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

3

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15 Keywords:

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

18 **Abstract**

19 The effect of forest management practices on carbon quality is poorly documented.

20 To assess changes in the quality and stability of soil organic carbon (SOC) of a

21 temperate forest upon human activities, we investigated soil from forests (i)

22 developed following natural regeneration after clearcutting 20 and 40 years ago, (ii)
23 developed following afforestation on an abandoned crop area 40 years ago and (iii) in
24 an area where regular clear-cut (with wood residues input) was conducted 40 years
25 ago. Topsoil and subsoil layers were collected (0-20 cm and 50-80 cm). Soil organic
26 matter (OM) was characterized by elemental analysis (total carbon and total nitrogen),
27 thermal analysis (Rock-Eval®) and thermochemolysis (i.e., Py-GC/MS in the
28 presence of tetramethylammonium hydroxide (TMAH)). In addition, a size
29 fractionation to separate the labile coarse fraction (50-2000 µm) from the fine fraction
30 (<50 µm) was performed. These fractions were analyzed by thermal analysis.

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

41

42 **1. Introduction**

43 Forest ecosystems cover about 4 billion hectares and account for 31% of the
44 global land area (Keenan et al., 2015; Bastin et al., 2017). They store around 861 Pg C,
45 44% in soil and 5% in litter) (Janzen, 2004; Heimann and Reichstein, 2008; Pan et al.,
46 2011) and are considered crucial in global change mitigation as a potential carbon
47 sink (Janssens Ivan et al., 2003). However, human activities – such as deforestation –
48 constitute a threat to forest ecosystems, and lead to large loss of organic carbon stored
49 into the soils (Guo and Gifford, 2002; Stockmann et al., 2013; Lal, 2016; Mayer et al.,
50 2020). Depending on these activities, forest soils could represent either a C source or
51 a C sink for atmospheric CO₂ (Jandl et al., 2007; Adamczyk et al., 2019; Mayer et al.,
52 2020). For example, forest management practices can positively or negatively impact
53 soil organic carbon (SOC).

54 Conservation of old forest and afforestation are some examples of practices
55 able to modify SOC stocks (Mayer et al., 2020). For example, Ameray et al. (2021)
56 have reported different management strategies to maintain or increase carbon in forest
57 soil, from conservation of old forest to afforestation. Clear-cut harvesting is another
58 common management of forest. Depending on intensity, it can influence SOC stocks
59 for up to 100 years (Jandl et al., 2007). Clear-cut induces a SOC stocks decrease
60 mainly through the drastic decrease in litter input and faster SOC decomposition due
61 to higher insolation and warmer soil temperature (Kulmala et al., 2014; Mayer et al.,
62 2017) and compaction effect on microbiome and soil function (Hartmann et al., 2014).
63 In a meta-analysis study with a database of 945 responses to harvest from 112
64 publications, James and Harrison (2016) evidenced different magnitudes in C loss
65 depending on the considered soil depth. Conversely, reforestation and afforestation,
66 also in areas relatively resilient to clear-cut, SOC recover over one to seven decades

67 depending on the soil taxonomic order (Poeplau et al., 2011; Bárcena et al., 2014;
68 James and Harrison, 2016). Although SOC concentration has been measured to reach
69 similar or even higher level than before the imposed management practice, few
70 studies have investigated the chemical composition and the biogeochemical stability,
71 i.e., bioavailability or bioaccessibility to decomposition by microorganisms as well as
72 chemical stability, of this SOC (Angst et al., 2016; Mustafa et al., 2022). Indeed, SOC
73 before clear-cut and after recovery could have different stabilities, and finally, the
74 recovery at decennial to centennial time scale would not balance the carbon losts due
75 to reduced residence time (Hamburg et al., 2019). The changes in residence time can
76 be interpreted as a consequence of SOC stabilization mechanisms driven by changes
77 in SOC chemical composition and through greater reactivity with minerals (organo-
78 mineral adsorption) and physical protection by incorporation into aggregates
79 (Lehmann and Kleber, 2015). Thus, evaluation of the quality and stability of SOC is
80 required to integrate the intrinsic stability of OM (i.e., the energy input to break down
81 organic molecules) and the stabilization of SOM through interactions with soil
82 minerals (i.e., the energy required to break the bonds between SOM and mineral
83 particles).

