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1 **The application of DFRC method for the analysis of carbohydrates in a peat bog:**
2 **validation and comparison with conventional chemical and thermochemical degradation**
3 **techniques**

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10 **Abstract**

11 Traditional acid hydrolysis is the main method used for carbohydrates characterization in
12 soils and sediments. It has been used to cleave the glycosidic bond, yielding monomers that
13 compose polysaccharides of the non-cellulosic pool (free, ligno-cellulosic and hemicellulosic
14 carbohydrates). In this study, the widely used chemical and thermochemical degradation
15 procedures have been compared with the derivatisation followed by reductive cleavage (DFRC)
16 method. This method presented efficiency in specifically cleaving the aryl ether bonds, yielding
17 for sugar moieties linked to aromatic structures (ligno-cellulosic fraction). Once applied for peat
18 samples, some discrepancies were observed. DFRC method revealed some information about the
19 origin of carbohydrates that was hidden from the traditional chemical and thermochemical
20 degradation methods. Several patterns of carbohydrates have been obtained with DFRC
21 indicating difference in the origin and/or degradation rates between monosaccharides. For
22 xylose, it showed an increase in the catotelm (deepest layer of the peatland), indicating an
23 angiosperm vegetation input at the early stage of the peatland formation. Ribose, commonly
24 known as a microbial indicator, showed the same profile as for hexoses and deoxyhexoses, with
25 acid hydrolysis. The difference in the profile yielded in the case of DFRC method, revealed its
26 microbial input. These information were hindered from acid hydrolysis and thermochemolysis,
27 as the first showed nearly the same profile for all monosaccharides and the second one didn't
28 have the capacity to analyze arabinose and ribose.

29 Generally, higher similarity was obtained between DFRC and acid hydrolysis than
30 between DFRC and thermochemolysis. This is due to the difference of mechanisms involved in
31 chemolytic and thermochemolytic methods. For the first group, both methods shared acid
32 conditions and milder temperature conditions if compared to the second group. In addition
33 thermochemolysis is performed under alkaline conditions.

34 **1. Introduction**

35 Northern peatlands are estimated to store up to a third of the terrestrial carbon as peat
36 (Vitt et al., 2000); the continental C as peat is estimated for 20% while covering up only 3% of
37 the Earth's surface (Gorham, 1991). These very complex ecosystems impact both carbon cycle
38 and water at local as well as global scale. Since peat is progenitor of coal, a better understanding

39 of the molecular fingerprint of different pools in peats will improve our insight in the formation
40 of coal.

41 Carbohydrates are the major pool of terrestrial vegetation and represent the main form of
42 photosynthetically assimilated organic matter (OM) in the biosphere. Understanding
43 carbohydrates dynamics in peatlands will allow a better understanding of coal formation. Given
44 their ubiquitous character, carbohydrates potentially represents a powerful tool for elucidating
45 processes, sources and diagenetic pathways of biologically important OM in sediments (Jia et al.,
46 2008). Although in most settings (soils and sediments) carbohydrates composition have shown
47 high sensitivity to diagenetic alterations, the OM-rich character of peat deposits and the potential
48 for preservation below the acrotelm makes *ombrotrophic* peatlands a suitable environment for
49 also exploring the potential of carbohydrates as paleovegetation proxies (Bourdon et al., 2000;
50 Comont et al., 2006; Delarue et al., 2011; Estournel-Pelardy et al., 2013; Younes et al., 2017).

51 The analysis of OM at the molecular level commonly requires several techniques and
52 each technique targets all patterns of one of the three major pools. Most studies dealing with
53 lignin analysis were conducted using cupric oxide under alkaline conditions (CuO-NaOH)
54 (Hedges and Ertel, 1982). For lipid fraction analysis, various organic solvents have been used
55 (Bligh and Dyer, 1959). For carbohydrates analysis in soils and sediments, it is mostly performed
56 with acid hydrolysis (Bourdon et al., 2000). The high heat and concentration of acid used,
57 contributes to the cleavage of all glycosidic bonds (relative to non-cellulosic/cellulosic fractions)
58 (Younes and Grasset, 2018). Derivatisation Followed by Reductive Cleavage (DFRC) method
59 showed a high efficiency for cleaving α and β -aryl ethers and is therefore suitable for ligno-
60 cellulose materials. Therefore, all studies conducted for the DFRC method were done for lignin
61 subunits analysis (Del Río et al., 2007; Grasset et al., 2010; Lu and Ralph, 1998; Matsui and
62 Ohira, 2013; Peng et al., 1999).

63
64 The only attempt to use this technique in a geochemistry study was done by Grasset et al.
65 (2010). The authors applied this technique to characterize lignin in gymnosperm wood, and in
66 soils and sediments formed from degraded gymnosperm wood, in order to assess the supply of
67 terrestrial OM to marine sediments. The purpose of applying this method is a need for finding
68 efficient and more selective method for lignin α - and β -aryl ether cleavage. It was evident that
69 the overall lignin yield and the compound ratios used as plant source proxies have been found to
70 be considerably different from those yielded from the mostly used lignin degradation techniques.

71
72 For our knowledge, this is the first attempt to analyze the carbohydrate pool in peat samples
73 with the DFRC method. One of the advantages of using peatland samples to conduct this
74 comparison is the bilayer character of this ecosystem. The upper half is the “living” part where
75 fresh OM input and microbial activity occur. The bottom half is the “dead” part where
76 decomposed OM is preserved making the peats a reference for paleoenvironmental
77 interpretations (see Younes et al. (2017)). In this study, the DFRC method was applied for
78 characterizing carbohydrates in a French peatland. In order to reveal the extent of DFRC method,
79 carbohydrates yielded moieties were compared with lignin input from CuO-NaOH oxidation and
80 the thermally assisted hydrolysis and methylation (THM) method. In case of discrepancy in
81 carbohydrates profile, this will give more insight into the molecular aspect of OM and the

82 occurrence of a carbohydrate fraction that was hindered from the acid hydrolysis and THM
83 method. PCA was used to compare between the employed chemical and thermochemical
84 degradation methods.
85

86 **2. Materials and methods**

87

88 *2.1. Sampling*

89

90 Samples were collected from a peatland site in the Limousin, France. Settings of the studied
91 site was developed in a previous study by Younes et al. (2017). Samples of a 1m peat core were
92 were divided into 4 cm thick slices and then freeze-dried.
93

