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To cite this version:

Zheng Sun, Katell Quénéa, Frédéric Delarue, Christelle Anquetil, Herman Ravelojaona, et al.. Combining Rock-Eval® thermal analysis and thermochemolysis to evaluate the influence of forest management on soil organic matter quality and stability. Organic Geochemistry, 2023, 179, pp.104585. 10.1016/j.orggeochem.2023.104585 . hal-04047790

HAL Id: hal-04047790
https://hal.sorbonne-universite.fr/hal-04047790v1

Submitted on 27 Mar 2023

Combining Rock-Eval® thermal analysis and thermochemolysis to evaluate the influence of forest management on soil organic matter quality and stability

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Keywords:

Rock-Eval® thermal analysis; Py-GC/MS analysis; Forest management; Organic matter stability.

Abstract

The effect of forest management practices on carbon quality is poorly documented. To assess changes in the quality and stability of soil organic carbon (SOC) of a temperate forest upon human activities, we investigated soil from forests (i) developed following natural regeneration after clearcutting 20 and 40 years ago, (ii) developed following afforestation on an abandoned crop area 40 years ago and (iii) in an area where regular clear-cut (with wood residues input) was conducted 40 years ago. Topsoil and subsoil layers were collected (0-20 cm and 50-80 cm). Soil organic matter (OM) was characterized by elemental analysis (total carbon and total nitrogen), thermal analysis (Rock-Eval®) and thermochemolysis (i.e., Py-GC/MS in the presence of tetramethylammonium hydroxide (TMAH)). In addition, a size fractionation to separate the labile coarse fraction (50-2000 µm) from the fine fraction (<50 µm) was performed. These fractions were analyzed by thermal analysis.

Despite no measurable differences in carbon and nitrogen contents, the characterization of the OM by thermal analysis, and the relative quantification of OM compounds revealed differences in the composition in OM for the topsoil layers. The thermal analysis clearly distinguished sites with inputs of woody residues (higher HI) with a higher relative contribution of lignin and cutin/suberin compounds. However, the OM thermal stability seems mainly controlled by the organo-mineral interactions rather than chemical composition. Combination of Rock-Eval® thermal analysis and Py-GC/MS suggests that thermal stability cannot be used as an indicator of stability in specific contexts where pedogenetic processes are deeply modified by regular and extensive anthropogenic inputs of woody residues.

1. Introduction

Forest ecosystems cover about 4 billion hectares and account for 31% of the global land area (Keenan et al., 2015; Bastin et al., 2017). They store around 861 Pg C, 44% in soil and 5% in litter) (Janzen, 2004; Heimann and Reichstein, 2008; Pan et al., 2011) and are considered crucial in global change mitigation as a potential carbon sink (Janssens Ivan et al., 2003). However, human activities – such as deforestation – constitute a threat to forest ecosystems, and lead to large loss of organic carbon stored into the soils (Guo and Gifford, 2002; Stockmann et al., 2013; Lal, 2016; Mayer et al., 2020). Depending on these activities, forest soils could represent either a C source or a C sink for atmospheric CO_2 (Jandl et al., 2007; Adamczyk et al., 2019; Mayer et al., 2020). For example, forest management practices can positively or negatively impact soil organic carbon (SOC).

Conservation of old forest and afforestation are some examples of practices able to modify SOC stocks (Mayer et al., 2020). For example, Ameray et al. (2021) have reported different management strategies to maintain or increase carbon in forest soil, from conservation of old forest to afforestation. Clear-cut harvesting is another common management of forest. Depending on intensity, it can influence SOC stocks for up to 100 years (Jandl et al., 2007). Clear-cut induces a SOC stocks decrease mainly through the drastic decrease in litter input and faster SOC decomposition due to higher insolation and warmer soil temperature (Kulmala et al., 2014; Mayer et al., 2017) and compaction effect on microbiome and soil function (Hartmann et al., 2014). In a meta-analysis study with a database of 945 responses to harvest from 112 publications, James and Harrison (2016) evidenced different magnitudes in C loss depending on the considered soil depth. Conversely, reforestation and afforestation, also in areas relatively resilient to clear-cut, SOC recover over one to seven decades depending on the soil taxonomic order (Poeplau et al., 2011; Bárcena et al., 2014; James and Harrison, 2016). Although SOC concentration has been measured to reach similar or even higher level than before the imposed management practice, few studies have investigated the chemical composition and the biogeochemical stability, i.e., bioavailability or bioaccessibility to decomposition by microorganisms as well as chemical stability, of this SOC (Angst et al., 2016; Mustafa et al., 2022). Indeed, SOC before clear-cut and after recovery could have different stabilities, and finally, the recovery at decennial to centennial time scale would not balance the carbon losts due to reduced residence time (Hamburg et al., 2019). The changes in residence time can be interpreted as a consequence of SOC stabilization mechanisms driven by changes in SOC chemical composition and through greater reactivity with minerals (organo-mineral adsorption) and physical protection by incorporation into aggregates (Lehmann and Kleber, 2015). Thus, evaluation of the quality and stability of SOC is required to integrate the intrinsic stability of OM (i.e., the energy input to break down organic molecules) and the stabilization of SOM through interactions with soil minerals (i.e., the energy required to break the bonds between SOM and mineral particles).