84 A common approach to study the quality and stability of SOC is based on a
85 relative estimation of pools with different stability and their respective responses to
86 land use (Sahoo et al., 2019; Liu et al., 2020). However, the quantification of the C
87 from these different pools is challenging and different methods are used. A method
88 widely applied is soil physical fractionation (von Lützow et al., 2007) which provides
89 insights into SOM bioaccessibility (Rovira et al., 2010; Moni et al., 2012). The coarse
90 fraction mainly contains particulate organic matter and plant residues, with fast
91 turnover times, whereas organic matter from fine fraction is intimately associated to

92 mineral phases and exhibit lower turnover. A less time consuming method, that could
93 be applied is thermal analysis. Indeed, more recently, thermal analysis of forest litter
94 or topsoil samples revealed that OC with higher stability will combust at higher
95 temperatures and provide less energy than labile OM (Plante et al., 2009; Barré et al.,
96 2016). As underlined by extensive literature (Disnar et al., 2003; Plante et al., 2011;
97 Derenne and Quénéa, 2015; Sebag et al., 2016), thermal analyses appear promising in
98 the evaluation of SOC stability. Several thermal analysis approaches have been used
99 to estimate the stability of carbon pools in soils from various environments (Disnar et
100 al., 2003; Gregorich et al., 2015; Sebag et al., 2016; Cécillon et al., 2018, 2021;
101 Thoumazeau et al., 2020). Among them, Rock-Eval® thermal analysis, has been used
102 to characterize soil organic matter (SOM) (Disnar et al., 2003) and its dynamics
103 (Sebag et al., 2016). Several Rock-Eval® parameters were also defined to reflect the
104 SOM stability (Saenger et al., 2013; Soucémariadin et al., 2018). Hence, Saenger et
105 al. (2015), Soucémariadin et al. (2018) and Sebag et al. (2022) studied different soil
106 size fractions and compared results to assess the efficiency of Rock-Eval® to evaluate
107 the thermal stability of SOM. Whatever the soil studied, the thermal stability was
108 lower for coarse fractions. Forest managements influences SOC however generally
109 only its quantitative impact is considered. In this study, we compared the effect of
110 afforestation, natural reforestation and regular clear-cut on SOC concentration and
111 also their effect on organic matter quality thermochemolysis analyses and on stability
112 by Rock Eval® analysis.

113 2. Materials and methods

114 2.1 Study site

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

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

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

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

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

138 **2.2. Soil physico-chemical properties**

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

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

151 **2.3. Soil size fractionation**

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

158

159 **2.4. Rock-Eval® thermal analysis**

160 Crushed (100 mg) sample was analyzed by Rock-Eval® 7S analyser (Vinci
161 Technologies). The samples were first pyrolyzed in an inert N₂ atmosphere at
162 temperatures from 200 to 650 °C with a heating rate of 25 °C min⁻¹. Then the
163 remaining samples was combusted in N₂/O₂ (80/20) atmosphere from 200 to 850 °C at
164 20 °C min⁻¹. The resulting thermograms were used to calculate the standard
165 parameters (including Total Organic Carbon (TOC in wt%) and Mineral Carbon
166 (MINC in wt%) by integrating the amounts of HC, CO, and CO₂ within the defined
167 temperature limits (Lafargue et al., 1998; Behar et al., 2001). Because carbonates
168 were not present in studied soils, the SOC measurements were equal to the sum TOC
169 + MINC. Those values were positively correlated ($R^2 = 0.99$) to TOC measured with a
170 CHN analyzer. The Hydrogen Index (HI in mg HC g⁻¹ SOC) corresponds to the
171 amount of HC released during pyrolysis relative to SOC. In soil samples, decrease in
172 HI along soil profiles is related to the dehydrogenation of organic compounds during
173 pedogenesis (Disnar et al., 2003; Barré et al., 2016). The Oxygen Index (OI in mg
174 CO₂ g⁻¹ SOC) corresponds to the amount of CO₂ released during pyrolysis relative to
175 SOC. In soil samples, increase in OI along soil profiles is related to relative
176 oxygenation (Disnar et al., 2003).

177 In addition to these standard parameters, previous studies have proposed using the
178 shape of thermograms to obtain additional information about SOM quality. In the
179 present study, we used R-index derived from the integrated S2 areas above 400 °C,
180 usually interpreted as specific threshold of the thermal stability of organic compounds,
181 separating the thermolabile, and thermoresistant C pools (Disnar et al., 2003; Sebag et
182 al., 2006; Saenger et al., 2013; Saenger et al., 2015). Thus, R-index measures the
183 contribution of the thermoresistant pool of the most reactive fraction of SOM (i.e.,

184 richer in H bonds), which is directly involved in short-term changes in soil C
185 dynamics. However, as highlighted by Schiedung et al. (2017), this thermal index
186 does not consider the various labile pools. As such, it does not reflect biological
187 stability, but focus on SOM biogeochemical stability in the studied environmental
188 context.