93

94 *2.2. Derivatization followed by reductive cleavage (DFRC)*

95

96 The samples were subjected to DFRC analysis according to the procedure of Lu and Ralph,
97 (1998), as dried peat samples were treated with 5 mL of acetyl bromide/acetic acid (8% v/v),
98 stirred at 50 °C for 2 h, then evaporated under reduced pressure until dry. Zinc powder (500 mg)
99 and 25 mL of dioxane/acetic acid/water (5 :4 :1, v/v/v) were added and stirred for 30 min at 50
100 °C. Saturated aqueous ammonium chloride (50 mL) were added, whereupon the solution was
101 acidified to pH 3 with HCl. The mixture was then extracted with CHCl₃ 2 times (total 50 mL).
102 The chloroform layer was dried over magnesium sulfate, then evaporated. Degradation products
103 were acetylated with acetic anhydride (2 mL), acetic acid (0.1 mL) and 1-methylimidazole (0.1
104 mL) at room temperature for 10 min and analyzed by GC (Shimadzu GC-2010) with an FID and
105 by GC-MS and 50 µL of nanodecanoic acid methyl ester was added as an internal standard.
106

106

107 *2.3. Methods used for validation and comparison*

108 *2.3.1. CuO- NaOH oxidation for lignin characterization*

109

110 The release of lignin monomers was performed by alkaline CuO-NaOH oxidation. This is the
111 classical chemical degradation method used for lignin characterization. About 10 mg of the dried
112 peat samples was oxidized for 2 h at 170 °C in sealed Teflon vials, following the procedure
113 developed by Hedges and Ertel, (1982). CuO-NaOH oxidation yields a suite of one ring phenols
114 (H, *p*-hydroxyphenol; V, vanillyl; S, syringyl and C, cinnamyl; Table. 1) with their aldehyde,
115 ketone and acid counterparts (for further information see Younes et al. (2017) and Younes and
116 Grasset, (2018)).
117

117

118 *2.3.2. Acid hydrolysis*

119

120 Acid hydrolysis was carried out with HCl for 200-300 mg of the dried peat samples at 100 °C
121 for 3 h, the obtained monosaccharides (check Table. 1 for symbols) was derivatized into alditol
122 acetates following the procedure of Rumpel and Dignac, (2006) and well developed in a previous
123 study on the same site by Younes et al. (2017).
124

124

125 2.3.3. *Thermally Assisted Hydrolysis and Methylation (THM) method*

126

127 The THM method is based on the offline procedure developed by Grasset and Amblès,
128 (1998). About 50 mg of the dried peat samples were mixed with 4 ml of tetramethylammonium
129 hydroxide (TMAH) and heated at 400 °C (for further information see Younes and Grasset,
130 (2018)). THM method yields a suite of monosaccharides and one ring phenols (check Table. 1
131 for symbols).

132 2.4. *Gas Chromatography (GC) analysis*

133

134 GC measurements were performed with a SHIMADZU GC-2010 Plus gas
135 chromatograph equipped with a flame ionization detector at 300 °C. Separation of the
136 compounds was achieved with a 30 m fused silica capillary column (Supelco Equity 5%, 0.25
137 mm internal diameter, 0.25 µm film thickness), and helium as carrier gas. The oven temperature
138 was programmed from 60 to 300 °C at 5 °C min⁻¹ and held for 15 min.

139

140 2.5. Data normalization

141 The relative contribution of monosaccharide with depth were scaled and centered
142 following the formula below:

$$143 \text{ Normalized } P_i(\%) = \frac{P_i - P_{\min}(\%)}{P_{\max}(\%) - P_{\min}(\%)}$$

144 Where:

145 $P_{\min}(\%)$ = smallest $P_i(\%)$ for each monosaccharide along peatland depth records,

146 $P_{\max}(\%)$ = biggest $P_i(\%)$ for each monosaccharide along peatland depth records.

147

148 2.6. PCA

149 PCA is used in order to reduce dimensionality of dataset by producing linear
150 combinations of original variables. PCA breaks down the matrix of initial data, X, to express
151 them as a least-square model (Geladi et al., 2003):

$$152 X = A \times F + U$$

153 Where, X is the original data matrix, A is the matrix of loadings of the original
154 variables in the new reduced space, F is the matrix of scores of objects or samples and U is the
155 matrix of residuals. PCA aims to reduce the dimensionality of datasets containing many
156 interrelated variables, while retaining as much as possible of the systematic variation in the
157 original data (Jolliffe and Morgan, 1992). In the analysis, the original variables are replaced by a
158 few principal components (PCs) variables. The PCs are totally independent with each other and
159 ordered so that PC₁ contains most of the original variation and PC₂ contains the second largest
160 amounts, etc. PCA was carried out using XLSTAT 2014.5 software. The peat depth samples
161 were considered as the observations and normalized data of monosaccharide and lignin as the
162 variables.

163

164 3. Results and Discussion

165 3.1. *Validation of the DFRC method for carbohydrates molecular characterization :*

166 In order to validate the DFRC method as an efficient technique for the molecular
167 characterization of carbohydrates, the relative abundance of total carbohydrates yielded with
168 DFRC has been compared to lignin content yielded by CuO-NaOH oxidation and THM method

169 in one hand and to the total carbohydrates yielded with HCl hydrolysis and THM method in
170 another hand (Fig. 1).

171 3.1.1. Carbohydrates vs. Lignin input

172 Contrary to monosaccharides, lignin biomarkers provide more reliable proxies for vascular
173 plant input (Filley et al., 2006; Wysocki et al., 2008; Mason et al., 2009; Abbott et al., 2013;
174 Younes and Grasset, 2018). The relative abundance of carbohydrates can be examined using the
175 ratio of carbohydrates yield (mg carbohydrate / 1 g OC; Jia et al., 2008) at a given depth in the
176 peat core. Fig. 1 presents bi-dimensional plots of total carbohydrates yielded with DFRC, HCl
177 hydrolysis and THM method as a function of “ Λ ” proxies, yielded by CuO-NaOH oxidation and
178 THM method. Lignin proxies were used in Younes and Grasset, (2018) as described by Filley et
179 al. (2006) with all phenols normalized to 1 g OC. $\Lambda_{\text{THM}} = G_4 + G_5 + G_6 + S_4 + S_5 + S_6 +$
180 $G_{18} + P_{18}$ and $\Lambda_{\text{CuO-NaOH}} = S + V + C$, indicates the relative lignin yielded by THM method and
181 CuO-NaOH oxidation, respectively.

182 Because THM primarily cleaves β -O-4 bonds and CuO-NaOH oxidation presumably cleaves
183 β -O-4 as well as some C-C bond, the latter accesses a larger part of the unaltered non-oxidized
184 lignin structure (Filley et al., 2006; Wysocki et al., 2008; Younes and Grasset, 2018).