A common approach to study the quality and stability of SOC is based on a relative estimation of pools with different stability and their respective responses to land use (Sahoo et al., 2019; Liu et al., 2020). However, the quantification of the C from these different pools is challenging and different methods are used. A method widely applied is soil physical fractionation (von Lützow et al., 2007) which provides insights into SOM bioaccessibility (Rovira et al., 2010; Moni et al., 2012). The coarse fraction mainly contains particulate organic matter and plant residues, with fast turnover times, whereas organic matter from fine fraction is intimately associated to mineral phases and exhibit lower turnover. A less time consuming method, that could be applied is thermal analysis. Indeed, more recently, thermal analysis of forest litter or topsoil samples revealed that OC with higher stability will combust at higher temperatures and provide less energy than labile OM (Plante et al., 2009; Barré et al., 2016). As underlined by extensive litterature (Disnar et al., 2003; Plante et al., 2011; Derenne and Quénéa, 2015; Sebag et al., 2016), thermal analyses appear promising in the evaluation of SOC stability. Several thermal analysis approaches have been used to estimate the stability of carbon pools in soils from various environments (Disnar et al., 2003; Gregorich et al., 2015; Sebag et al., 2016; Cécillon et al., 2018, 2021; Thoumazeau et al., 2020). Among them, Rock-Eval® thermal analysis, has been used to characterize soil organic matter (SOM) (Disnar et al., 2003) and its dynamics (Sebag et al., 2016). Several Rock-Eval® parameters were also defined to reflect the SOM stability (Saenger et al., 2013; Soucémarianadin et al., 2018). Hence, Saenger et al. (2015), Soucémarianadin et al. (2018) and Sebag et al. (2022) studied different soil size fractions and compared results to assess the efficiency of Rock-Eval® to evaluate the thermal stability of SOM. Whatever the soil studied, the thermal stability was lower for coarse fractions. Forest managements influences SOC however generally only its quantitative impact is considered. In this study, we compared the effect of afforestation, natural reforestation and regular clear-cut on SOC concentration and also their effect on organic matter quality thermochemolysis analyses and on stability by Rock Eval® analysis.

2. Materials and methods

2.1 Study site

Soil samples were collected from different sites located in Ferrières forest (3000 ha) (France, (48°48'36.9"N 2°39'57.7"E)) in January 2021. The dominant species are deciduous trees, oak (*Quercus spp*) (>80%) and chestnut (*Castanea sp*).

In this study area, the soils developed on loess plateau, on carbonate rock, and are acidic silty loam. The soil is classified as Alfisols by USDA soil taxonomy. Five replicates were sampled in five different sites in the forest selected to reflect differences in human activities (Table 1):

- A control site (control) where tree growth was unmanaged for more than 80 years; the tree density is around 1000 per ha.
- Two sites where the forest has been subjected to clearcut and harvest prior to the natural regeneration of the forest started 20 (C20) and 40 (C40) years ago, respectively. The tree density is around 10000 per ha.
- A former cropland site which was afforested 40 years ago (A40). The tree density is around 2500 per ha.
- The last site (B40) was regularly cleared for 40 years. Every three years, the trees were cut, crushed and wood residues left on the topsoil, thereafter trees regrowth naturally. The last clear-cut was in autumn 2020, so there were no trees when the samples were collected

The soil samples were collected on topsoil (0-20cm) and subsoil layers (50-80 cm), oven-dried at 40 °C and sieved at 2 mm.

The annual carbon input was estimated after sampling of the litter on the different sites, weighting of leaves and branches. Then carbon was calculated after mean C content in oak leaves and branches data from Lajtha and Jones, (2018).

2.2. Soil physico-chemical properties

Dissolved organic carbon (DOC) content and pH were measured on fresh samples. Soil pH was measured with a pH meter at a ratio of 1:5 soil: water (w:v; g:ml). Deionized water was used to extract soil DOC at a ratio of 1:5 soil: water (w:v). The mixture was shaken for two hours before centrifugation and filtered using a 0.45 µm filter. Dissolved carbon content was measured with a Shimadzu TOC-TN analyzer (Shimadzu Corp., Kyoto, Japan).

Several analyses were performed by the Soil Analysis Laboratory of Arras, (LAS, INRA, Arras, France) on air-dried samples after sieving at 2 mm. Soil texture was obtained after sieving (according to AFNOR X31 107 standard), CEC by Metson method (NF X 31-130 (1993)), whereas carbon (according to ISO 1423) and total nitrogen (according to ISO 13878) were obtained by dry combustion and quantification by GC equipped with a catharometer.

2.3. Soil size fractionation

A 15g aliquot of each sample was size fractionated to separate coarse (50 µm-2 mm) and fine (<50 µm) fractions. Soil was disaggregated in 100 ml of deionized water into a spin rotator for 12h with 10 4 mm-diameters glass beads (Rovira et al., 2010). The dispersed mixture was passed through a 50 µm sieve with water flowing, thus isolating the coarse and fine fractions. Both size fractions finally were dried at 40 ℃, weighed, and ground. The total mass recovery of both fractions was 98.02±0.09%.

2.4. Rock-Eval® thermal analysis

Crushed (100 mg) sample was analyzed by Rock-Eval® 7S analyser (Vinci Technologies). The samples were first pyrolyzed in an inert N_2 atmosphere at temperatures from 200 to 650 °C with a heating rate of 25 °C min^{-1}. Then the remaining samples was combusted in N_2/O_2 (80/20) atmosphere from 200 to 850 ℃ at 20 °C min^{-1}. The resulting thermograms were used to calculate the standard parameters (including Total Organic Carbon (TOC in wt%) and Mineral Carbon (MINC in wt%) by integrating the amounts of HC, CO, and CO_2 within the defined temperature limits (Lafargue et al., 1998; Behar et al., 2001). Because carbonates were not present in studied soils, the SOC measurements were equal to the sum TOC + MINC. Those values were positively correlated ($R^2 = 0.99$) to TOC measured with a CHN analyzer. The Hydrogen Index (HI in mg HC g^{-1} SOC) corresponds to the amount of HC released during pyrolysis relative to SOC. In soil samples, decrease in HI along soil profiles is related to the dehydrogenation of organic compounds during pedogenesis (Disnar et al., 2003; Barré et al., 2016). The Oxygen Index (OI in mg CO_2 g^{-1} SOC) corresponds to the amount of CO_2 released during pyrolysis relative to SOC. In soil samples, increase in OI along soil profiles is related to relative oxygenation (Disnar et al., 2003).