189 **2.5. Thermochemolysis**

190 Thermochemolyses were carried out with a pyrolyzer (Pyroprobe 6250, CDS) coupled
191 to a gas chromatograph (7890B, Agilent) and to a mass spectrometer (5977B, Agilent).
192 Samples were pyrolyzed at 650 °C for 15 s under a helium flow of 1 mL/min, in the
193 presence of tetramethylammonium hydroxide (TMAH 25 % methanol) (Quénéa et al.,
194 2005a). This thermochemolysis resulted in thermal cleavage concurrent with the
195 chemical cleavage of ester and ether bonds of the OM, and simultaneously to the
196 alkylation of acidic functional groups improving detection of polar compounds. The
197 released pyrolysis products were separated using a non-polar GC column Rxi5Sil MS
198 (30m*0.25mm*0.5µm, Restek) and an oven ramp (initial temperature of 50 °C
199 maintained 10min, raised at 2 °C/min until 320 °C, the final temperature, maintained
200 13min). The mass spectrometer was operated in electron impact and SCAN modes.
201 The molecules were ionized and fragmented in an electron impact source (70 eV;
202 230 °C), and analyzed with a quadrupole mass spectrometer, working at 2 scans/s
203 from 35 to 650 m/z.

204 Each compound was identified based on literature and using the NIST library, and
205 classified into one of seven categories (Grandy et al., 2007; Kaal et al., 2007)
206 comprising: 1) aromatics compounds (e.g. benzene, xylene and alkylbenzenes); 2)
207 cutin and suberin compounds (alkanes, alkenes, fatty acids and methyl ketones); 3)
208 lignin compounds (known products of coniferyl, syringyl, and coumaryl moieties); 4)

209 phenolic compounds (e.g. phenol and methyl phenols); 5) nitrogen-containing
210 compounds; 6) polyaromatic (PAH) compounds; and 7) polysaccharide products
211 (mainly furans and levoglucosan) (Table S2). The relative contribution of each
212 category was calculated as the sum of the area of each compound in the category,
213 divided by the total area of identified compounds.

214

215 **2.6. Statistical analysis**

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

222

223 **3. Results**

224 **3.1. Bulk soil analyses**

225 **3.1.1. Soil organic carbon and total nitrogen content**

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

231 The TN contents were highest in topsoil soils. They ranged from 0.79 ± 0.23 to
232 2.26 ± 0.33 g kg⁻¹ in topsoil layers. Sites A40 and B40 had a significantly higher TN
233 content than the other sites in topsoil layers. Total nitrogen concentrations were low
234 and closer in subsoil soil, around 0.30 g kg⁻¹, except for the A40 site which contained
235 twice the other sites (0.73 g kg⁻¹) (Table 2).

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

240

241 **3.1.2. Rock-Eval® parameters**

242 The HI were higher in topsoil layers than in subsoil layers, with all HI values in the
243 topsoil layers higher than 100 mg HC g⁻¹ TOC and all HI lower than 100 mg HC g⁻¹
244 TOC in the subsoil layers (Fig. 1). The HI ranged from 119 ± 14 to $264\pm$ and 22 ± 19 to
245 72 ± 7 mg HC g⁻¹ TOC in topsoil and subsoil layers, respectively. In the topsoil layers,
246 the HI of B40 was significantly higher ($p<0.05$) than in the other sites. The mean HI
247 of C20 and C40 were close and slightly lower than the soil control, though some C20
248 replicates presented HI closer to the values of the control soil. The HI in C40 and A40
249 showed no differences ($p>0.05$) and both of them were lower than C20 ($p<0.05$). In
250 the subsoil layers, no significant differences ($p>0.05$) in HI index between C20, C40,
251 B40 and control could be evidenced but A40 exhibited higher value of HI.

252 The OI were below 250 mg CO₂ g⁻¹ TOC in topsoil layers (from 148 ± 4 to 188 ± 10 mg
253 CO₂ g⁻¹ TOC) and comprised between 335 ± 23 to 448 ± 87 mg CO₂ g⁻¹ TOC with no

254 significant differences in subsoil layers (Fig. 1). In the topsoil layers, the OI of B40
255 was significantly lower than the other sites.

256 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
257 topsoil and subsoil layers, respectively (Fig. 2). In the topsoil layers, the R-index in
258 C20, C40 and A40 showed no differences ($p>0.05$) from natural forest, and the R-
259 index in B40 was significantly lower. In the subsoil layer, only the R-index in A40
260 was significantly lower than in natural forest ($p<0.05$).

