 emissions released during a 6h incubation period in a microtiter plate (Campbell et al., 2003)

- **Sample storage, preparation and quantity** :

Microbial and enzymatic activity should be carried out as soon as possible after sample collection. If longer storage (> 7 days) is necessary, samples can be stored at -20°C according to ISO/FDIS 18400-206. Depending on the soil type and origin, air-drying can also be an adapted method for basal respiration measurements (Fromin et al., 2024).

Soil is sieved (mesh size <2 mm), the water holding capacity adjusted (60-80%) and small amounts (<1 g) of are dispensed in the wells of a microtiter plate.

- **Implementation**

About 0,3 - 0,5 g of soil are loaded into the wells of a 96-well microtiter plate. The precise weight is recorded. A loading device made of a 300 µl microtiter plate from which the bottom has been replaced by a Perspex sliding base (Campbell et al., 2003) should be used. The plate is subsequently sealed with a gasket and a detection plate (Fig. X). The latter contains a CO_2 detection gel in each well (2.5 mM $NaHCO_3$, 150 mM KCl, 12.5 ppm (wt/wt) Cresol Red and 1 % Noble agar). The detection plates are conserved in a wet paper towel and with soda lime to prevent desiccation and reaction with atmospheric CO_2. For measurement of soil respiration, the plates are then incubated at 25°C for 6h. Before and after incubation, the absorbance is measured at 590 nm using a microtiter plate reader.
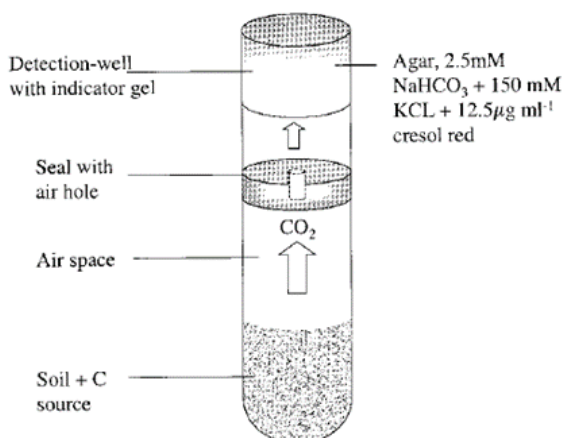

Fig. X : Schematic presentation of a deep well of the Microresp device connected to the detection well (from Campbell et al., 2003)

- **Parameters output**

The absorbance difference is converted to % CO_2 using a calibration curve that had been established by incubation of gel-containing wells in the presence of gas mixtures containing well determined CO_2 concentrations, prepared by a gas mix device.

- **Advantages compared to other methods**

Rapid and cost-effective method to measure soil respiration

Standardized conditions (temperature, humidity) limit bottlenecks restraining microbial activity, such as nutrient availability or water content, and are therefore better suited for sample comparisons than field methods.

- **Limits**

Small sample volumes require thorough mixing and sieving.

As for the SituResp, the MicroResp method delivers only an estimation of CO_2 production. 90 replicates are recommended to get reliable results.

Edaphic characteristics have a lower impact on the outcome of results in laboratory methods, but as for all respiration-based methods, readily convertible organic matter can be underestimated due to the presence of toxic compounds or pollutants.

Extrapolation of the BSR data to soil emissions and consequently amounts of labile soil organic carbon depends on the assumptions, such as soil density and horizon thickness.

- **Operator and delivery time**

Results can be obtained in 48h, including soils sampling, transport and soil preparation. The cost for necessary devices is low.

- **On going R&I**

The MicroResp was not previously used for soil carbon stability studies, but rather soil health or toxicity assessments (Onica et al., 2018). However, as for the SituResp method, we propose that it can be used for this application, possibly in combination with other methods.

B. Interpretation and associated tools

If total carbon has been assessed, the results can be expressed as the mineralizable fraction of total carbon.

- **Time scale sensitivity**

BSR rapidly responds to changes in the availability of easily degradable organic carbon.

- **On going R&I**

C. Examples of application

Examples of applications of the MicroResp for other purposes than carbon stability measurements can be found in Onica et al., 2018.

D. References

Campbell C.D., S.J. Chapman, C.M. Cameron, M.S. Davidson and J.M. Potts 2003, A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl. Environ. Microbiol. 69(6):3593–9. doi: 10.1128/AEM.69.6.3593–3599.2003

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❖ **Microbial soil respiration according to method ISO 16072**

This document describes methods to measure microbial respiration in aerobic soils, by either determining the oxygen consumption or CO_2 production. The document gives indications about which method to

choose in which case. For the purpose of determining organic carbon stability of a given soil, only basal respiration is here of relevance (in contrast to induced respiration).

- **Sample storage, preparation and quantity**

Microbial and enzymatic activity should be carried out as soon as possible after sample collection. If longer storage (> 7 days) is necessary, samples can be stored at -20°C according to ISO/FDIS 18400-206. Depending on the soil type and origin, air-drying can also be an adapted method for basal respiration measurements (Fromin et al., 2024).

- **Implementation**

- **Parameters output**

- **Advantages**

The advantages are the same as for the MicroResp method, but more expensive devices are needed in some cases (e.g. gas chromatograph, infrared spectrometer). However, CO_2 and O_2 are quantified with higher precision and a lower number of replicates are needed (three replicates can be sufficient)

- **Limits**

A higher amount of soil is needed than for the miniaturized method (10-200 g per replicate). Set-up and gas analyses are also more tedious and time-consuming.

- **Operator and delivery time**

2-3 days

Depending on the method chosen, cost-level is low to medium

- **On going R&I**

Enzymatic activities

CM, colorimetric method, ISO 20130
FM, fluorogenic method, ISO/TS 22939

A. Analysis

- **Principle**

Measurement of carbohydrate hydrolysis capacity of soil samples (mainly issued from microbial activity) in microwell plates

- **Sample storage, preparation and quantity**

CM: 4 g of sieved soil (5 mm) suspended in 25 mL water, stirred for 10 min, and 150 µL suspension added to each micro-well with reactants, incubation of 1 or 2 hours at 37°C, absorbance reading

FM: 4 g of sieved soil (5 mm) suspended in 120 mL buffer, homogenized for 3 min, dilution, and 200 µL diluted suspension added to each micro-well with reactants, incubation for 3 hours at 30°C, fluorescence reading

- **Implementation**

Incubation of soil suspensions under controlled temperature conditions

- **Parameters output**

Amounts of p-nitrophenol (CM) or 4-methylumbelliferone (FM) formed, quantified by comparison to a standard curve.

- **Advantages**

CM: sensitive (validated: international ring-test), simple, cheap, can be automated (LoD around 0.01 mU/g soil)

FM: very sensitive

Both: experimental conditions are well controlled

Each method allows the measurement of specific patterns of activities, which are complementary. Combining the two methods provides information on a large set of enzymes considered.

- **Limits**

FM: more complex to perform and more expensive than CM

FM: presence of inhibitors or interfering compounds in soils can lead to quenching and irrelevant measurement of activities

- **Operator and delivery time**

- **On going R & I**

B. **Interpretation and associated tools**

➔ Enzymatic activities involved in the carbon cycle
➔ CM: method   used   by   private   laboratories,   numerous   data   available

- **Time scale sensitivity**

- **On going R&I**

C. Examples of application

Enzymatic activities are used to assess the impact of chemical contaminants or agricultural practices on soil functioning, including the carbon cycle (Sun et al., 2023, Jacquiod et al., 2024).

D. References

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Summary of methods

The following two synthetic tables compare the methods described in this document, including the main information.

→ SEE ATTACHED EXCEL TABLE

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