

1 **Molecular characteristics of permanganate and dichromate oxidation resistant soil**
2 **organic matter from a Black C rich colluvial soil**

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19

20 **Abstract**

21 Samples from a colluvial soil rich in pyrogenic material (Black C, BC) in NW Spain
22 were subjected to $K_2Cr_2O_7$ and $KMnO_4$ oxidation and the residual soil organic matter
23 (SOM) was NaOH-extracted and analyzed using analytical pyrolysis–gas
24 chromatography–mass spectroscopy (Py-GC/MS) and solid-state ^{13}C cross
25 polarization–magic angle spinning–nuclear magnetic resonance (^{13}C CP MAS-NMR) in
26 order to study the susceptibility of different SOM fractions (fresh, degraded/microbial,
27 BC and aliphatic) towards these oxidizing agents. NaOH extracts of untreated samples
28 were also analyzed. Py-GC/MS and ^{13}C NMR indicated that $KMnO_4$ promotes the
29 oxidation of carbohydrate products, mostly from degraded/microbial SOM and
30 lignocellulose, causing a relative enrichment of aliphatic and aromatic structures.
31 Residual SOM after $K_2Cr_2O_7$ oxidation contained BC, N-containing BC and aliphatic
32 structures. This was corroborated by a relatively intense resonance of aromatic C and
33 some signal of alkyl C in ^{13}C NMR spectra. These results confirm that dichromate
34 oxidation residues contain a non-pyrogenic fraction mainly consisting of aliphatic
35 structures.

36 1. Introduction

37 Soil organic matter (SOM) is a complex mixture of plant, animal and microbial
38 tissue, both fresh and at different stages of decomposition (Stevenson 1994; Tabatabai
39 1996). The molecular structures present in SOM have been a source of debate for many
40 decades due the analytical difficulties inherent to SOM characterization (Piccolo 1996).
41 A wide set of methodologies have been used to study SOM composition, including 1)
42 fractionation methods such as (i) chemolytic techniques (i.e. application of acid-
43 hydrolysis or extracting agents) that are coupled with colorimetric and/or GC/MS
44 analyses to identify specific SOM components (polysaccharides, lignin-derived
45 compounds, amino sugars, extractable- lipids or hydrolysable proteins) (Kögel-Knabner
46 1995); (ii) physical fractionation into organo- mineral fractions based on particle size
47 and/or density yields (Christensen 1992; Six et al. 2002); (iii) wet oxidation with
48 potassium permanganate (KMnO₄) (Loginow et al. 1987; Tirol-Padre and Ladha 2004),
49 H₂O₂ (Eusterhues et al. 2005), Na₂S₂O₈ (Eusterhues et al. 2003), NaOCl (Kleber et al.
50 2005), and K₂Cr₂O₇ (Skjemstad and Taylor 1999); and 2) analytical techniques
51 including (i) spectroscopic techniques, such as infrared (IR) spectroscopy and solid-
52 state ¹³C nuclear magnetic resonance (¹³C NMR) (Wilson et al. 1981; Fründ et al. 1993)
53 and (ii) pyrolysis-GC/MS (Py-GC/MS) (Sáiz-Jiménez and the Leeuw 1994a). Whereas
54 IR and ¹³C NMR provide information on the environment of carbon atoms (functional
55 groups) (Baldock and Smernik 2002), more detail on the molecular chemistry is
56 obtained by pyrolysis-GC/MS.

57 Py-GC/MS is based on thermal degradation in an inert atmosphere (pyrolysis)
58 and subsequent separation (GC) and identification (MS) of the pyrolyzate, from which
59 information on macromolecular structures can be extracted (Moldoveanu 1998). For
60 example, it allows the identification of different sources and degradation states of plant

61 detritus, secondary/microbial material, Black C (BC), and the estimation of their
62 relative proportions (Nierop et al. 2005; Buurman et al. 2007). Nonetheless, Py-GC/MS
63 is a semi-quantitative method because of differences in the pyrolyzability of different
64 organic matter components, not all pyrolysis products are amenable and detectable by
65 GC, and differences in relative response factors by MS (Sáiz-Jiménez 1994a)

66 The Walkley-Black dichromate oxidation, as modified by Heanes (1984), is a
67 relatively simple and rapid procedure with minimal equipment needs (Nelson and
68 Sommers 1996) that has long been used to estimate the organic C (OC) content of soils.
69 Its major disadvantage is that it incompletely oxidizes soil OC (Gillman et al. 1986),
70 and has different oxidation efficiencies for different soils (Tabatabai 1996), which
71 produce considerable and unpredictable deviations from 'true' soil OC content.
72 Probable causes of deviations in recoveries are (i) spatial inaccessibility of organic
73 substrates to the oxidation agent (Skjemstad et al. 1996; Six et al. 2002), (ii) binding
74 with inorganic phases (Eusterhues et al. 2005) and (iii) the presence of chemically
75 recalcitrant SOM fractions such as BC (Six et al. 2002). In fact, the difference between
76 total OC and dichromate-oxidizable OC has been used to estimate the BC content of
77 soils. BC is defined as the product resulting from incomplete thermal combustion of
78 vegetation and/or fossil fuels, and is relatively resistant to decomposition (Schmidt et al.
79 2001). However, an unknown portion of BC is actually oxidized while some non-
80 pyrogenic SOM may survive the oxidation, e.g. non-hydrolyzable aliphatic compounds
81 that resist aqueous dichromate oxidation (Knicker et al. 2007). This implies that the
82 properties of the oxidation-resistant residue must be assessed in order to obtain
83 meaningful estimations of BC content using dichromate oxidation (Knicker et al. 2007).
84 The stability of BC towards these reagents is of significant interest considering its
85 upcoming use as a soil amendment (as biochar).