261

262 **3.2. Soil fraction analyses**

263 The range of the HI indices in the different fractions did not differ from the range in
264 the bulk soils and followed the same trend between topsoil and subsoil layers
265 (Supplementary table 1). In topsoil layers, the HI indices ranged from 126 ± 21 to
266 272 ± 25 mg HC g⁻¹ TOC in coarse fraction and from 129 ± 10 to 305 ± 39 mg HC g⁻¹
267 TOC in fine fraction. In subsoil layers, the HI indices ranged from 40 ± 18 to 81 ± 24 mg
268 HC g⁻¹ TOC in coarse fraction and 44 ± 8 to 85 ± 1 mg HC g⁻¹ TOC in fine fraction.

269 There was higher OI in subsoil layer than in topsoil layers, the OI indicated no
270 differences between sites and between fractions (Supplementary table 1). The O-index
271 ranged in coarse fractions from 117.0 ± 3.7 to 126.0 ± 12.4 mg CO₂ g⁻¹ TOC and from
272 153.4 ± 15.5 to 192.3 ± 20.6 mg CO₂ g⁻¹ TOC in the topsoil and subsoil layers,
273 respectively. In the fine fractions, the O-index ranged from 110.6 ± 10.0 to 140.0 ± 9.8
274 and 158.0 ± 12.1 to 285.4 ± 68.4 mg CO₂ g⁻¹ TOC in the topsoil and subsoil layers,
275 respectively.

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

280

281 **3.3. Molecular characterization of SOM**

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

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

295

296 **4. Discussion**

297 **4.1. Effect of forest management on soil carbon and nitrogen in soil layers**

298 Clear-cut is a common disturbance in managed forests. Clear-cut leads to a decrease
299 in leaf and wood litter fall input and also to higher rate of OM decomposition
300 resulting from higher temperature, lower moisture content, light promotion, and
301 decreasing nutrient levels (Achat et al., 2015; Baker and Allison, 2015; Wang et al.,
302 2015; Taylor et al., 2017; Yan et al., 2017). Hence, clear-cut has often been shown to
303 decrease total SOC of up to 8% in topsoil and subsoil layers of hardwood forests
304 (Johnson and Curtis, 2001; Nave et al., 2010). It is worth mentioning that logically,
305 the topsoil of soils is more affected by clear-cut as illustrated by the decrease in SOC
306 of ca. 20% and of ca. 3% in organic layer and topsoil, respectively (0-15 cm; James
307 and Harrison (2016); Achat et al. (2015)). After an initial short term loss of SOC
308 following forest clearance, SOC tend to increase and could recover initial carbon
309 stock within 20 to 100 years depending on the soil taxonomy (Nave et al., 2010;
310 James and Harrison, 2016). In the present study, sites that were subjected to clear-cut
311 20 and 40 years ago (C20 and C40) did not display any significant difference in their
312 SOC and N content (Table 2). This could indicate that the forest development after
313 clear-cut did not change SOC or alternatively, has already enabled the full recovery of
314 the SOC after 20 years.

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

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

326 In contrast to SOC, the effects of forest management practices on the modifications of
327 total nitrogen (TN) remain poorly documented (Chang et al., 2014). As with SOC, the
328 TN means do not distinguish the soils from control and regenerated forest area (Table
329 1). In comparison to them, A40 and B40 topsoil layers exhibit higher TN (2.23 and
330 1.53 g kg⁻¹, respectively; Table 2). Nonetheless, A40 and B40 are unlikely sharing a
331 similar N source in their respective topsoil layers. As illustrated by Py-GC/MS (Fig.
332 7), the A40 topsoil layer is relatively enriched in N organic compounds in comparison
333 to all other studied areas. This suggests that the high TN measured in A40 is mostly
334 driven by organic N (N_{org}). A direct legacy of former agriculture practices is unlikely
335 as N inputs mainly would consist in inorganic N fertilizer in conventional agriculture
336 (Van Meter et al., 2016). Moreover, afforestation has been demonstrated to enhance
337 N_{org} (Xu et al., 2016) as a consequence of the rise in bacterial diversity and abundance
338 (Ren et al., 2016; Turley et al., 2020). In this study, N-containing compounds such as
339 pyrrole, benzonitrile and pyridine are the pyrolysis product of amino acids or other
340 polypeptides (Buurman et al., 2007; 2009; Buurman and Roscoe, 2011) and other N-
341 containing pyrolysis products such as indole and diketodipyrrole have been associated
342 with relatively fresh organic matter (Buurman and Roscoe, 2011). In addition, the
343 higher polysaccharide pyrolysis products in A40 associated to high contribution of
344 nitrogen organic compounds point to a larger contribution of microorganisms to SOM