185 Following Fig. 1, high correlation were obtained between input of carbohydrates yielded by
186 DFRC method and HCl hydrolysis, from one side, and lignin yielded by CuO-NaOH oxidation
187 from the other side (67.45% and 69.57% for DFRC method and HCl hydrolysis, respectively).
188 These trends indicate the high correlation of hemicellulosic and ligno-cellulosic fraction along
189 the peat core. The low correlation between the input of carbohydrates yielded by DFRC and HCl
190 hydrolysis from one side, and lignin yielded by THM from another side (4.56% and 2.43% for
191 DFRC and HCl hydrolysis, respectively) indicates that the ligno-cellulosic fraction of the
192 investigated peat core is bonded to the intact non-oxidized lignin that is most likely revealed by
193 CuO-NaOH oxidation. Surprisingly, even the total carbohydrates yielded with THM method
194 showed a low correlation with Λ_{THM} . Its higher correlation with carbohydrates yielded with
195 DFRC indicates that the DFRC method is a more reliable technique for carbohydrates analysis as
196 it reveals both the free and ligno-cellulosic fraction rather than only the free and terminal
197 carbohydrates, in the case of THM method. This is due to a higher efficiency of ether cleavage
198 under acidic conditions (Acetic acid used) in case of DFRC than basic conditions (pK_b of TMAH
199 = 4.2) in case of THM (Wade, 2012).

200 3.1.2. Relative carbohydrate abundance: DFRC vs. Acid Hydrolysis & THM

201 The total non-cellulose sugar content varied from 6 mg/g at the top to 1 mg/g at the bottom of
202 the peat core, in the case of DFRC method. For HCl hydrolysis and THM method, it varied from
203 26 mg/g at the top to 4 mg/g at the bottom of the peat core and from 227 mg/g in the acrotelm to
204 29 mg/g at the bottom of the peat core, respectively (see Supplementary Information). The
205 difference in the yielded amounts shows the capacity of HCl hydrolysis to target the whole non-
206 cellulose fraction (free sugars, sugars bonded to polyphenols and hemicellulose); as for DFRC
207 method, it targets only a specific pool of free sugars and sugars bonded to polyphenols. For THM
208 method, it has showed the capacity of targeting free and terminal carbohydrates (Younes and

209 Grasset, 2018; Estournel-Pelardy et al., 2011) which is more likely a smaller pool compared to
210 hemicellulose and ligno-cellulose. The higher abundance yielded for THM method is related to
211 the semi-quantitative approach used in this case. This was not the case of DFRC method and HCl
212 hydrolysis, as full quantitative approach is used (response factor of each monosaccharide is
213 identified with calibration curves).

214 Fig. 2 shows that depth amounts of the nine investigated monosaccharides was higher in the
215 acrotelm (0 to -20 cm) than the mesotelm (-20 to -50 cm) and catotelm (-50 to -100 cm), for all
216 three methods, indicating degradation of carbohydrates with depth (Jia et al., 2008). High
217 correlation between DFRC method and HCl hydrolysis has been obtained (between 65% and
218 87%, Fig. 2). This shows a high consistency between the two methods and proves the findings of
219 the comparison between carbohydrates amounts and lignin input (Fig. 1) as hemicellulosic and
220 ligno-cellulosic pools occurs in a synchronous fashion in the investigated peat core. Lower
221 correlation has been obtained between DFRC and THM methods (between 32% and 41%, Fig.
222 2). Even know that these methods share the free sugar pools, but this pool is more likely less
223 frequent in a terrigenous sediment as peatland, if compared with the occurrence of ligno-
224 cellulose and hemicellulose (Clymo, 1991).

225 More detailed investigations revealed several patterns. For acid hydrolysis, two main patterns
226 of variation in concentration of each group of monosaccharides were revealed. The first
227 composed of Ara)_{HCl} and Xyl)_{HCl}, showed a rapid decrease from the first depth of the acrotelm (0
228 to -20 cm), and concentration was approximatively constant down core. The second pattern
229 composed of Deoxyhexoses (Rha + Fuc)_{HCl}, Rib)_{HCl} and hexoses (Glu + Man)_{HCl} and Gal)_{HCl})
230 showed a comparatively slower decline down core (for further details see Younes et al. (2017)).
231 For THM method, the highest input was observed at -20 cm, indicating a free and/or terminal
232 carbohydrate occurrence. The interface between the mesotelm and the catotelm (around -50 cm)
233 showed an increase in non-cellulosic carbohydrates. This indicates free carbohydrates input at
234 the interface of these ecological layers and a probable microbial deposition during peatland
235 accumulation. In the catotelm, the higher input of Xyl)_{THM} indicates a past vegetation changes
236 and/or past humification processes (Cowie and Hedges, 1984), due to the alkali attack of TMAH
237 (for further details see Younes and Grasset, 2018).

238 For DFRC method, different monosaccharides showed a significant difference and the
239 decreasing trend was skewed by small increases at particular positions. For pentose pattern
240 (Ara)_{DFRC} and Xyl)_{DFRC}, it showed a different decreasing profile, Ara significantly decreases
241 along depth as for Xyl it showed a more stable input in the acrotelm and an increase at the
242 interface between mesotelm and catotelm and at -70 cm in the catotelm. These trends indicate
243 some difference in the origin between pentoses and/or degradation rate that was hindered by HCl
244 hydrolysis (same profile for Xyl)_{HCl} and Ara)_{HCl}; Fig. 2) and THM (no detection of Ara)_{THM}; Fig.
245 2). For the acrotelm and mesotelm, the higher decrease of Ara)_{DFRC} from the very first depth
246 records indicates a higher degradation rate, once compared with Xyl)_{DFRC}. In the catotelm, the
247 increase of Xyl)_{DFRC}, at particular depths, indicates the preservation of a ligno-cellulosic fraction
248 downcore and an input of vascular vegetation. Several studies have shown a higher input of Xyl
249 in angiosperms than gymnosperms (Wicks et al., 1991). In our case, the angiosperm input in the

250 first stages of the Sagnes peatland formation was shown by an S/V value of 1 by lignin analysis
251 (Younes et al., 2017). These trends indicate that differential degradation between
252 monosaccharides occurred and suggests that Ara and Xyl are components of polysaccharides that
253 are more degradable than other carbohydrates. Differences in degradation among carbohydrates
254 have been observed in several investigations (Jia et al., 2008). Thus the preferential degradation
255 of Ara and Xyl, compared to deoxyhexoses and hexoses, suggests that these monosaccharides
256 were in a less refractory form. The difference of depth profile, in the case of DFRC method, and
257 the absence of increases in the case of Ara indicates that this monosaccharide presented a higher
258 degradation rate, in comparison with Xyl.

259 For Deoxyhexoses (Rha)_{DFRC} and Fuc)_{DFRC}) and hexoses (Gal)_{DFRC} and Glu + Man)_{DFRC}), it
260 showed approximatively the same profile (Fig. 2). The highest input was around -20 cm at the
261 interface between acrotelm and mesotelm. Unlike the decreasing trend in the case of HCl
262 hydrolysis, monosaccharides of this pattern showed their highest input in the mesotelm. This
263 indicates most likely their belonging to the same origin.