86 The permanganate-oxidizable fraction has been used as a proxy for the labile
87 fraction of SOM (Loginow et al. 1987, Lefroy et al. 1993), based on the assumption that
88 the oxidative capacity of KMnO_4 on SOM is comparable to that of soil microbial
89 enzymes (Conteh et al. 1997). However, some studies indicated that KMnO_4 - oxidizable
90 C may not be a reliable measure of the proportion of labile C because, even though it
91 efficiently degrades lignin (van Soest and Wine 1986), it has little effect on several
92 SOM components that are widely recognised as easily degraded by soil
93 microorganisms, e.g. structural carbohydrates, sugars and amino acids (e.g. Tyrol-Padre
94 and Ladha 2004). Furthermore, its ability to react with charcoal was also stated
95 (Skjemstad et al. 2006).

96 SOM is thermodynamically unstable in well-aerated soils (Macías and Camps-
97 Arbestain 2010). However, SOM stabilized by specific mechanisms can remain as
98 meta-stable forms in soils for hundreds to thousands of years (Six et al. 2002). These
99 non-ideal conditions for SOM decay are associated to physical and chemical protection
100 mechanisms offered by the soil matrix that either impede the access of enzymes to SOM
101 (e.g., within microaggregates or by creating hydrophobicity) or increase the energy
102 needed to degrade SOM through interactions with minerals (Eusterhues et al. 2003). In
103 addition, enhanced SOM preservation may occur when environmental conditions are
104 not adequate for microbial growth, e.g. the presence of free Al and Fe, low soil pH
105 and/or due to low nutrient availability (Buurman and Roscoe 2011). Furthermore,
106 intrinsic recalcitrance of specific SOM components may increase its longevity in soil.
107 This is most likely the main process responsible for the long turn-over time of highly
108 condensed aromatic compounds present in BC (Harvey et al. 2012).

109 The nature of permanganate- and dichromate-oxidation resistant SOM is still
110 poorly understood. Here we study the molecular properties of KMnO_4 - and $\text{K}_2\text{Cr}_2\text{O}_7$ -

111 oxidation-resistant SOM from a BC-rich colluvial soil, by Py-GC/MS and solid-state
112 ^{13}C NMR, which may add to our knowledge on the stability of specific organic
113 compounds against soil microbial oxidative enzymes.

114

115 **2. Material and methods**

116 *2.1. Study site and sample descriptions*

117 Soil PRD-4 is a 2.4 m thick Haplic Umbrisol (humic/alumic) according to the
118 IUSS Working Group (2006) and Humic Pachic Dystrudept according to the SSS
119 (1998). This soil type is traditionally referred to as Atlantic Ranker (Carballas et al.
120 1967). Radiocarbon dating showed that the soil gradually accumulated through
121 colluviation during the last ca. 13,000 yr (Kaal et al. 2011). Soil PRD-4 has a deep
122 black color owing to a combination of high SOM content and abundance of BC (Table
123 1). For the present study, three samples were selected from this soil, corresponding to
124 three periods with radically different ecosystems and hypothetically also different SOM
125 compositions: S1 (5-10 cm depth) corresponds to recent material (<150 y BP),
126 evidenced by ^{14}C dating and the presence of pollen of exotic species *Eucalyptus* sp.
127 (López-Merino et al. 2012) (Table 1). This soil layer contains considerable amounts of
128 “fresh” (non- or slightly degraded) SOM and root fragments, as described by Kaal and
129 van Mourik (2008). The vegetation corresponding to **this sample** is a mosaic of
130 shrubland (dominated by Ericaceae), pasture and exotic tree species. Sample S2 (95-
131 100 cm depth) contains large amounts of charcoal from palaeofires (Kaal et al. 2011)
132 that occurred ca. 5,000 yr ago. Anthracological analysis showed that most charcoal
133 originates from deciduous *Quercus* sp. This sample is thought to correspond to an oak-
134 dominated woodland under substantial fire and grazing pressure. Sample S3 (190- 195
135 cm depth) corresponds to an Early- Holocene phase (ca. 9,700 yr BP) that preceded the

136 colonization of the area by deciduous forest, with “steppe-like” vegetation dominated
137 by *Betula* sp. (birch), shrubs of the Fabaceae family and herbaceous species.

138

139 2.2. Determination of organic C fractions

140 Potassium dichromate-oxidizable organic C (OC_{dichro}) was determined following
141 the Walkley-Black oxidation method as modified by Wolbach and Anders (1989) and
142 Knicker et al. (2007). 0.5 g of dry soil (< 2 mm) were oxidized in triplicate with 20 ml
143 of 0.2 M $K_2Cr_2O_7$ and 20 ml of concentrated H_2SO_4 at 60 °C in a water bath for 6 h.
144 **Control samples without soil were also analyzed.** After the reaction, excess dichromate
145 was determined by titration against 0.033 M $FeSO_4$. The amount of dichromate
146 consumed by the soil was used to calculate the amount of dichromate-oxidizable
147 organic C (OC_{dichro}) assuming that (i) the oxidation state of soil OC is zero (C^0) and (ii)
148 complete oxidation to C^{+4} occurs.

149 Potassium permanganate-oxidizable organic C (OC_{per}) was determined, in
150 triplicate, using 25 ml of 33 mM $KMnO_4$ solution added in 50 ml centrifuge tubes
151 containing an amount of dry soil (<2 mm) equivalent to 15 mg organic C (Tirol-Padre
152 and Ladha 2004). After 24 h shaking, the tubes were centrifuged for 5 min at 2600 g
153 and the supernatant diluted in distilled water (1:25 v:v). Absorbance was read on a split
154 beam spectrophotometer at 565 nm. Blanks and a standard soil were analyzed before
155 each run. For calculation purposes, it was assumed that three moles of C (e.g.
156 carbohydrates) are oxidized for every four moles of Mn^{+7} reduced (Tirol-Padre and
157 Ladha 2004).