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

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

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

363 **4.2. Impact on SOC quality and thermal stability**

364 Hydrogen Index values are related to the contribution of hydrocarbon compounds to
365 the SOC. This compositional index presents the highest values in organic layers rich in
366 fresh plant debris and decreases during OM decomposition in the topsoils (Disnar et
367 al., 2003; Sebag et al., 2016) and in carbon-rich samples like peats (Delarue et al.,
368 2013). This gradual decrease appears to be linked to the mineralization of the most

369 chemically labile and bioavailable organic compounds (Barré et al., 2016; Poeplau et
370 al., 2019). In this study, HI values were highly heterogeneous between sites in the
371 topsoil layers consistent with those reported by Soucémariadin et al. (2019) for
372 other forest topsoils (Fig. 1). This heterogeneity can be due to differences in
373 composition or quantity of fresh organic inputs. SOM composition is a major
374 influence on OM quality in forest organic layers (Marschner et al., 2008; Angst et al.,
375 2021). However, as oak is the main tree species in all studied areas, distinct chemical
376 compositions in leaf and branches are unlikely. Therefore, these differences in
377 quality/HI can be partially explained by differences in early decomposition of fresh
378 inputs related to soil environmental conditions and to quantities of inputs driven by
379 forest management practices (light, moisture, temperature). Alternatively, the source
380 of OM can also explain the discrepancies in HI. As illustrated in B40, higher HI
381 values are correlated with the relative abundance of cutin and suberin ($r= 0.60$; $p <$
382 0.05 ; $n=25$; Fig. 5), known as highly aliphatic (Quénéa et al., 2005b) and provided by
383 regular clear-cut and wood residues inputs, including bark and cork rich in suberin, in
384 the B40 topsoil layers. This interpretation is congruent with low R-index values in
385 B40 topsoil layers indicating a weak thermal stability related to poorly decomposed
386 SOM (Sebag et al., 2016; Sebag et al., 2022).

387 Cutin and suberin are known to be chemically stable in litter (Feng et al., 2008) and in
388 bark and cork. The relative higher proportion of cutin, suberin and lignin in B40
389 samples likely reflects an accumulation of these compounds, and plays a key role in
390 the stability of OM from B40. The low R index therefore reflects the chemical
391 composition of studied OM inputs and/or preferential decomposition of more
392 biodegradable biochemicals such as polysaccharides (Melillo et al., 1982; Feng et al.,
393 2010; Córdova et al., 2018). Despite having a high proportion of lignin, cutin and

394 suberin, B40 presented a low R-index, reflecting low OM thermal stability (Sebag et
395 al., 2016). This apparently contradictory result between low R-index and abundance
396 of chemically stable compounds could indicate that thermal stability is not only
397 related to chemical stability is also also dependent on organo-mineral interactions.

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

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

419 undergone different human activities, not only the quantity of SOC, but also its
420 stability should be taken into account (Cotrufo et al., 2019).

421 The evaluation of physically separated fractionations can help to better distinguish the
422 respective effects of the preferential decomposition of the most labile organic
423 compounds and of organo-mineral interactions. The coarse fraction (>50 μ m) is
424 enriched in particulate OM and is considered a more labile fraction mainly constituted
425 of plant residues (Cotrufo et al., 2019), while the fine fraction (<50 μ m) has more
426 enriched mineral associated OM and is considered as the more stable fraction through
427 physical protection and organo-mineral interaction (Christensen, 2001; Jastrow et al.,
428 2007; Kögel-Knabner et al., 2008). Coupled to thermal analyses, the grain-size
429 fractions were already used to distinguish labile and stable C pools related to
430 environmental drivers (Saenger et al., 2015).

431 In the present study, lower thermal stability (R-index values) in coarse fractions (Fig.
432 3) are in line with results from Sebag et al. (2022) that demonstrated the ability of
433 fractionation to isolate weakly decomposed and more degradable OM. The samples
434 B40 and A40 exhibited lowest thermal stability despite very different HI values. This
435 confirms that quality and thermal stability are not always related in samples enriched
436 in plant debris. On the other hand, no significant differences were observed in the fine
437 fractions. This suggests that the composition of SOM is more homogeneous in the
438 fine fraction of subsoil layers. Thus, in the presence of mineral rich soil matrix, the
439 organo-mineral interactions play a more important role on the stability of the SOM
440 independently of its quality. The source of SOM in subsoil layers and fine fractions is
441 often attributed to more processed OM and microbial residues prone to organo-
442 mineral interaction (Miltner et al., 2012; Rumpel et al., 2012; Cotrufo et al., 2013).
443 Thus, the chemical composition from thermal analysis reflects this more

444 homogeneous (Grandy and Neff, 2008) and more stable source of carbon and could
445 explain the close values of the fine fractions. Similar results obtained by Sebag et al.
446 (2022) in different size fractions have been attributed to the plant-microbe-soil
447 continuum concept, and evidenced the role of interaction with the mineral matrix.