In addition to these standard parameters, previous studies have proposed using the shape of thermograms to obtain additional information about SOM quality. In the present study, we used R-index derived from the integrated S2 areas above 400 °C, usually interpreted as specific threshold of the thermal stability of organic compounds, separating the thermolabile, and thermoresistant C pools (Disnar et al., 2003; Sebag et al., 2006; Saenger et al., 2013; Saenger et al., 2015). Thus, R-index measures the contribution of the thermoresistant pool of the most reactive fraction of SOM (i.e., richer in H bonds), which is directly involved in short-term changes in soil C dynamics. However, as highlighted by Schiedung et al. (2017), this thermal index does not consider the various labile pools. As such, it does not reflect biological stability, but focus on SOM biogeochemical stability in the studied environmental context.

2.5. Thermochemolyis

Thermochemolyses were carried out with a pyrolyzer (Pyroprobe 6250, CDS) coupled to a gas chromatograph (7890B, Agilent) and to a mass spectrometer (5977B, Agilent). Samples were pyrolyzed at 650 °C for 15 s under a helium flow of 1 mL/mn, in the presence of tetramethylammonium hydroxide (TMAH 25 % methanol) (Quénéa et al., 2005a). This thermochemolysis resulted in thermal cleavage concurrent with the chemical cleavage of ester and ether bonds of the OM, and simultaneously to the alkylation of acidic functional groups improving detection of polar compounds. The released pyrolysis products were separated using a non-polar GC column Rxi5Sil MS (30m*0.25mm*0.5µm, Restek) and an oven ramp (initial temperature of 50 °C maintained 10min, raised at 2 °C/min until 320 °C, the final temperature, maintained 13min). The mass spectrometer was operated in electron impact and SCAN modes. The molecules were ionized and fragmented in an electron impact source (70 eV; 230 °C), and analyzed with a quadrupole mass spectrometer, working at 2 scans/s from 35 to 650 m/z.

Each compound was identified based on literature and using the NIST library, and classified into one of seven categories (Grandy et al., 2007; Kaal et al., 2007) comprising: 1) aromatics compounds (e.g. benzene, xylene and alkylbenzenes); 2) cutin and suberin compounds (alkanes, alkenes, fatty acids and methyl ketones); 3) lignin compounds (known products of coniferyl, syringyl, and coumaryl moieties); 4) phenolic compounds (e.g. phenol and methyl phenols); 5) nitrogen-containing compounds; 6) polyaromatic (PAH) compounds; and 7) polysaccharide products (mainly furans and levoglucosan) (Table S2). The relative contribution of each category was calculated as the sum of the area of each compound in the category, divided by the total area of identified compounds.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was used to test if the means calculated for the differences between treatments are equal. Differences were considered significant at $p < 0.05$ by the Duncan test. A Pearson correlation was performed to identify the relationship of the chemical properties in all soil samples. Statistical analyses were conducted using the SPSS 25.0 (IBM SPSS Statistics 20) software package. The figures were drawn using GraphPad Prism 9.3.

3. Results

3.1. Bulk soil analyses

3.1.1. Soil organic carbon and total nitrogen content

In the different sites, the TOC contents ranged from 14.25±5.03 to 31.24±5.33 g kg^{-1} in topsoil layers and from 4.11±1.71 to 7.15±0.62 g kg^{-1} in subsoil layers (Table 2). As expected, the TOC contents were higher in the topsoil layers than in subsoil layers. Site A40 and B40 had significantly higher TOC content than the other sites in the topsoil layer. In the subsoil layer, A40 had a higher TOC content than other sites.

The TN contents were highest in topsoil soils. They ranged from 0.79±0.23 to 2.26±0.33 g kg^{-1} in topsoil layers. Sites A40 and B40 had a significantly higher TN content than the other sites in topsoil layers. Total nitrogen concentrations were low and closer in subsoil soil, around 0.30 g kg^{-1}, except for the A40 site which contained twice the other sites (0.73 g kg^{-1}) (Table 2).

The C/N ratio ranged from 11.76±0.31 to 20.67±1.94 in the topsoil layers, which is higher than subsoil layers which ranged from 9.82±0.17 to 13.77±2.88. Site B40 had a highest C/N ratio in both topsoil and subsoil layers. A40 had a significantly lower C/N ratio (11.76) compared with the other sites in both topsoil and subsoil layers.

3.1.2. Rock-Eval® parameters

The HI were higher in topsoil layers than in subsoil layers, with all HI values in the topsoil layers higher than 100 mg HC g^{-1} TOC and all HI lower than 100 mg HC g^{-1} TOC in the subsoil layers (Fig. 1). The HI ranged from 119±14 to 264± and 22 ±19 to 72±7 mg HC g^{-1} TOC in topsoil and subsoil layers, respectively. In the topsoil layers, the HI of B40 was significantly higher ($p<0.05$) than in the other sites. The mean HI of C20 and C40 were close and slightly lower than the soil control, though some C20 replicates presented HI closer to the values of the control soil. The HI in C40 and A40 showed no differences ($p>0.05$) and both of them were lower than C20 ($p<0.05$). In the subsoil layers, no significant differences ($p>0.05$) in HI index between C20, C40, B40 and control could be evidenced but A40 exhibited higher value of HI.

The OI were below 250 mg CO_2 g^{-1} TOC in topsoil layers (from 148±4 to 188±10 mg CO_2 g^{-1} TOC) and comprised between 335±23 to 448±87 mg CO_2 g^{-1} TOC with no significant differences in subsoil layers (Fig. 1). In the topsoil layers, the OI of B40 was significantly lower than the other sites.

The R-index ranged from 0.61±0.04 to 0.69±0.008 and 0.66±0.03 to 0.79±0.01 in the topsoil and subsoil layers, respectively (Fig. 2). In the topsoil layers, the R-index in C20, C40 and A40 showed no differences (p>0.05) from natural forest, and the R-index in B40 was significantly lower. In the subsoil layer, only the R-index in A40 was significantly lower than in natural forest (p<0.05).