158

159 2.3. Isolation of SOM fractions

160 The non-oxidized (NO) SOM extraction was considered the control treatment,
161 consisting of SOM extraction by NaOH as described by Buurman et al. (2007). Briefly,
162 5 g soil (air- dried fine earth <2 mm) was extracted with 50 ml of 1 M NaOH and
163 shaken for 24 h under N₂ to prevent oxidation/saponification. The suspension was
164 centrifuged at 2600 g for 1 h and the extract decanted, after which the extraction was
165 repeated. The two extracts were combined and the residues discarded. The extracts were
166 then acidified to pH 1 with concentrated HCl to protonate SOM. One ml of concentrated
167 HF was added to dissolve silicates and increase C content of the extracted fraction. The
168 acid mixture was shaken for 48 h, after which it was dialyzed to neutral pH against
169 distilled water to remove excess salt. Finally, the suspension was freeze- dried.

170 Dichromate oxidation-resistant SOM (CR): 12.5 g soil was oxidized in 500 ml
171 of 0.2 M K₂Cr₂O₇ and 100 ml of concentrated H₂SO₄ for 6 h at 60 °C using a water
172 bath. Once cooled, the suspension was centrifuged at 2600 g for 1 h and the supernatant
173 decanted, after which the sediment was washed with distilled water until the solution
174 was colorless. The suspension was discarded and the dichromate oxidation-resistant
175 SOM extracted by 200 ml of 1 M NaOH for 24 h under N₂. The resultant suspension
176 was centrifuged at 2600 g for 1 h and the supernatant decanted. This extraction was
177 repeated twice. Thereafter, the three extracts were combined and acidified to pH 1 with
178 concentrated HCl. One ml of concentrated HF was added to dissolve silicates and
179 increase the content of organic C of the extracted fraction. This acid mixture was shaken
180 for 48 h, dialyzed against H₂O to neutral pH and finally freeze- dried.

181 Permanganate oxidation-resistant SOM (MN): 1000 ml of 33 mM KMnO₄ were
182 added to 4.8, 8.2 and 7.0 g dry soil (<2 mm) for samples S1, S2 and S3, respectively,
183 aiming to add 25 ml of KMnO₄ per 15 mg of organic C (calculated from OC_{dichro}
184 values). After 24 h shaking, the suspension was centrifuged at 2600 g for 1.5 h and the

185 extract decanted, after which the sediment was washed with distilled water until the
186 supernatant was colorless. The MN in the residue was isolated and purified using 200
187 ml of 1 M NaOH for 24 h under N₂ atmosphere, analogous to the extraction of CR
188 described above.

189

190 2.4. Py-GC/MS

191 Platinum filament Py-GC-MS was performed with a Pyroprobe 5000 (CDS
192 Analytical Inc., Oxford, USA) coupled to a 6890 GC and 5975 MS (Agilent
193 Technologies, Palo Alto, USA). The non-oxidized (NO) and oxidation-resistant SOM
194 fractions (MN and CR) were pyrolyzed at 750 °C for 10 s (heating rate 10 °C/ms).
195 Analyses of sample S2 of the CR series was repeated, first at 400 °C and then at 750 °C,
196 to distinguish between evaporation and pyrolysis products from volatile and
197 macromolecular components, respectively. The pyrolysis interface was set at 300 °C
198 and the GC inlet at 325 °C. The oven of the GC was heated from 50 to 325 °C at 10
199 °C/min and held isothermal for 5 min. The GC/MS transfer line was held at 325 °C, the
200 ion source (in electron impact mode, 70 eV) at 230 °C and the quadrupole detector at
201 150 °C, measuring fragments in the *m/z* 50-500 range. The GC was equipped with a
202 (non-polar) HP-1 100% dimethylpolysiloxane column. Helium was used as the carrier
203 gas (constant gas flow, 1 ml/min). The major peaks in the total ion current of all
204 samples were listed and, if possible, identified using the NIST '05 library and Py-
205 GC/MS literature (Appendix A). Quantification of these pyrolysis products, 172 in total,
206 was obtained by using the peak area of the major fragment ions (*m/z*). The sum of these
207 peaks, i.e. total quantified peak area (TQPA) was set as 100% and the relative
208 proportions of the pyrolysis products were calculated as the % of TQPA. This is a semi-
209 quantitative estimate that allows for better comparison among samples than visual

210 inspection of pyrolysis chromatograms (pyrograms) alone, and produces a dataset that
211 can be treated statistically.

212

213 2.5. ^{13}C NMR spectroscopy

214 Solid-state NMR spectroscopy experiments were performed with cross-polarization
215 magic angle spinning (CPMAS) at 298 K in a 17.6 T Varian Inova-750 spectrometer
216 (operating at 750 MHz proton frequency) equipped with a T3 Varian solid probe
217 [Varian, Inc, USA]. Solid NMR samples were prepared in 3.2 mm rotors with an
218 effective sample capacity of 22 μL which corresponds to approximately 30 mg of the
219 powdered sample. Spectra were processed and analyzed with MestreC software
220 (Mestrelab Research Inc.). Carbon chemical shifts were referred to the carbon
221 methylene signal of solid adamantane at 28.92 ppm. This sample was also used for the
222 calibration of the 1D CPMAS experiments. 1D CPMAS spectra were acquired for the
223 samples with the following conditions: the inter-scan delay was set to 0.5 s, the number
224 of scans was 24000 and the MAS rate was 20 kHz. Heteronuclear decoupling during
225 acquisition of the FID was performed with Spinal-64 with a proton field strength of 70
226 kHz. The cross polarization time was set to 1 ms. During cross polarization, the field
227 strength of the proton pulse was set constant to 75 kHz and that of the ^{13}C pulse was
228 linearly ramped during a 10% of the duration with a 20 kHz ramp near the matching
229 sideband. Spectra were divided into different regions of chemical shift following
230 Knicker et al (2005). Relative abundances of the various C groups were determined by
231 integration of the signal intensity in their respective chemical shift regions. The region
232 between 0– 45 ppm is assigned to alkyl C corresponding to terminal methyl groups and
233 methylene groups of aliphatic moieties. The *O*-alkyl C region, typically assigned to
234 carbohydrate-derived structures, between 45– 95 ppm. Here, between 45– 60 ppm, *N*-