264 3.2. Geochemistry of Carbohydrates

265 3.2.1. Source identification

266 Carbohydrate moieties can come from different plant and microbial sources. Therefore, their
267 compositional patterns can be useful to differentiate between terrestrial and microbial origin, as
268 well as some specific tissue or type of organisms within each of these categories (Cowie and
269 Hedges, 1984; Popper and Fry, 2003; Comont et al., 2006; Jia et al., 2008; Delarue et al., 2011;
270 Younes et al., 2017; Younes and Grasset, 2018). Carbohydrate monomers have been investigated
271 to a lesser extent than lignin moieties. This is due to the rapid degradation of carbohydrates and
272 their incorporation in the so called “humic fraction” (Estournel-Pelardy et al., 2013; Younes and
273 Grasset, 2017; Younes and Grasset, 2018).

274

275 Despite the limited number of combinations that can be done with carbohydrate moieties, the
276 variation in the compositions of hemicelluloses from one species to another can provide a plant
277 source signature (Comont et al., 2006). Xyl and Ara are indicators of vascular plant input (Cowie
278 and Hedges, 1984). Popper and Fry (2003) mentioned the occurrence of high proportions of Gal,
279 Rha and Man in the hemicelluloses of bryophyte primary cell walls. Comont et al. (2006)
280 showed that these monosaccharides are indicators of moss in ombrotrophic peat bogs.
281 Furthermore, Fuc and Rha could originate from *Sphagnum* species, given the work of Jia et al.
282 (2008). Other studies have attributed the contribution of these monosaccharides to microbial
283 biomass (Cowie and Hedges, 1984). In our case, deoxyhexoses (Rha + Fuc)_{DFRC} and hexoses
284 (Gal)_{DFRC} and Glu + Man)_{DFRC}) showed a similar pattern between them and a different one with
285 Rib)_{DFRC} (Fig. 2). The latter is considered to have a microbial origin (Jia et al., 2008). Delarue et
286 al. (2011) identified Rib as marker of bacterial and/or fungal activity in peatlands. Following the
287 present data and given the previous studies, hexoses and deoxyhexoses are considered as a
288 *Sphagnum* indicator and Rib a microbial indicator.

289 3.2.2. Carbohydrate proxies :

290 In order to assess the origin of carbohydrates, two ratios were commonly employed for
291 monosaccharides (Amelung et al., 1996; Nierop et al., 2001; Rumpel and Dignac, 2006), the
292 C_6/C_5 (Gal + Man / Xyl + Ara) and the Deoxy/ C_5 (Rha + Fuc / Xyl + Ara).

293 Bi-dimensional plots of carbohydrates ratios obtained with DFRC method as a function of
294 their HCl hydrolysis and THM method homologues are presented in Fig. 3. For HCl hydrolysis,
295 both ratios showed roughly the same profile with depth (Fig. 3), as their values increased from 0
296 to -20 cm and decreased slightly at the remainder of the core. This indicates a probable microbial
297 input occurred in the mesotelm (Younes et al., 2017). In the case of DFRC method, smaller
298 values of both ratios were obtained (between 0.96 and 0.20 for DFRC against 0.85 and 3.21 for
299 HCl hydrolysis, in the case of C_6/C_5 ; and between 0.03 and 0.18 for DFRC against 0.08 and 0.47
300 for HCl hydrolysis, in the case of Deoxy/ C_5 ; Fig. 3). This difference of magnitude between the
301 two methods, could be explained by the higher concentration of Ara and Xyl yielded with DFRC
302 method, as for the HCl hydrolysis both pentoses and hexoses yielded concentrations of the same
303 magnitude. Both ratios showed two increases, one located in the mesotelm and the other one in
304 the catotelm. For the mesotelm, it could be an indicator of microbial and/or *Sphagnum*
305 dominance in the upper half of the peat core. The latter is more probable as a microbial
306 dominance is discarded in this case. For the catotelm, it could be an indicator of an old microbial
307 deposit that was hidden from the mostly used acid hydrolysis method. Once DFRC and acid
308 hydrolysis were compared, high correlation were obtained for Deoxy/ C_5 (67.88 %; Fig. 3) and a
309 low correlation for C_6/C_5 (15.2 %; Fig. 3). This indicates the occurrence of a hemicellulosic
310 fraction of hexoses (Gal and Man) that is inaccessible to the DFRC method. Although small
311 correlation was obtained between $(C_6/C_5)_{HCl}$ and $(Gal/C_5)_{DFRC}$, this comparison have allowed to
312 split the peat core into the three ecological layers. The acrotelm is characterized by small values
313 of both ratios, indicating the input of Xyl and Ara from the Sedges vegetation growing the
314 uppermost depth (Younes et al., 2017). The mesotelm is characterized by low values of
315 $(Gal/C_5)_{DFRC}$ and high values of $(C_6/C_5)_{HCl}$, indicating the occurrence of a hemicellulosic fraction
316 mainly composed of Gal and Man that was hindered from the DFRC method. The catotelm
317 characterized by high values of both ratios due to the higher rate of degradation of pentoses
318 compared to hexoses (Jia et al., 2008; Younes et al., 2017).

319 $(Xyl/Glu)_{THM}$ has been used to distinguish between angiosperm and gymnosperm occurrence
320 (Grasset et al., 2009; Younes and Grasset, 2018). Xyl is an indicator of plant input and it is
321 produced in a more significant way by angiosperms than gymnosperms vegetation (Cowie and
322 Hedges, 1984). Glu is characterized by its ubiquity in the living kingdom and is commonly
323 present at high levels in vascular vegetation (Cowie and Hedges, 1984). Comparison of this ratio
324 with it's DFRC homologue showed a low correlation (5.7 %; Fig. 3), even know both methods
325 showed a decreasing profile of Xyl is the upper half (acrotelm and mesotelm; Fig. 2) and an
326 increase in the bottom half (catotelm; Fig. 2). Minor correlations between DFRC and THM
327 methods ratios is due to the difference of mechanisms occurred between these two methods.
328 DFRC is a chemolytic method in acid conditions yet THM is a high temperature
329 thermochemolysis under alkaline conditions. This allow the latter method to access the so-called
330 "Humic Fraction" that will be hindered from molecular degradation methods under acid
331 conditions.

332 *3.3. Mechanistic and technical implications*

333 The DFRC degradation method includes two key steps: (a) solubilization of lignin by
334 bromination and acetylation with AcBr and (b) reductive cleavage catalyzed by solid zinc (Zn;
335 Lu and Ralph, 1998). Since ligno-cellulosic fraction is the matter of interest of this study,
336 adjacent phenolic structures at the α - and β - positions were substituted by monosaccharides
337 (highlighted in blue) (Fig. 4).