448

449 **5. Conclusions**

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

469 higher SOC in topsoil layers, this increase could be offset by a lower stability of the
470 SOC from the residue input. In consequence this study reinforces the necessity to
471 separately assess both SOC chemical composition and stability to estimate the long-
472 term effect of forest management.

473

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481

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Fig. captions

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

780

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

783

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

787

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

793

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

796

797 Table 1: Soil characteristics and description of the 5 sites

Site description	Layer	Mean annual carbon		CEC (cmol kg^{-1})	Texture			Water content (%)
		input (kg ha^{-1})			Clay%	Silt%	Sand%	
		Litter	Branch					
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)

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800

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

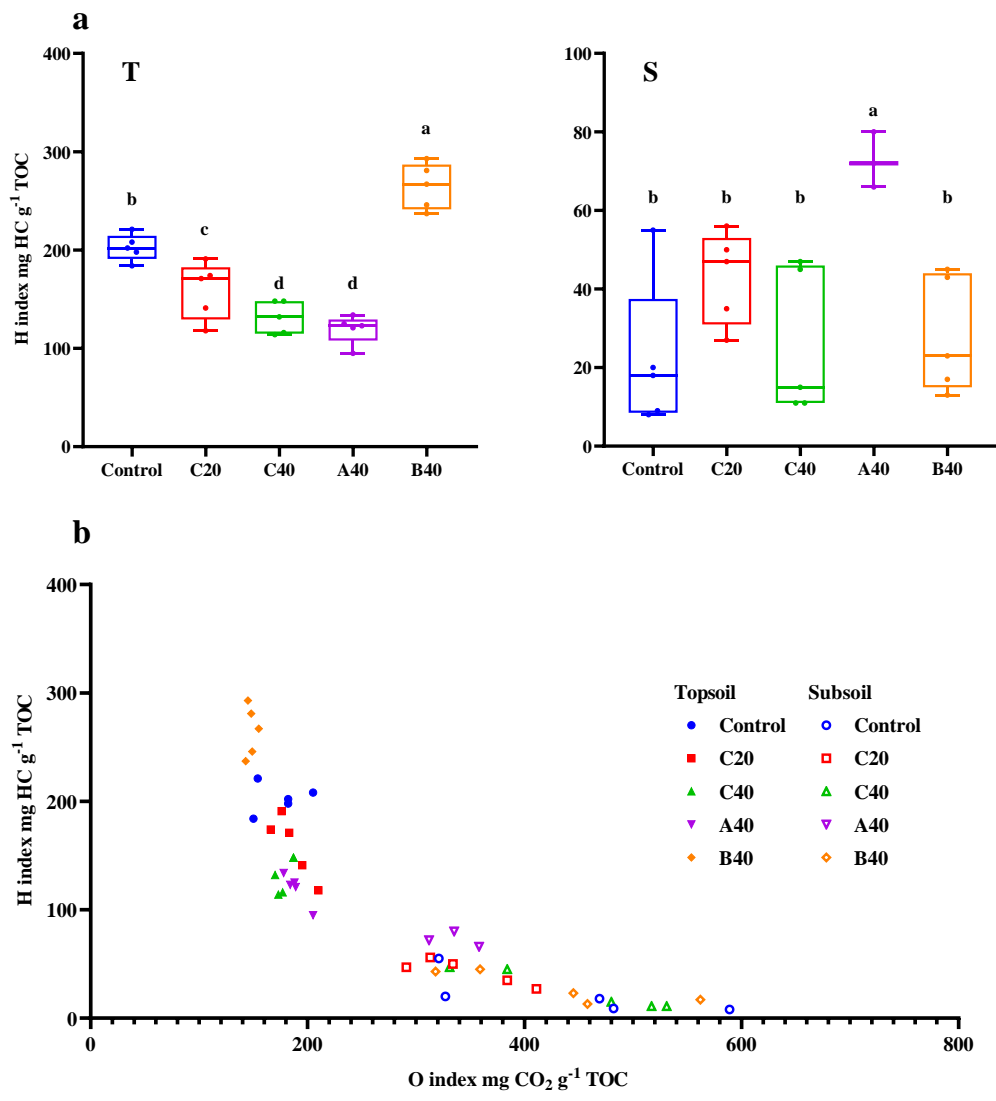
Sample's name	Topsoil			Subsoil		
	TOC (g kg ⁻¹)	TN (g kg ⁻¹)	C/N	TOC (g kg ⁻¹)	TN (g kg ⁻¹)	C/N
Control	16.31 (4.98)b	0.87 (0.30)c	18.98 (1.34)ab	4.11 (1.71) b	0.33 (0.08) b	12.06 (1.79) ab
C20	14.25 (5.03)b	0.79 (0.23)c	17.76 (1.84)b	4.24 (1.51) b	0.33 (0.07) b	12.38 (1.91) ab
C40	17.05 (1.65)b	0.91 (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