235 alkyl C (i.e. in amino sugars and peptide structures) can contribute to the signal.
236 Between 90 and 160 ppm resonance lines of olefins and aromatic C are detected. The
237 regions from 160 to 220 ppm and from 220 to 245 are assigned to carbonyl C separated
238 into carboxyl/amide and aldehyde/ketone groups, respectively. Although often assumed
239 that solid- state ^{13}C NMR underestimate BC, recent studies demonstrated that most
240 charcoals have an atomic H/C ratio > 0.5 and thus provide sufficient protonation for
241 efficient cross polarization and reliable NMR spectra (Knicker et al. 2005). Because of
242 the limited sample availability some samples required Al-oxide to fill the rotor, causing
243 some signal quality deterioration. Samples MN-2, MN-3 and CR-3 were not analyzed
244 for that reason.

245

246 *2.6. Factor analysis*

247 The relative proportions of pyrolysis products were subjected to factor analysis
248 using Statistica Version 8 (Statsoft, Tulsa, USA). Factor analysis proved useful in the
249 interpretation of Py-GC/MS datasets, especially with respect to the sources and
250 degradation states to which the pyrolysis products correspond.

251

252 **3. Results and discussion**

253 *3.1. Py-GC/MS: source allocation*

254 The pyrolysis products were grouped according to their chemical structure into
255 the following classes: (i) aliphatic compounds (homologous series of *n*-alkanes and *n*-
256 alkenes, and branched alkenes), (ii) lignin-derived methoxyphenols, (iii) phenols, (iv)
257 monocyclic aromatic compounds (MAHs), (v) polycyclic aromatic hydrocarbons
258 (PAHs), (vi) N-containing compounds, (vii) carbohydrate-derived pyrolysis products

259 and (viii) unidentified compounds. Appendix A is a list of the pyrolysis products
260 identified.

261 *Aliphatic compounds.* *n*-alkane/*n*-alkene pairs, ranging from C₁₀ to C₂₈, originate
262 largely from aliphatic biopolymers (Eglinton and Hamilton 1967). The other short- and
263 mid-chain (ca. C₁₀-C₂₀) alkenes, not from the homologous series and most of which are
264 probably branched, are considered significant products of charred aliphatic matter
265 according to recent studies, even though they are not produced exclusively from
266 pyrolysis of BC (Eckmeier and Wiesenberg 2009; Kaal et al. 2012a). Several *n*-fatty
267 acids (mainly C₁₆ and C₁₈) seemed to increase disproportionately upon chemical
268 oxidation, especially in CR. This might be explained by the enrichment of aliphatic
269 structures upon dichromate oxidation due to their hydrophobicity (Knicker et al. 2007).
270 However, the use of a HP-1 column, which has a larger internal diameter than
271 frequently used non-polar columns for Py-GC/MS, may have affected the
272 'chromatographic mobility' and the relative proportions of these compounds, thus
273 making its interpretation difficult. Therefore, these compounds were not included in the
274 statistical analyses neither.

275 *Lignin-derived methoxyphenols.* Methoxyphenols (guaiacyl- and syringyl-based)
276 are typical products of coniferyl and sinapyl lignin, respectively (Boerjan et al. 2003).
277 4-vinylphenol was also added to this group because it has frequently been shown to be
278 marker of coumaryl lignin and the non-lignin coumaric acid in grasses (Sáiz-Jiménez
279 and de Leeuw 1986). An unknown proportion of 4-vinylphenol and 4-vinylguaiacol
280 may originate from non-lignin phenolic acids as well (Schellekens et al. 2012), but that
281 does not influence the interpretation of results here as statistically they behave as the
282 lignin markers (see below).

283 *Phenols.* The other phenols have multiple origins: phenol and C₁-C₂-
284 alkylphenols may originate from any phenolic precursor including lignin, tannin,
285 proteinaceous biomass, weakly-charred BC and carbohydrates (Tegelaar et al. 1995;
286 Stuczynski et al. 1997), while lignin, tannin or thermally demethylated lignin (Kaal et
287 al. 2012b) are the most likely precursors of 1,2-benzenediol (catechol).

288 *Monocyclic aromatic hydrocarbons.* MAHs include benzene, toluene, styrene,
289 dimethylbenzenes, linear C₂-C₄-alkylbenzenes, a dimethylstyrene and a dimethyl-
290 methylethylbenzene compound. MAHs are formed from many aromatic and some non-
291 aromatic precursors (Schulten et al. 1991) but BC is known to produce an exceptionally
292 high proportion of especially benzene (Kaal et al. 2012a). Indeed, the analysis of the
293 products of incomplete combustion by Py-GC/MS showed that MAHs and also PAHs
294 are major pyrolysis products of Black C (Pastorova et al. 1994, Almendros et al. 2003).
295 Besides a renewed interest in the detection of burning residues in SOM established that
296 BC is a major source of SOM and that its oxidation products could be a potential source
297 of highly aromatic humic acids (Hatcher et al. 1989; Skjemstad et al. 1996; Shindo et
298 al., 2004). On the other hand, the alkylstyrenes most likely originate from the
299 monoterpenes present in *Eucalyptus globulus* litter (see below).