338 For the free monosaccharides, the lone pairs of electrons of the hydroxyl groups will attack
339 the carbonyl function of the acetyl bromide. This attack is enhanced by the fact that bromide is
340 considered as a good leaving group. For the ligno-cellulosic bond, the bromide will perform a
341 nucleophilic substitution (SN) reaction, releasing monosaccharides directly bonded to the
342 aromatic structure. The substitution is of SN₂ since the monosaccharide is bonded to the aryl
343 group by the primary alcohol (Wade, 2012). Once the monosaccharide is released, it will
344 undergo acetylation. The Zn is well known as a reducing agent (Wade, 2012), its function in this
345 case is to reduce the α,β -Carbon bond. This reduction will enhance the elimination of the β -
346 glycosidic bond and the yielded monosaccharide will attack the carbonyl group of an acetyl
347 bromide, thereby performing acetylation.

348 Following this mechanism, several assumptions can be made about the efficiency of DFRC
349 as a degradation technique, thereby confirming or infirming its efficiency in the molecular
350 characterization of carbohydrates in sediments:

- 351 (1) An exclusive cleavage to lignin moieties as proposed by Lu and Ralph, (1998).
- 352 (2) Higher yield for DFRC due to the higher corrosive effect and higher affinity of acetic
353 acid and acetyl bromide compared with a mineral acid the HCl (since the investigated
354 matrix is OM).
- 355 (3) Lower yield but higher selectivity for DFRC due the milder reaction conditions once
356 compared with the conditions of HCl hydrolysis (See Materials and Methods).

357 Assumption (1) is directly discarded since the carbohydrates has been analyzed via DFRC.
358 Assumption (2) can also be discarded as the DFRC yielded lower amounts for all
359 monosaccharides. Assumption (3) can be accepted, since a very good correlation is obtained for
360 the relative amount of monosaccharides between the two methods ($52\% < R^2 < 87\%$; Fig. 2).
361 HCl is considered as a strong acid, its capacity to cleave ether and ester type bonds has been
362 proven (Wade, 2012), especially if manipulated at high temperatures (> 100 °C). Therefore, we
363 consider that all non-cellulosic bonds are cleaved with the followed HCl procedure. The highest
364 correlation between DFRC and HCl hydrolysis were yielded for Xyl and Ara (Fig. 2). These
365 monosaccharides are considered as a vascular vegetation indicator. The obtained trends can show
366 the efficiency of AcBr in cleaving the ligno-cellulosic bonds and that pentoses are the major
367 carbohydrate components of ligno-cellulose. Lower correlations for the other monosaccharides
368 can be related to the occurrence of hexoses and deoxyhexoses in the free, hemicellulose and
369 ligno-cellulose compartments.

370 At the technical level, DFRC method is an all-in-one method for the characterization of
371 various classes of soil OM, such as carbohydrates, lignin and lipids. Several studies have used

372 this procedure or similar ones in order to analyse lignins (Lu and Ralph, 1998; Matsui and Ohira,
373 2013) and aromatic compounds in fossilized sediments (Grasset et al., 2010). In this study,
374 although we were interested in revealing the capacity of this degradation technique to analyze
375 carbohydrates, it yielded aromatic compounds, fatty acids and ω -hydroxy fatty acids. These
376 families of molecules will be investigated in upcoming studies.

377 If one considers the time and temperature it takes for the different methods (2 h and 50 °C for
378 DFRC method, 3 h and 100 °C, for acid hydrolysis and 30 min and 400 °C for THM method),
379 DFRC is faster and/or requires milder conditions.

380 Beyond the yielded molecules, DFRC extract varied in weight from 300 mg/g to 500 mg/g of
381 the freeze-dried weighted peat samples. This reveals, not only the degradation capacity, yet the
382 extraction capacity of this method. A consideration of a double-shot procedure, like DFRC
383 treatment followed by acid/alkaline hydrolysis could be interesting (*study under progress*).

384 In its mechanistic behavior, the acetyl bromide and acetic acid combination employed in
385 DFRC, has the capacity to degrade, in a specific way, ligno-cellulose fraction and/or free
386 carbohydrates entrapped in aromatic macromolecular structures. The “softer” acidic conditions
387 for DFRC method, compared to acid hydrolysis, and the difference of the temperatures used, will
388 make the cleavage of the strong glycosidic bond less probable, in the case of DFRC method.

389 3.4. Statistical analysis of method comparison

390 The application of PCA in geochemistry falls into three categories. (1) Determine OM
391 sources based on biomarker specificity (terrestrial or marine) and trace the fate of OM in the
392 oceanic environment (Hu et al., 2006). (2) Revealing similarities and dissimilarities among
393 samples (Pearson et al., 2008). (3) Uncovering relationships among variables by developing
394 summary statistics (i.e. “degradation indices”) (Boheme et al., 2004; Younes et al., 2017). Other
395 studies have, specifically, focused on the application of multidimensional statistical analysis for
396 interpreting molecular fingerprints of peat. Schellekens et al. (2009, 2011, 2012, 2015 a and b)
397 used the factor analysis method to extract parameters that might be used to interpret the
398 vegetation change, anaerobic and aerobic decomposition and fire incidence of several peat bogs.
399 In our case, PCA was used in order to compare between samples and between two methods of
400 degradation.

401 Some of the strengths of PCA is its ability to analyze variables with interactive relationships
402 as they vary with respect to other variables. This method removes intercorrelation between
403 variables by creating independent ones (PC₁, PC₂ ...). It also presents an “all in one” data
404 visualization method as one can assess geochemical relationships among different compound
405 classes (Younes and Grasset, 2017). In our analysis, two PCAs has been performed in order to
406 statistically validate the comparison between DFRC and HCl hydrolysis and CuO-NaOH
407 oxidation, from one side, and DFRC and THM methods from another side (Fig. 5). This division
408 is done following the difference of mechanisms between the employed methods. As DFRC,
409 CuO-NaOH oxidation and HCl hydrolysis are qualified as “Chemical Degradation Methods” and
410 THM is qualified as a “Thermochemical Method”.

411 PC₁ and PC₂ of the chemical methods (left plot; Fig. 5) accounted for 86.70% of the total
412 variance. Lower but a high variance was obtained once DFRC and THM methods data was
413 investigated (PC₁ and PC₂ accounted for 76.31% of the total variance; Fig.5, right plot). These

414 values indicate that the comparison of the employed parameters is statistically meaningful for all
415 methods and reliable trends can be concluded from this study.

416 For the chemical methods (left plot, Fig. 5), PCA analysis showed all four ratios (Deoxy/C₅
417 and C₆/C₅) plotted together, indicating a stronger relationship than suggested by simple
418 correlation. Ara and Xyl in DFRC and HCl hydrolysis were closer to $\Lambda_{\text{CuO-NaOH}}$ than other
419 monosaccharides. This indicates that the ligno-cellulosic fraction is more likely composed of
420 pentoses than hexoses proving the higher Xyl contribution in the case of DFRC method (Fig. 2).
421 For the PCA of DFRC and THM methods (right plot, Fig. 5), lower clustered were yielded than
422 the case of chemical methods. Although carbohydrate moieties of the two methods were
423 clustered together, Λ_{THM} was clustered by itself in the negative side of both PCs.