802 Different letters indicate significant differences between different treatments by

803 Duncan test (p < 0.05).

804

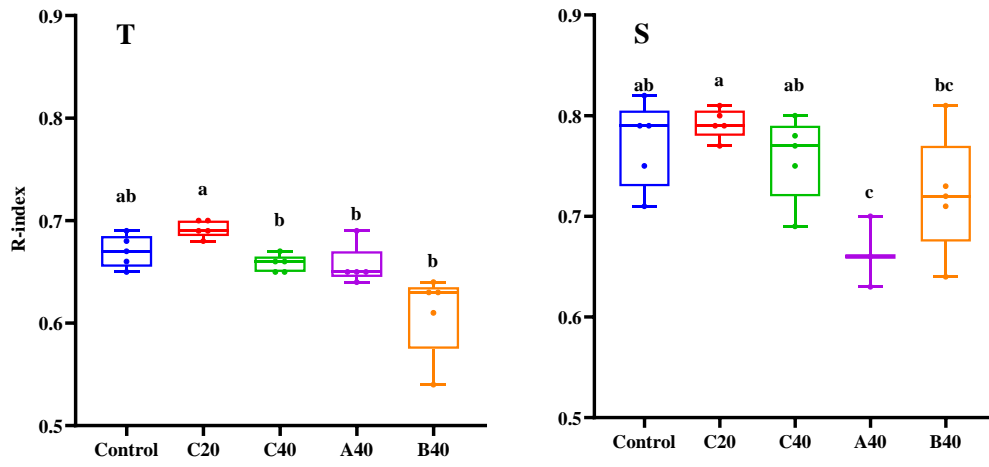
805 Figure 1



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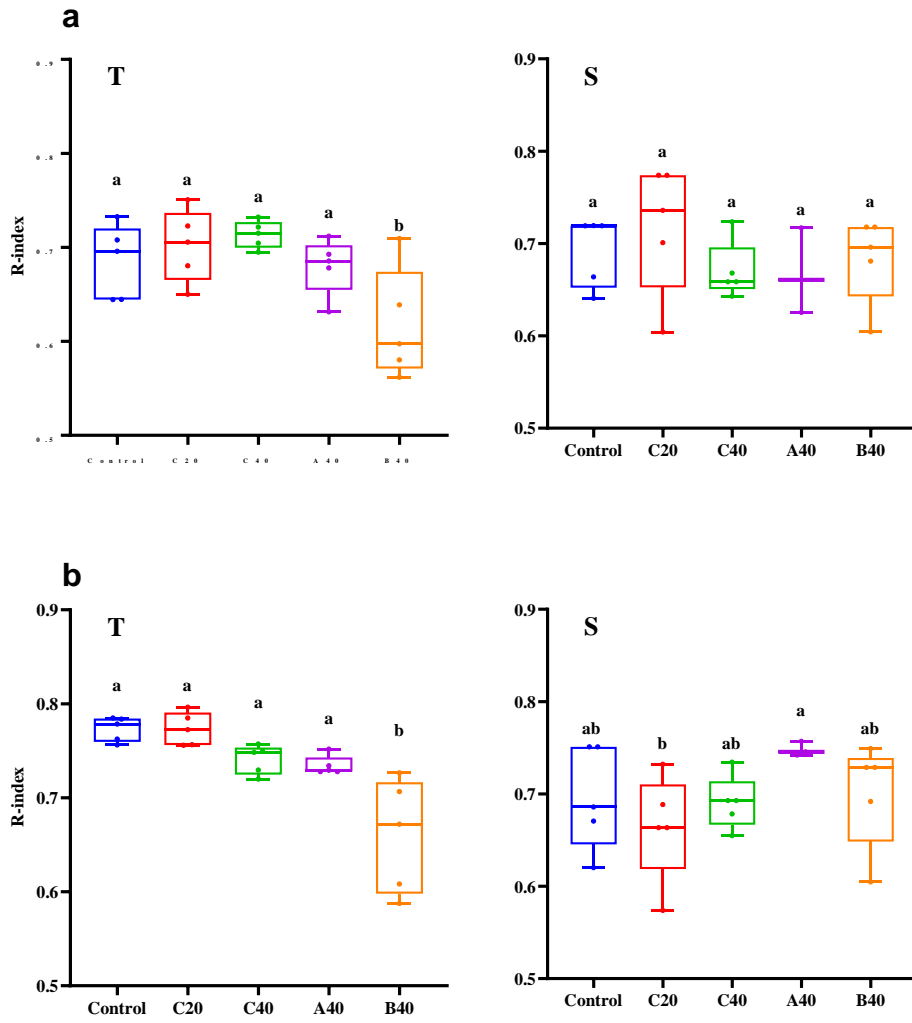
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808 Figure 2