300 *Polycyclic aromatic hydrocarbon compounds.* The origin of PAHs in SOM
301 pyrolyzates have been the subject of considerable debate. They were sometimes
302 considered actual building blocks of humic substances formed upon condensation
303 reactions during humification (Schulten et al. 1991), or more frequently interpreted as
304 analytical artefacts because of evidence of their formation during pyrolysis of aliphatic
305 compounds through cyclization and aromatization (Sáiz-Jiménez 1994b). More
306 recently, PAHs and particularly the non-alkyl-substituted and >2 ring PAHs (Rumpel et
307 al. 2007) are considered indicative of (but not markers of) BC in SOM (Kaal and

308 Rumpel 2009; Song and Peng 2010). In the present study, unsubstituted PAHs (indene,
309 naphthalene, fluorene, biphenyl, phenanthrene and anthracene) and C₁-C₂ alkyl
310 analogues of these PAHs were abundant. In addition, a series of C₃-C₄
311 alkylnaphthalenes and C_{5:0} and C_{5:1} alkylnaphthalenes probably originate from
312 evaporation and pyrolysis of monoterpenes (e.g. pinene, phellandrene, eucalyptol) and
313 sesquiterpenoids (aromadendrene, globulol), respectively, present in *Eucalyptus* sp. oil.
314 Indeed, these compounds were identified in *Eucalyptus globulus* litter (a mixture of
315 leaves, cortex and branches) pyrolyzates (data not shown). Some of the polysubstituted
316 PAHs (C₃-indene, C₂-C_{5:0}- and C_{5:1}-naphthalene) provided the largest contributions to
317 the pyrograms of sample S1 (contrary to many unsubstituted or C₁- alkylsubstituted
318 PAHs, largely from BC); this suggests that a significant portion of these compounds
319 originate from fresh *Eucalyptus* sp. litter. Overall, it should be noted that, in this study,
320 where distinguishing between BC and non-pyrogenic SOM components will appear to
321 be important, the interpretation of MAHs and PAHs relies more strongly on the growing
322 body of knowledge on BC's pyrolysis fingerprints (Kaal et al. 2012a; Fabbri et al. 2012;
323 Song and Peng 2010) than on previous studies on the structural characteristics of humic
324 acids (Schulten et al. 1991; Sáiz-Jiménez 1994a).

325 *N*-containing products. Of the 24 N-containing pyrolysis products identified,
326 benzonitrile and C₁-benzonitriles were recently proposed as the main products of N-
327 containing groups in BC (Schnitzer et al. 2007; Song and Peng 2010). In addition,
328 isoquinoline, phenylpyridine, benzenedicarbonitriles and pyridinecarbonitriles can be
329 considered as markers of 'Black N' (BN) (Knicker 2007; Kaal et al. 2009). Note that
330 absence of these products does not imply absence of BN: these compounds can
331 probably only be detected in high-quality pyrograms of exceptionally BN-rich samples.
332 Pyrroles, pyridines and indoles are potential products of BN as well but these

333 compounds are common in the pyrolyzates of non-pyrogenic N-moieties. Several
334 markers of chitin (acetamide and a compound tentatively identified as trianhydro-2-
335 acetamido-2-deoxyglucose; van der Kaaden et al. 1984; Stankiewicz et al. 1996) and
336 chitin-entangled protein (diketopiperazine) probably originate from fungal cell walls
337 and/or arthropod exoskeleta, either way serving as an indication of biologically re-
338 assimilated (“secondary”) remains in SOM (Gutierrez et al. 1995). Finally, for
339 picolinamide, cyanobenzoic acid and phthalimide-based compounds no specific origin
340 has been identified yet.

341 *Carbohydrate compounds.* Of the carbohydrate products identified, levoglucosan,
342 dianhydro- α -glucopyranose, pyranones and dianhydrorhamnose largely originate from
343 “fresh” or well-preserved polysaccharides (Stuczynski et al. 1997; Poirier et al. 2005;
344 Nierop et al. 2005). On the other hand, cyclopentenediones, furans, furfurals,
345 levoglucosenone and dibenzofuran originate from fresh and/or degraded carbohydrates
346 (Buurman and Roscoe 2011). This degradation may be either biological or thermal in
347 nature, the latter especially for the furans, furaldehydes and dibenzofuran (Pastorova et
348 al. 1994; Boon et al. 1994).

349 *Unidentified compounds.* An unsaturated non-aromatic cyclic compound (U1) was
350 identified only in the pyrolyzates from S1 (NO-1 and MN-1), which also contained the
351 pollen of *Eucalyptus* sp. It probably corresponds to α -phellandrene, which is abundant
352 in eucalyptus oils (Samaté et al. 1998). Furthermore, several polymethyl-substituted
353 polycyclic compounds (U3-U6), also detected in the aforementioned fresh eucalyptus-
354 litter pyrolyzate, probably derived from *Eucalyptus* sp. Finally, a methylated
355 cyclohexane (U2) of unknown origin was tentatively identified.