3.2. Soil fraction analyses

The range of the HI indices in the different fractions did not differ from the range in the bulk soils and followed the same trend between topsoil and subsoil layers (Supplementary table 1). In topsoil layers, the HI indices ranged from 126±21 to 272±25 mg HC g^{-1} TOC in coarse fraction and from 129±10to 305±39 mg HC g^{-1} TOC in fine fraction. In subsoil layers, the HI indices ranged from 40±18to 81±24 mg HC g^{-1} TOC in coarse fraction and 44±8to 85±1 mg HC g^{-1} TOC in fine fraction.

There was higher OI in subsoil layer than in topsoil layers, the OI indicated no differences between sites and between fractions (Supplementary table 1). The O-index ranged in coarse fractions from 117.0±3.7 to 126.0±12.4 mg CO_2 g^{-1} TOC and from 153.4±15.5 to 192.3±20.6 mg CO_2 g^{-1} TOC in the topsoil and subsoil layers, respectively. In the fine fractions, the O-index ranged from 110.6±10.0 to 140.0±9.8 and 158.0±12.1 to 285.4±68.4 mg CO_2 g^{-1} TOC in the topsoil and subsoil layers, respectively.

In the topsoil layers, the R-indices of the coarse fractions were lower (from 0.61±0.06 to 0.71±0.015 in coarse fraction and 0.66±0.06 to 0.77±0.02 in fine fractions (Fig.3). In the subsoil layers, the R-indices were very close between coarse and fine fractions, which were 0.66±0.04 to 0.71±0.07 and 0.66±0.06 to 0.748±0.01, respectively.

3.3. Molecular characterization of SOM

The soil organic matter was characterized by Py GC/MS in the presence of TMAH, a process commonly referred to as thermochemolysis. The list of the identified compounds and an assignated structural category are displayed in the supplementary table 2. The relative quantification of each category was calculated (Fig. 4) and statistical analyses were performed to compare the relative contribution of each group between the different sites (supplementary tables 3 and 4). In the topsoil layer, the pyrochromatograms are dominated by aromatic compounds for each site, except B40 which was dominated by cutin and suberin compounds. The A40 sites exhibited a more specific molecular composition with lower cutin and suberin, lignin compounds and a higher contribution of N-containing and polysaccharide compounds ($p < 0.05$).

The molecular composition of the subsoil OM was more similar. The pyrochromatograms were again dominated by aromatic compounds but also by N-containing compounds.

4. Discussion

4.1. Effect of forest management on soil carbon and nitrogen in soil layers

Clear-cut is a common disturbance in managed forests. Clear-cut leads to a decrease in leaf and wood litter fall input and also to higher rate of OM decomposition resulting from higher temperature, lower moisture content, light promotion, and decreasing nutrient levels (Achat et al., 2015; Baker and Allison, 2015; Wang et al., 2015; Taylor et al., 2017; Yan et al., 2017). Hence, clear-cut has often been shown to decrease total SOC of up to 8% in topsoil and subsoil layers of hardwood forests (Johnson and Curtis, 2001; Nave et al., 2010). It is worth mentioning that logically, the topsoil of soils is more affected by clear-cut as illustrated by the decrease in SOC of ca. 20% and of ca. 3% in organic layer and topsoil, respectively (0-15 cm; James and Harrison (2016); Achat et al. (2015)). After an initial short term loss of SOC following forest clearance, SOC tend to increase and could recover initial carbon stock within 20 to 100 years depending on the soil taxonomy (Nave et al., 2010; James and Harrison, 2016). In the present study, sites that were subjected to clear-cut 20 and 40 years ago (C20 and C40) did not display any significant difference in their SOC and N content (Table 2). This could indicate that the forest development after clear-cut did not change SOC or alternatively, has already enabled the full recovery of the SOC after 20 years.

Contrary to the negligible SOC impact observed in the regenerated forest (C40), areas afforested 40 years ago (A40) and regularly cleared for 40 years (B40) both showed an increase in SOC, of 55.8% and 83.2%, respectively (Table 1). In the B40 site, regular clear-cut with wood residues restitution is likely implying an accumulation of C in the soil top layer. The high SOC of A40 is in line with results showing that the conversion of cropland to forests can yield a SOC increase (Post and Kwon, 2000;

Schulp and Veldkamp, 2008) of up to 48% after 40 years of afforestation (Poeplau et al., 2011). Here, the high SOC cannot be explained by higher carbon inputs through leaf and branch falling compared to the other studied sites (Table 1). But high water content in A40 was recorded and may highlight seasonal water-logging conditions implying a lower decomposition rate of SOC compared to other studied soils.

In contrast to SOC, the effects of forest management practices on the modifications of total nitrogen (TN) remain poorly documented (Chang et al., 2014). As with SOC, the TN means do not distinguish the soils from control and regenerated forest area (Table 1). In comparison to them, A40 and B40 topsoil layers exhibit higher TN (2.23 and 1.53 g kg^{-1}, respectively; Table 2). Nonetheless, A40 and B40 are unlikely sharing a similar N source in their respective topsoil layers. As illustrated by Py-GC/MS (Fig. 7), the A40 topsoil layer is relatively enriched in N organic compounds in comparison to all other studied areas. This suggests that the high TN measured in A40 is mostly driven by organic N (N_{org}). A direct legacy of former agriculture practices is unlikely as N inputs mainly would consist in inorganic N fertilizer in conventional agriculture (Van Meter et al., 2016). Moreover, afforestation has been demonstrated to enhance N_{org} (Xu et al., 2016) as a consequence of the rise in bacterial diversity and abundance (Ren et al., 2016; Turley et al., 2020). In this study, N-containing compounds such as pyrrole, benzonitrile and pyridine are the pyrolysis product of amino acids or other polypeptides (Buurman et al., 2007; 2009; Buurman and Roscoe, 2011) and other N-containing pyrolysis products such as indole and diketodipyrrole have been associated with relatively fresh organic matter (Buurman and Roscoe, 2011). In addition, the higher polysaccharide pyrolysis products in A40 associated to high contribution of nitrogen organic compounds point to a larger contribution of microorganisms to SOM

(Huang et al., 1998). These results suggest a larger contribution of microbial biomass in the topsoil of A40.