424

425 **Conclusion**

426 This study presents the application of a recently developed and less used molecular analytical
427 technique for the characterization of the carbohydrate pool in peat samples, the DFRC method.
428 Acetyl bromide method is previously used to digest aromatic macromolecular structures, for
429 analytical and industrial purposes. Following this principle, an application of this degradation
430 technique to the OM matrix of a peatland will allow the access of a specific carbohydrate pool
431 linked to lignin/polyphenols and/or entrapped into macromolecular aromatic structures.

432 In order to validate the DFRC method, a comparison have been held with lignin input. The
433 high correlation between total carbohydrates yielded with DFRC method and acid hydrolysis
434 from one side and lignin input yielded with CuO-NaOH oxidation, from another side, indicates
435 that hemi-cellulosic and ligno-cellulosic pools of carbohydrates occurs simultaneously along the
436 investigated peat core. It also shows that the ligno-cellulosic fraction is more likely linked to the
437 intact non-oxidized lignin (yielded with CuO-NaOH oxidation).

438 Lower carbohydrates abundance, in the case of DFRC method, indicates its capacity to only
439 target a portion of the carbohydrate fraction which is the free and ligno-cellulosic pools. For
440 carbohydrates amounts, all three methods (acid hydrolysis and DFRC and THM methods)
441 showed a decreasing trend from acrotelm, mesotelm and catotelm indicating the degradation of
442 carbohydrates with depth.

443 Unlike acid hydrolysis, the DFRC method showed several patterns. This indicates some
444 difference in the origin of the yielded monosaccharides. DFRC showed some difference in the
445 origin and/or degradation rate between Xyl and Ara. It also allowed the identification of Rib as a
446 microbial indicator. These information were hindered from acid hydrolysis and THM method, as
447 the first showed nearly the same profile for all monosaccharides and the second one didn't have
448 the capacity to analyze Ara and Rib.

449 Briefly, higher correlation (therefore higher similarity) was obtained between DFRC and acid
450 hydrolysis than between DFRC and THM methods. This is due to the difference of mechanisms
451 involved in chemolytic and thermochemolytic methods. For the first group, both methods shared
452 acid conditions and milder temperature conditions if compared to the second group. In addition
453 thermochemolysis is performed under alkaline conditions. These differences allow the
454 application of chemical degradation methods to more recent less oxidized sediments and

455 thermochemical methods to ancient more oxidized sediments. In order to confirm this scope of
456 applications, additional analyses should be performed on multiple soils and sediments samples.
457 Therefore, we caution against making direct comparison of carbohydrates data yielded with the
458 three methods because of different mechanisms involved in each process. Yet DFRC shows as a
459 prominent method for the molecular characterization of carbohydrates in soils and sediments.

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Fig. 1. Total Carbohydrates (normalized per 1 g OC) yielded by DFRC method compared to total lignin yielded by CuO-NaOH oxidation and THM method.

Fig. 2. Depth record of relative carbohydrate abundance (mg carbohydrate / 1 g OC) with DFRC method, acid hydrolysis and THM method (mg carbohydrate / 1 g OC). Small boxes in HCl hydrolysis and THM method charts present the relationship between relative carbohydrate abundance of these methods from one side and DFRC method from other side (R^2 is the coefficient of determination).

Fig. 3. DFRC carbohydrate proxies compared to their homologues yielded by acid hydrolysis (HCl) and THM method.

Fig. 4. Comparison of the Lu and Ralph mechanism with the proposed mechanism

Fig. 5. PCA of carbohydrate and lignin parameters analyzed using DFRC and CuO-NaOH and HCl (left plot) and DFRC and THM (right plot).