356

357 3.2. Py-GC/MS: quantification and interpretation

358 Pyrograms of chemical oxidation resistant SOM fractions and non-oxidized
359 samples are represented in Fig. 1. The relative contributions to TQPA for identified
360 groups in the different soil horizons studied are presented in Table 2. In NO-1,
361 carbohydrate-derived pyrolysis products accounted for 30% of TQPA, with
362 levoglucosan (Ps13) from intact polysaccharide (Stuczynski et al. 1997; Poirier et al.
363 2005) being dominant. The presence of 4-hydroxy-5,6-dihydro-(2H)-pyranone (Ps6)
364 and dianhydrorhamnose (Ps7) confirms the existence of fresh (or well-preserved)
365 polysaccharides in NO-1 (Nierop et al. 2005). Of the samples studied, these compounds
366 showed the largest contribution to sample NO-1. The same pattern was observed for
367 many other indicators of fresh plant material, including the aliphatic compound
368 producing m/z 83+280, diketodipyrrole, and the lignin-derived products (Suárez-
369 Abelenda et al. 2011). The large proportion of phenols in the pyrolyzate of this sample
370 may be explained by the abundance of lignin. Samples NO-1 and MN-1 had the highest
371 contributions of probably eucalyptus-derived moieties ($C_{5:0}$ -, $C_{5:1}$ -alkylnaphthalenes and
372 α -phellandrene and C_3 - naphthalenes). It is concluded that the SOM of NO-1 is
373 characterized by a large fraction of well-preserved polysaccharides and lignin, with an
374 additional contribution of specific eucalyptus-derived substances, and relatively small
375 proportions of microbial and pyrogenic SOM. The latter is supported by the low
376 benzene/alkyl-benzenes and PAH/alkyl-PAHs ratios (Table 2), which are indicative of a
377 low contribution of strongly charred BC to the MAHs and PAHs of these samples (Kaal
378 and Rumpel 2009; Kaal et al. 2012a).

379 Unsurprisingly, sample NO-2 (ca. 5,000 yr old), produced fewer pyrolysis
380 products from fresh SOM than NO-1. More specifically, in comparison with NO-1,
381 among the carbohydrate markers there was a strong increase of furans, furaldehydes,
382 levoglucosenone and acetic acid, while levoglucosan, pyranones and

383 dianhydrorhamnose diminished, which is a clear indication of a shift of fresh
384 polysaccharide to degraded/microbial carbohydrates (Sáiz-Jiménez and de Leeuw 1986;
385 Buurman and Roscoe 2011). Lignin markers were virtually absent. NO-2 sample
386 produced many N-compounds, including those from chitin (N3 and N22), pyridine (N1,
387 often associated with microbial SOM; Buurman et al. 2007) and BN (e.g. aromatic
388 carbonitriles and phenylpyridine). It also gave higher proportions of MAHs and BC-
389 derived PAHs than the NO-1 sample. It is concluded that the SOM of sample NO-2 was
390 predominantly composed of degraded/microbial and pyrogenic material.

391 The pyrolyzate of sample NO-3 was dominated by carbohydrate markers, with
392 acetic acid, 3/2-furaldehyde, 5-methyl-2-furaldehyde, dianhydro- α -glucopyranose, a
393 furanone and 4-acetylfuran accounting for 62% of TQPA (Table 2). These pyrolysis
394 products are frequently ascribed to SOM with large proportions of microbial biomass
395 (Sáiz-Jiménez and de Leeuw 1986; Buurman and Roscoe 2011). The small relative
396 proportions of MAHs and PAHs suggest that BC accounts for only a minor portion of
397 the SOM in NO-3. This is supported by the low ratios of benzene/alkyl-benzenes and
398 PAH/alkyl-PAH (Table 2).

399 In general, the differences in pyrolyzate compositions between NO-samples and
400 MN-samples were small, yet some are worth mentioning. For sample S1, oxidation with
401 KMnO_4 (which promoted a decreased of 2.3 mg g^{-1} of OC; Table1) caused an increase
402 in MAHs (from 16.4% in NO-1 to 30.0% in MN-1) and decrease in carbohydrates (from
403 30.4% to 18.4%) and lignin (from 7.4% to 3.0 %) (Table 2). These results can be
404 explained by the partial oxidation of fresh SOM (van Soest and Wine 1986; Tirol-Padre
405 and Ladha 2004) and the relative enrichment of pyrogenic (Almendros et al. 1990) and
406 aliphatic SOM (González-Vila and Martín 1985; Almendros et al. 1989). Besides,
407 permanganate oxidation concentrates pyrolysis products from aromatic structures

408 present in humic acids in general (Polvillo et al. 2009), even though cyclization of
409 aliphatic precursors may play a role as well (González-Vila and Martín 1985). In
410 sample S2, KMnO_4 oxidation (MN-2) (with a smaller decrease of OC; 1.3 mg g^{-1})
411 caused a strong decline in carbohydrate products (from 31.5% to 17.4% of TQPA in
412 NO-2 and MN-2, respectively) and an increase in aliphatic pyrolysis products (sum of
413 *n*-alkanes, *n*-alkenes and other aliphatic compounds from 5.3% in NO-2 to 23.1% in
414 MN-2). These changes are indicative of selective oxidation of (an unknown proportion
415 of) the degraded/microbial SOM and the relative enrichment of aliphatic precursors
416 probably from degraded root components (Kaal and van Mourik 2008). In addition,
417 MN-2 produced higher amounts of N-containing BC markers, as BC and BN are
418 relatively resistant against oxidation with this reagent. Skjemstad et al. (2006) found
419 that KMnO_4 may react significantly with BC, but no evidence of this was found here.
420 Finally, sample MN-3 (with a decrease of 1.7 mg g^{-1} of its OC content) contains a
421 smaller proportion of carbohydrates than NO-3 (17.7% vs. 62.2% of TQPA),
422 confirming that the degraded/microbial carbohydrate fraction is relatively susceptible to
423 this oxidation agent.