It is worth mentioning that a rise in SOC is usually associated with the accumulation of soil N due to a stoichiometric relationship (Kirkby et al., 2013). Here, C/N ratios were similar across all studied sites with the notable exception of A40 (Table 2). The similar C/N ratio and N-containing compounds of the top soils in B40 and the cleared (C20 and C40) and control samples indicating regular clear-cut with wood residues favors the accumulation of C and N without modifying their stoichiometric relationship, more in line with litter residues. The significant difference in C/N ratio of A40 suggests that afforestation has deeply modified the stoichiometric relationship between C and N according to N_{org} accumulation.

In contrast to topsoil layers, SOC in subsoil layers is far less sensitive to forest management and mainly depends on soil taxonomy (Nave et al., 2010). The A40 subsoil had the highest SOC and TN (Table 1). Nonetheless, in sharp contrast to topsoil layers, A40 exhibit a lower C/N ratio – ca. 9.8 (Table 1) - compared to other sites. Such low C/N ratio is in line with those usually assigned to French arable soils (Nicolardot et al., 2001) and may therefore still reflect former agriculture practices although they ceased 40 years ago.

4.2. Impact on SOC quality and thermal stability

Hydrogen Index values are related to the contribution of hydrocarbon compounds to theSOC. This compositional index presents the highest values in organic layers rich in fresh plant debris and decreases during OM decomposition in the topsoils (Disnar et al., 2003; Sebag et al., 2016) and in carbon-rich samples like peats (Delarue et al., 2013). This gradual decrease appears to be linked to the mineralization of the most chemically labile and bioavailable organic compounds (Barré et al., 2016; Poeplau et al., 2019). In this study, HI values were highly heterogeneous between sites in the topsoil layers consistent with those reported by Soucémarianadin et al. (2019) for other forest topsoils (Fig. 1). This heterogeneity can be due to differences in composition or quantity of fresh organic inputs. SOM composition is a major influence on OM quality in forest organic layers (Marschner et al., 2008; Angst et al., 2021). However, as oak is the main tree species in all studied areas, distinct chemical compositions in leaf and branches are unlikely. Therefore, these differences in quality/HI can be partially explained by differences in early decomposition of fresh inputs related to soil environmental conditions and to quantities of inputs driven by forest management practices (light, moisture, temperature). Alternatively, the source of OM can also explain the discrepancies in HI. As illustrated in B40, higher HI values are correlated with the relative abundance of cutin and suberin ($r= 0.60$; $p < 0.05$; $n=25$; Fig. 5), known as highly aliphatic (Quénéa et al., 2005b) and provided by regular clear-cut and wood residues inputs, including bark and cork rich in suberin, in the B40 topsoil layers. This interpretation is congruent with low R-index values in B40 topsoil layers indicating a weak thermal stability related to poorly decomposed SOM (Sebag et al., 2016; Sebag et al., 2022).

Cutin and suberin are known to be chemically stable in litter (Feng et al., 2008) and in bark and cork. The relative higher proportion of cutin, suberin and lignin in B40 samples likely reflects an accumulation of these compounds, and playis a key role in the stability of OM from B40. The low R index therefore reflects the chemical composition of studied OM inputs and/or preferential decomposition of more biodegradable biochemicals such as polysaccharides (Melillo et al., 1982; Feng et al., 2010; Córdova et al., 2018). Despite having a high proportion of lignin, cutin and suberin, B40 presented a low R-index, reflecting low OM thermal stability (Sebag et al., 2016). This apparently contradictory result between low R-index and abundance of chemically stable compounds could indicate that thermal stability is not only related to chemical stability is also also dependent on organo-mineral interactions.

The processes involved in the SOM quality and stability are different in the subsoil layers. Whereas amino acids and proteins considered highly labile, the contribution of N-containing compounds to OM is more important than in topsoils and R-index values were higher than topsoil ones. However, amino groups can be preferentially stabilized by mineral association (Kleber et al., 2015); i.e.organo-mineral interaction could modify the thermal stability of SOM (Saenger et al., 2013). In addition, lower C/N ratios and a high contribution of N-containing compounds in subsoil layers suggests increasing microbial inputs, as well as increased stabilisation by organo-mineral interaction (Kallenbach et al., 2016).

In consequence, the high SOC content observed with continuous input from plant residues in B40 could be only significant on a short term basis and would disappear when the input stopped. In A40, the microbial contribution was higher than other sites in topsoil layers and similar to subsoil layers and could reflect past activities. Indeed, agricultural management effects on microbial communities could last for decades (Turley et al., 2020). The R-index did not vary between topsoil and subsoil layers for this site. This could be evidence that the microbial necromass induced higher organo-mineral interaction even in topsoil layers, which would underline the role of chemical composition in this interaction and its consequences on OM stability (Kallenbach et al., 2016). Finally, afforestation can improve SOC content and control SOM quality by fresh labile organic inputs, but stability and residence-times could be more directly related to interactions with minerals. To assess the fate of SOC in sites having undergone different human activities, not only the quantity of SOC, but also its stability should be taken into account (Cotrufo et al., 2019).

The evaluation of physically separated fractionations can help to better distinguish the respective effects of the preferential decomposition of the most labile organic compounds and of organo-mineral interactions. The coarse fraction (>50µm) is enriched in particulate OM and is considered a more labile fraction mainly constituted of plant residues (Cotrufo et al., 2019), while the fine fraction (<50µm) has more enriched mineral associated OM and is considered as the more stable fraction through physical protection and organo-mineral interaction (Christensen, 2001; Jastrow et al., 2007; Kögel-Knabner et al., 2008). Coupled to thermal analyses, the grain-size fractions were already used to distinguish labile and stable C pools related to environmental drivers (Saenger et al., 2015).