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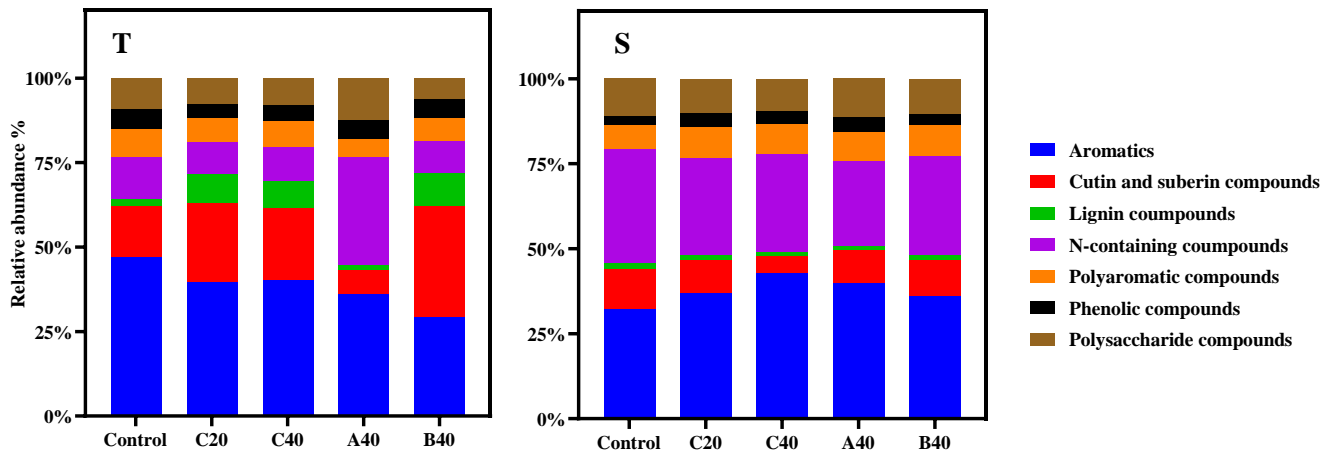
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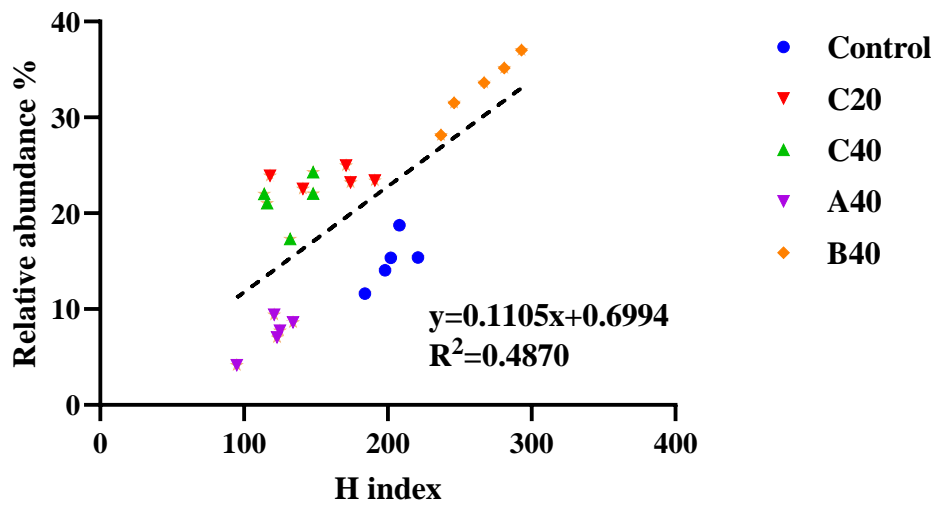
814 Figure 4



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818 Figure 5



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