424 The pyrolyzates obtained from the residues after dichromate oxidation were very
425 different from those of NO- and MN-samples. The CR-1 sample (27.5 mg g^{-1} of its OC
426 was oxidized by dichromate; Table 1) was strongly enriched in aliphatic pyrolysis
427 products (34% of TQPA), particularly of short-chain ($<C_{18}$) *n*-alkanes/*n*-alkenes
428 (located on the right side of the broken line in the aliphatic cluster; SE quadrant, Fig. 2)
429 and branched alkenes, and depleted in lignin-, carbohydrate- and eucalyptus-derived
430 pyrolysis products in comparison with NO-1 and MN-1. CR-1 also produced the highest
431 proportions of 3-ring PAHs and higher ratios of benzene/alkyl-benzenes and
432 PAH/alkyl-PAHs ratios (Table 2), suggesting that a large proportion of the MAHs and

433 PAHs from the CR-1 is pyrogenic. These results are indicative of the enrichment of
434 pyrogenic SOM in the fraction resistant to $K_2Cr_2O_7$ - oxidation. Indeed, the partial
435 resistance of BC to $K_2Cr_2O_7$ oxidation is well-documented (Knicker et al. 2007, 2008).
436 The same studies showed the existence of a $K_2Cr_2O_7$ -resistant alkyl fraction (Knicker et
437 al. 2007, 2008), which was also supported by the pyrolyzate composition of CR-1. The
438 increase was also observed for the *n*-fatty acids (data not shown), which are not
439 considered part of structural aliphatic plant material. These results support the
440 hypothesis that the enrichment of aliphatic material in the residual fraction of the
441 $K_2Cr_2O_7$ oxidation residues is produced by the hydrophobic nature of these constituents
442 (Knicker et al. 2007), possibly in combination with chemical recalcitrance of C-C bonds
443 in methylene chains. Finally, a decrease was observed for the intact terpene-like plant-
444 derived PAHs, clearly showing different origin for the un- and methyl-substituted PAHs
445 (mainly from BC) and the polyalkyl-substituted PAHs from eucalyptus litter. For
446 sample CR-2, dichromate oxidation oxidized less OC (12.3 mg g^{-1} for CR-2) than for
447 CR-1. It produced a further decrease in the proportion of lignin markers in comparison
448 with MN-2, and of microbial products such as acetamide and furans, while the BC and
449 BN fingerprints were relatively intense (e.g. benzene, unsubstituted PAHs, benzene
450 carbonitriles, isoquinoline and dibenzofuran). The largest proportion of these BC-
451 derived pyrolysis products coincides with the highest macroscopic charcoal content of
452 sample S2 (Table 1). These results confirm the accumulation of BC and BN in the
453 residues after $K_2Cr_2O_7$ oxidation. Similar to CR-1, sample CR-2 was enriched in
454 aliphatic pyrolysis products. Unexpectedly, significant amounts of the markers of well-
455 preserved polysaccharides such as levoglucosan, were detected in the pyrolyzates of
456 CR-2 and CR-3. With the information available at this moment, our best explanation to
457 this observation is the presence of an uncharred cellulose-containing core (Knicker et al.

458 2005) in incompletely charred particles and thereby protected against $K_2Cr_2O_7$
459 oxidation. Sample CR-3 (in which dichromate caused a loss of 14.3 mg g^{-1} of OC) was
460 also enriched in pyrogenic SOM with a high contribution of BN markers, and in an
461 aliphatic component with particularly high contributions of branched alkenes from an
462 aliphatic SOM fraction, possibly in part pyrogenic.

463

464 3.3. Py-GC/MS: factor analysis

465 The first four factors (F1-F4) explained 81% of the variation in the Py-GC/MS
466 dataset, with F1 and F2 combined accounting for 61%. The loadings of the pyrolysis
467 products, and the scores of the samples analyzed, are shown in F1-F2 factor space (Fig.
468 2).

469 *n*-Alkanes/ *n*-alkenes are predominantly represented in the SE quadrant with high
470 positive loadings on F1. Lower loadings on F1 were observed for the *n*-alkenes $>C_{20}$
471 than for C_{10} - C_{20} *n*-alkenes. Branched alkenes plot between their straight-chain
472 analogues and the pyrogenic SOM markers, supporting the hypothesis that these
473 branched alkenes are associated with charred aliphatic precursors (Eckmeier and
474 Wiesenberg 2009; Kaal and Rumpel 2009).

475 The N-containing compounds are spread throughout the F1-F2 factor space,
476 which is a result of the diverse origin of the members of this group. One cluster of N-
477 containing compounds in the NE quadrant is composed of benzonitrile, C_1 -
478 benzonitriles, benzene dicarbonitriles, cyanobenzoic acid, pyridine, C_1 -pyridine and
479 pyridinecarbonitrile, clearly reflecting a pyrogenic origin. Indeed, many non-alkyl-
480 substituted PAHs and dibenzofuran, also associated with BC (Pastorova et al. 1994),
481 plot in the same region. Chitin-derived N compounds (chitin markers such as
482 acetamide) and 4-acetylfuran are spread out in the NW quadrant together with microbial

483 polysaccharides whilst diketodipyrrole denotes the presence of fresh SOM in the SW
484 quadrant (see below). Indoles are spread throughout the SW and SE quadrants, which
485 may be indicative of a mixed origin.

486 The lignin-derived products (including free phenolic acids), i.e. 4-vinylphenol,
487 guaiacols and syringols), and catechol occur in the SW quadrant together with an
488 aliphatic marker of fresh plant material (A113). Most of the remaining pyrolysis
489 products in this region are associated with fresh or well-preserved SOM components as
490 well: phenol and alkylphenols (in this case from lignin), C_{5:0} and C_{5:1} alkylnaphthalenes
491 (from eucalyptus litter), dianhydrorhamnose (from polysaccharides) and diketodipyrrole
492 and indoles (typical N-containing pyrolysis products of fresh proteinaceous biomass;
493 Buurman and Roscoe 2011). Levoglucosan plots between fresh OM and charred
494 material; this may be attributed to the aforementioned protection of cellulose in interior
495 parts of charcoal particles in dichromate oxidation residues or an unknown alternative
496 levoglucosan source. The other carbohydrate products are spread along F2 because they
497 have multiple sources, most of which corresponding to degraded/microbial SOM. The
498 carbohydrate products of degraded/microbial SOM are probably those that plot in the
499 NW quadrant: furans (4-acetylfuran, 3/2-furaldehyde and 5-methyl-2-furaldehyde),
500 glucopyranose, which is microbial marker (Nierop et al. 2005), and acetic acid. This
501 interpretation is consistent with the presence of the markers of chitin in this region of
502 factor space.