In the present study, lower thermal stability (R-index values) in coarse fractions (Fig. 3) are in line with results from Sebag et al. (2022) that demonstrated the ability of fractionation to isolate weakly decomposed and more degradable OM. The samples B40 and A40 exhibited lowest thermal stability despite very different HI values. This confirms that quality and thermal stability are not always related in samples enriched in plant debris. On the other hand, no significant differences were observed in the fine fractions. This suggests that the composition of SOM is more homogeneous in the fine fraction of subsoil layers. Thus, in the presence of mineral rich soil matrix, the organo-mineral interactions play a more important role on the stability of the SOM independently of its quality. The source of SOM in subsoil layers and fine fractions is often attributed to more processed OM and microbial residues prone to organo-mineral interaction (Miltner et al., 2012; Rumpel et al., 2012; Cotrufo et al., 2013). Thus, the chemical composition from thermal analysis reflects this more homogeneous (Grandy and Neff, 2008) and more stable source of carbon and could explain the close values of the fine fractions. Similar results obtained by Sebag et al. (2022) in different size fractions have been attributed to the plant-microbe-soil continuum concept, and evidenced the role of interaction with the mineral matrix.

5. Conclusions

The quantification of soil carbon and nitrogen at sites having experienced different forest management regimes (from natural regeneration, afforestation to continuous clear-cut) showed two distinct patterns. In topsoil, the regenerated sites presented similar C and N content to the control site indicative of C recovery after clear-cut as described for other temperate forests. Conversely, the afforested site and the site with regular OM input had larger C and N contents. The molecular characterization of the OM reflected different sources of OM: in the afforested site, the N-containing compounds were abundant, pointing to a higher microbial source whereas in site with continuous input, the composition was clearly dominated by lignin, cutin and suberin compounds. R-indices from Rock-Eval® analyses show relatively lower thermal stability of the OM from continuous residue input despite a high contribution of chemically resistant lignin compounds. In contrast, high R-indices were determined when amino acids and proteins are stabilized owing to organo-mineral interactions. This suggests a limitation in assessing OM stability based on Rock-Eval® thermal analysis: when stability of OM is governed by its chemical stability, thermal stability is not a comparable proxy to evaluate OM stability. These results imply that caution is required when assessing OM stability – based solely on thermal stability – in soils where pedogenetic processes can be deeply modified by regular and extensive inputs of OM. Whereas, the afforested site and the site with continuous OM input displayed higher SOC in topsoil layers, this increase could be offset by a lower stability of the SOC from the residue input. In consequence this study reinforces the necessity to separately assess both SOC chemical composition and stability to estimate the long-term effect of forest management.

Acknowledgment

Rock-Eval® is a trademark registered by IFP Energies Nouvelles. The authors would like to G. Duquenoy for supplying sites' history and information, and N. Lefrançois for technical support. This work and Zheng Sun's PhD thesis were financially supported by the Institut Français du Pétrole Energies Nouvelles (IFPEN). The authors thank Dr. Paul Greenwood and an anonymous reviewer for their critical reading of our manuscript.

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Fig. captions

Fig.1: Boxplots of H-index (a) of samples from topsoil (T) and subsoil (S) and diagram of H-index vs O-index. Different letters indicate significant differences between different treatments by Duncan test ($p < 0.05$).

Fig.2: Boxplots of R-index of topsoil (T) and subsoil (S). Different letters indicate significant differences between different treatments by Duncan test ($p < 0.05$).

Fig.3: Boxplots of thermal stability (R-index) of the coarse fractions (a) and fine fraction (b) from 5 sites in topsoil (T) and subsoil (S) layers. Different letters in each indicate significant differences between different treatments by Duncan test ($p < 0.05$).

Fig. 4: Relative abundance of the main categories obtained by Py-GC/MS of samples from each site from topsoil layers (T) and subsoil layers (S): 1) aromatics compounds; 2) cutin and suberin compounds; 3) lignin compounds; 4) phenolic compounds; 5) N-containing compounds; 6) polyaromatic hydrocarbon (PAH) compounds; and 7) polysaccharide compounds (PS).

Fig.5 Linear regression of H-index and relative abundance of cutin and suberin relative abundance estimated by thermochemolysis (n=25), $p< 0.05$

Table 1: Soil characteristics and description of the 5 sites

Site description	Layer	Mean annual carbon input (kg ha^{-1})		CEC (cmol kg^{-1})	Texture			Water content (%)
		Litter	Branch		Clay%	Silt%	Sand%	
Unmanaged forest (control)	Topsoil	3362	1478	6.48	12.5	65.4	22.1	21.2 (2.4)
	Subsoil			7.20	17.5	60.6	21.9	20.9 (0.7)
Natural reforestation since 20 years (C20)	Topsoil	2737	358	5.35	12.3	65.8	21.9	23 (2.2)
	Subsoil			10.54	22.5	55.6	21.9	21.3 (0.9)
Natural reforestation since 40 years (C40)	Topsoil	2143	694	7.43	13.3	59.4	27.3	22.7 (2.6)
	Subsoil			11.95	22.8	54.3	22.9	22.1 (1.1)
Afforestation 40 years ago (A40)	Topsoil	1623	306	23.10	31.0	48.8	20.2	25.1 (4.2)
	Subsoil			15.01	26.1	52.3	21.6	20.9 (1.0)
Regular clearing since 40 years (B40)	Topsoil	4652		7.68	13.6	62.2	24.2	31.4 (4.9)
	Subsoil			12.18	25.5	56.2	18.3	29.1 (3.8)

Table. 2: TOC, TN, and C/N in the 5 sites

	Topsoil			**Subsoil**		
Sample's name	TOC (g kg^{-1})	TN (g kg^{-1})	C/N	TOC (g kg^{-1})	TN (g kg^{-1})	C/N
Control	16.31 (4.98)b	0.87 (0.30)c	18.98 (1.34)ab	4.11 (1.71) b	0.33 (0.08) b	12.06 (1.79) ab
C20	14.25 (5.03)b	0.79 (0.23)c	17.76 (1.84)b	4.24 (1.51) b	0.33 (0.07) b	12.38 (1.91) ab
C40	17.05 (1.65)b	0.91 (0.13)c	18.82 (0.98)ab	4.25 (2.15) b	0.31 (0.11) b	13.18 (2.16) a
A40	26.56 (3.36)a	2.26 (0.33)a	11.76 (0.31)c	7.15 (0.62) a	0.73 (0.05) a	9.82 (0.17) b
B40	31.24 (5.33)a	1.53 (0.36)b	20.67 (1.94)a	4.40 (1.79) b	0.31 (0.07) b	13.77 (2.88) a

Different letters indicate significant differences between different treatments by Duncan test ($p < 0.05$).