Fig.1

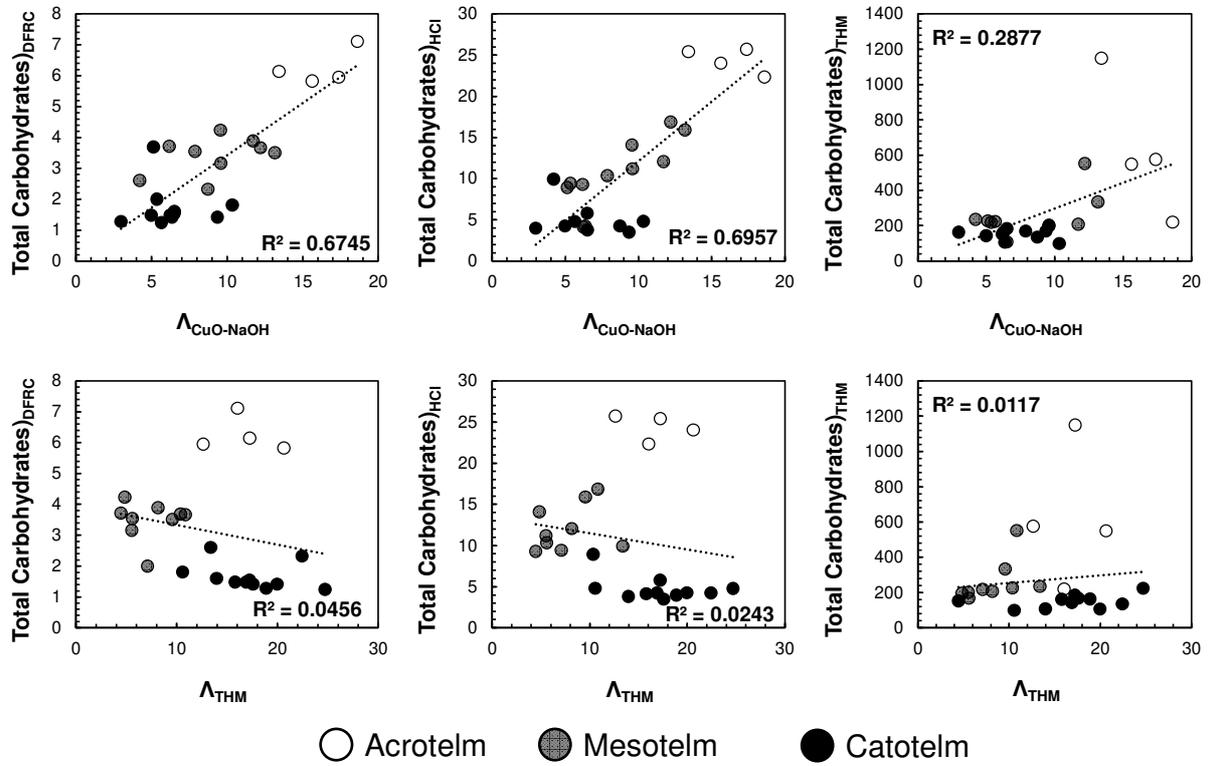


Fig. 2

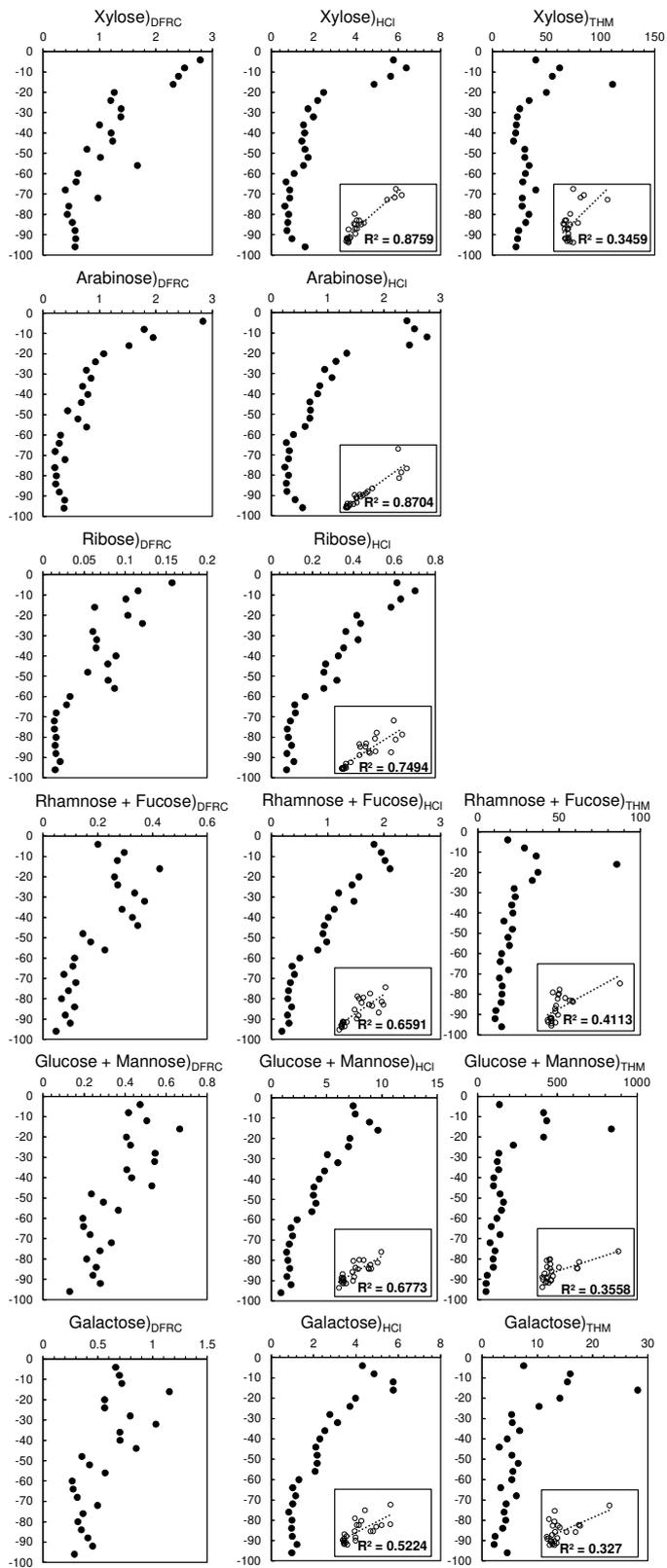


Fig. 3

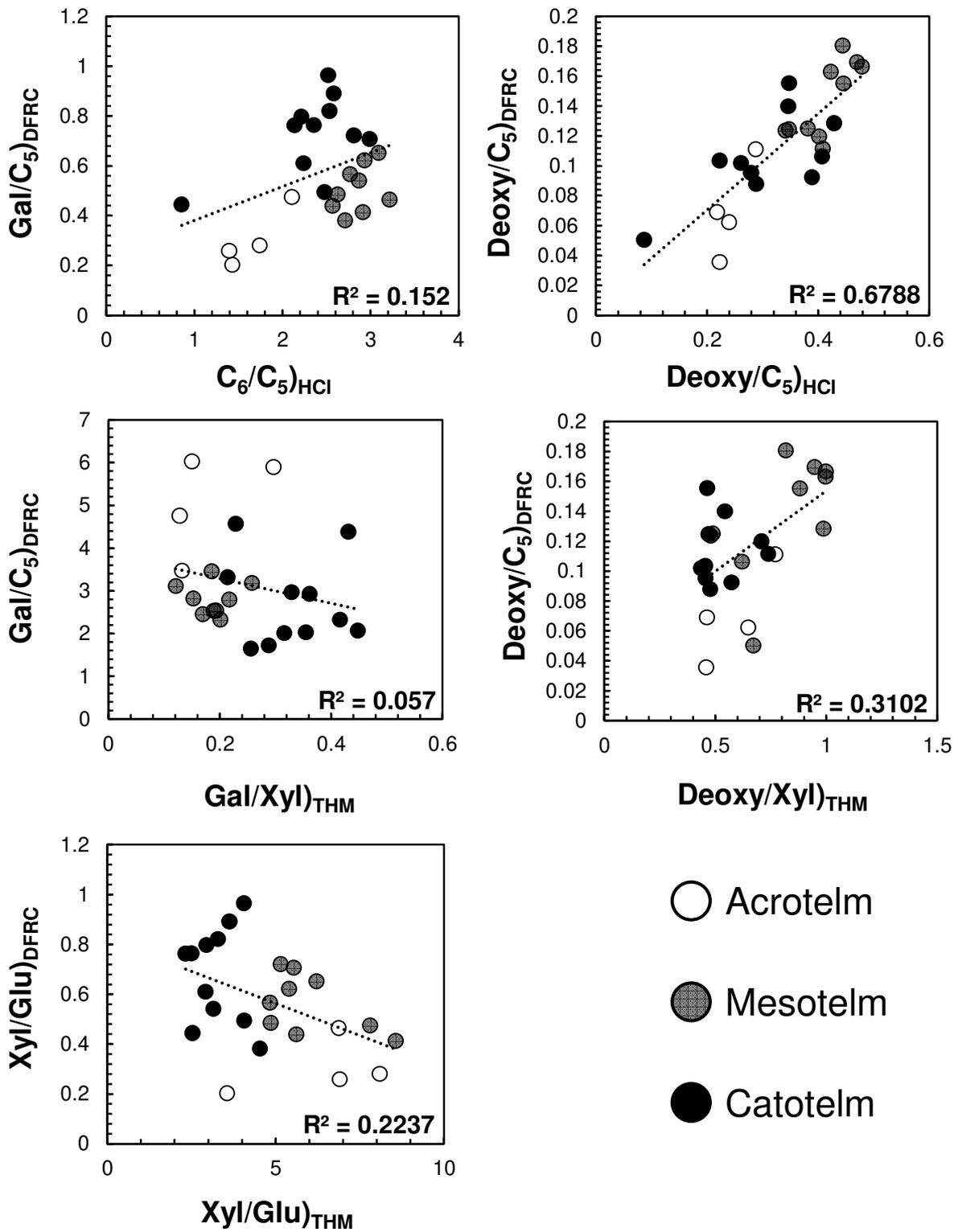


Fig 4

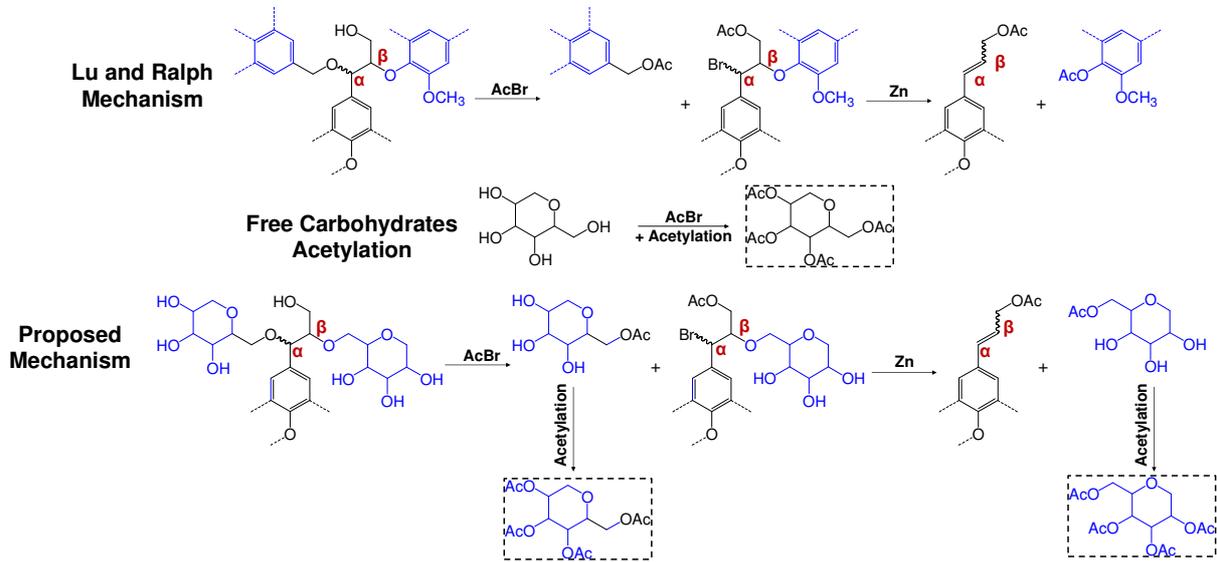


Fig. 5

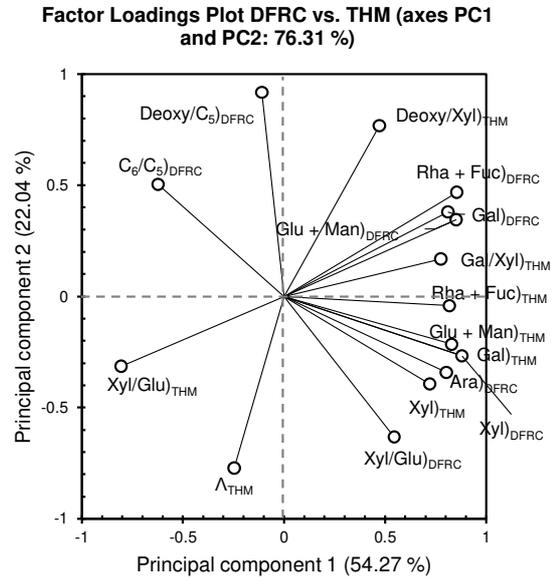
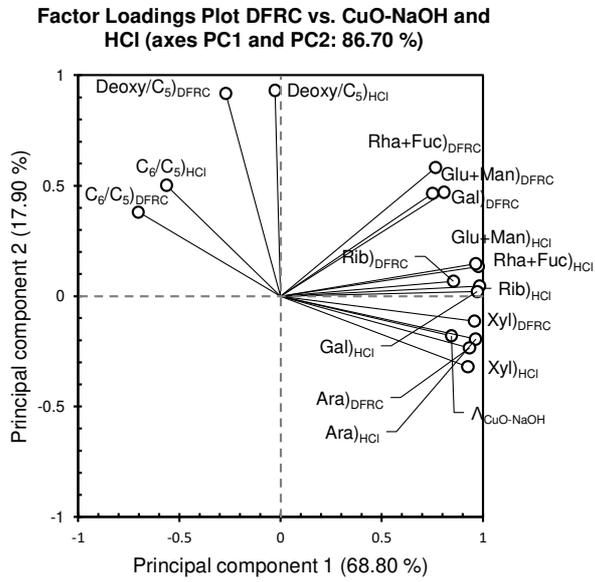


Table 1. Symbols of the investigated lignin and carbohydrate moieties.

Lignin monomers		Monosaccharides ^b	
THM ^a	CuO-NaOH oxidation		
G ₄ :	3,4-dimethoxybenzaldehyde	S: Syringyl	Xyl: Xylose
G ₅ :	1-(3,4-dimethoxyphenyl)ethanone	V: Vanillyl	Ara: Arabinose
G ₆ :	3,4-dimethoxybenzoic acid methyl ester	C: Cinnamyl	Rib: Ribose
G ₁₈ :	3-(3,4-dimethoxyphenyl)-2-propenoic acid methyl ester		Rha: Rhamnose
P ₁₈ :	3-(4-hydroxyphenyl)-2-propenoic acid methyl ester		Fuc: Fucose
S ₄ :	3,4,5-trimethoxybenzaldehyde		Man: Mannose
S ₅ :	1-(3,4,5-trimethoxyphenyl)ethanone		Gal: Galactose
S ₆ :	3,4,5-trimethoxybenzoic acid methyl ester		Glu: Glucose

^a The symbols used are the same as the ones used in Filley et al. (2006) and Younes and Grasset, (2018)

^b Symbols are followed by a subscript indicating the method the monosaccharides have been analyzed with (DFRC, THM or HCl).

Filley, T. R., Nierop, K. G., Wang, Y., 2006. The contribution of polyhydroxyl aromatic compounds to tetramethylammonium hydroxide lignin-based proxies. *Org. Geochem.* 37(6), 711-727.

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