Figure 1

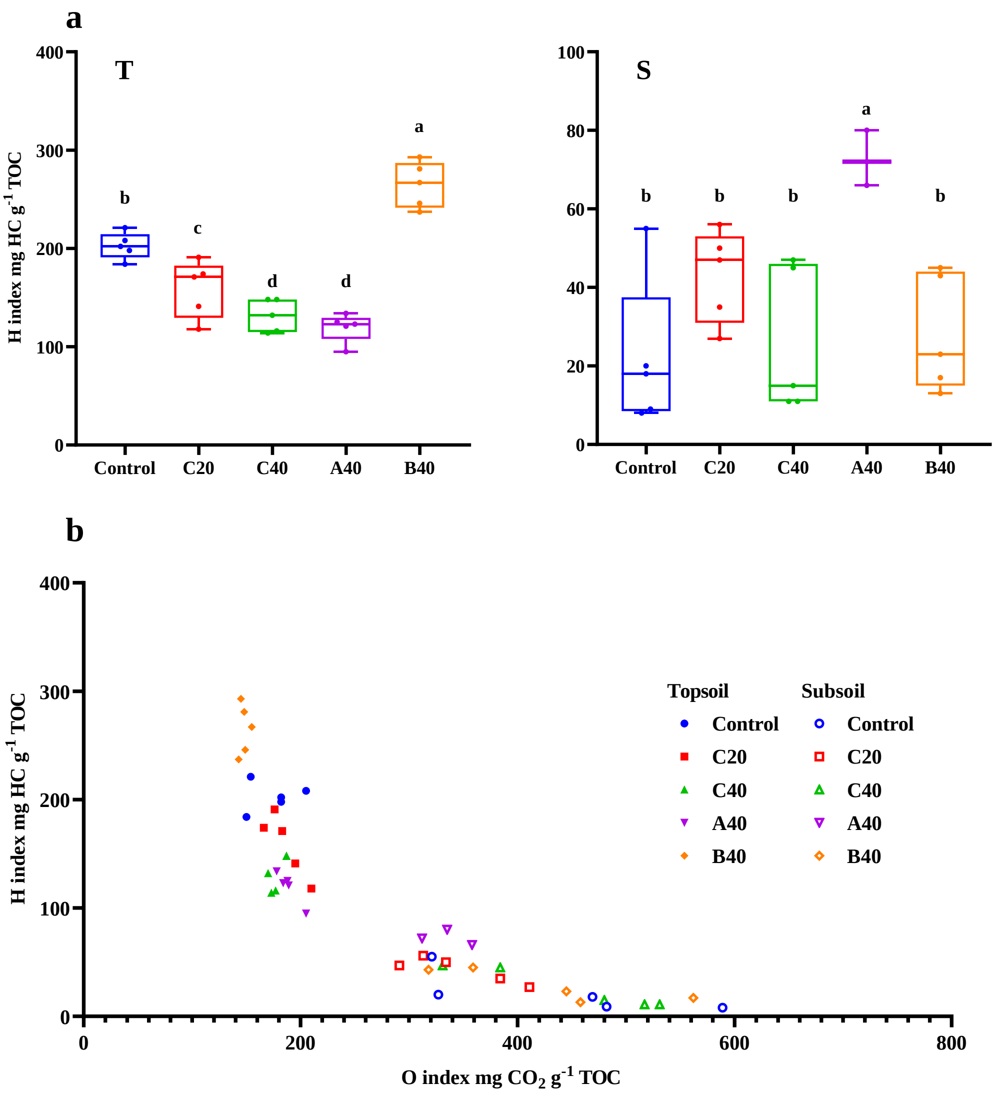
a
T
H index mg HC g^{-1} TOC
0
100
200
300
400
b
c
d
d
a
Control
C20
C40
A40
B40
S
0
20
40
60
80
100
b
b
b
a
b
Control
C20
C40
A40
B40
b
H index mg HC g^{-1} TOC
0
100
200
300
400
O index mg CO_2 g^{-1} TOC
0
200
400
600
800
Topsoil
● Control
■ C20
▲ C40
▼ A40
◆ B40
Subsoil
○ Control
□ C20
△ C40
▽ A40
◇ B40

Figure 2

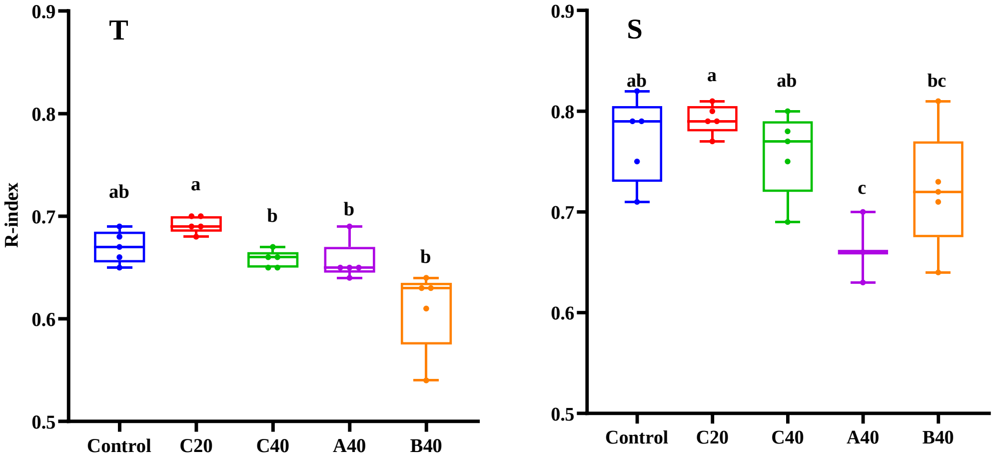
T
S
R-index
0.9
0.8
0.7
0.6
0.5
ab
a
b
b
b
ab
a
ab
c
bc
Control
C20
C40
A40
B40
Control
C20
C40
A40
B40

Figure 3

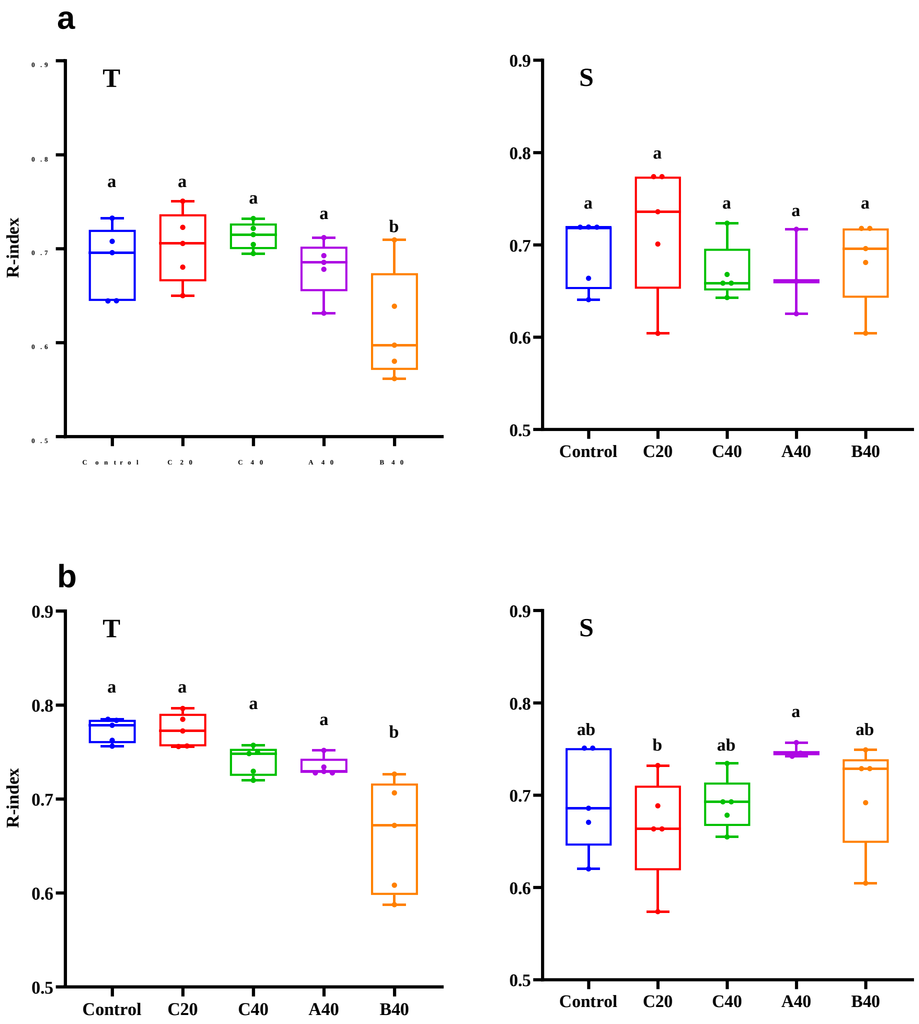
a
T
S
b
T
S
R-index
R-index
0.9
0.8
0.7
0.6
0.5
Control
C20
C40
A40
B40
a
a
a
a
b
a
a
a
a
a
a
a
a
a
b
ab
b
ab
a
ab

Figure 4

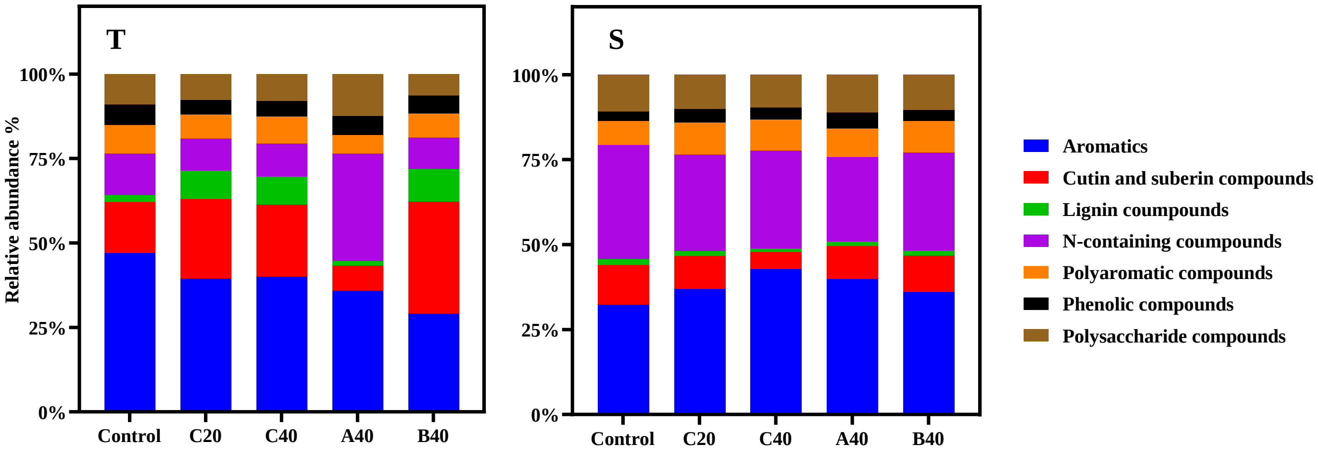

Figure 5