503 In summary, F1-F2 separates the pyrolysis products according to their principal
504 origin. Factor 1 separates the pyrogenic and aliphatic oxidation-resistant SOM fractions
505 (chemically stable) from the fresh and degraded SOM fractions (chemically labile),
506 while decomposed and pyrogenic SOM (strongly altered) are separated from fresh and

507 oxidation-resistant aliphatics (resembling plant material) according to their loadings on
508 F2.

509 The factor scores of the samples can be used to identify the main differences
510 between the samples analyzed (Fig. 2). As such, the samples with a large fraction of
511 fresh biomass (NO-1 and MN-1) plot in the SW quadrant. Sample CR-1 plots in the SE
512 quadrant, as dichromate oxidation eliminated most of the fresh SOM causing the
513 relative accumulation of aliphatic SOM and weakly charred material. Sample S2 is a
514 mixture of mainly degraded SOM and BC (with small contributions of fresh and
515 aliphatic material), which is why NO-2 plots in the NW region, dominated by
516 degraded/microbial markers, while MN-2 and CR-2 plot in the NE quadrant because of
517 relative enrichment of BC after chemical treatment. Sample S3 was also rich in
518 microbial SOM but has a lower content of chemically recalcitrant/hydrophobic aliphatic
519 SOM and BC, and higher proportion of degraded/microbial SOM; this explains why
520 NO-3 has a high F2 score while MN-3 and CR-3 plot in the NE quadrant reflecting BC
521 enrichment after the selective depletion of degraded/microbial SOM. The short distance
522 in factorial space between MN-3 and CR-3, and the large distance between these
523 samples and NO-3, suggests a that the abundant degraded/microbial biomass
524 (carbohydrates, chitin) in this sample is highly susceptible to permanganate and
525 dichromate treatment.

526 From these results some inferences on the effects of the oxidation agents on SOM
527 composition can be made. First, the minor differences between NO-1 and MN-1 can be
528 explained by the relatively small microbial contribution to sample S1. In contrast,
529 $K_2Cr_2O_7$ thoroughly modified the pyrolysis fingerprint obtained from the residues of S1
530 by eliminating lignin, polysaccharides and terpenes, and relative enrichment of aliphatic
531 and pyrogenic structures. In the older samples, where the aliphatic fraction is less

532 dominant while that of degraded/microbial and pyrogenic SOM prevail, chemically
533 oxidized samples (MN-2, MN-3, CR-2 and CR-3) had positive scores on F1 and F2
534 mainly because both oxidants concentrate BC, with $K_2Cr_2O_7$ being the stronger oxidant.

535

536 3.4. Solid- state ^{13}C NMR spectroscopy: results and comparison with Py-GC/MS

537 Samples are compared by relative intensity of the chemical shift regions. The
538 spectrum of NO-1 (Fig. 3) was characterized by a dominant signal at 21 ppm and a
539 shoulder at 29 ppm (combined 29%, Table 3) from alkyl C, which can be ascribed to
540 aliphatic structures in fatty acids, lipids, waxes, cutan, suberan, cutin and suberin
541 (Tegelaar et al. 1989) but also peptide structures and short alkyl side-chains. In the *O*-
542 alkyl C region (45–110 ppm) a broad peak at 75 ppm (with a contribution of 29%) was
543 detected, which is generally attributed to cellulose, hemicelluloses and pectins (Gramble
544 et al. 1994; Kögel-Knabner 1997). The peak at 55 ppm probably corresponds to
545 methoxyl groups in lignin structures (Kögel-Knabner 1997) but can also have
546 contributions of *N*-alkyl from amino sugars and peptides. The *O*-substituted aromatic C
547 between 140- 160 ppm may derive from lignin and oxidized BC (Knicker et al. 2005).
548 Resonance lines of aromatic C-H groups are detected in the chemical shift region
549 between 110 ppm and 140 ppm (14%) (Knicker and Lüdemann 1995). The chemical
550 shifts of carbon in carboxylic acids, esters and amides fall within the range between 160
551 ppm and 220 ppm and represents 15% of the total ^{13}C intensity. There are minor
552 contributions of carbonyl or aldehydes, giving signals between 220- 245 ppm.

553 The NMR spectra of NO-2 and NO-3 differ considerably from that of NO-1. In
554 NO-2, the *O*-alkyl C fraction has the highest values (26%), followed by alkyl C,
555 carboxyl/amide C and aromatic C. Considering that there is no clear signal in the *O*-
556 substituted C region (from 140– 160 ppm), this spectrum can be best explained with a

557 considerable contribution of oxidized charcoal. NO-3 presented the highest contribution
558 of non-lignin aromatic fraction, probably BC-derived (31%) and a large signal of
559 carboxyl/amide C (26%), which likely originates from microbial compounds.

560 The chemical oxidants had several effects on the NMR signal obtained from
561 sample S1. After the KMnO_4 treatment (MN-1), and comparable to results from Py-GC-
562 MS, a spectrum similar to that of NO-1 but with slightly higher relative intensities in the
563 alkyl C (34%) region was acquired, confirming other ^{13}C NMR studies (Tirol-Padre and
564 Ladha 2004) in that cellulose is largely resistant to permanganate oxidation. Dichromate
565 oxidation of sample S1 caused an increase of the relative contribution of aromatic C
566 (sum of aromatic C-H and aromatic C-O-R) (from 17% in NO-1 to 25% in CR-1) with a
567 concomitant depletion of methoxyl C/N-alkyl C and O-alkyl C (see Table 3). Note that
568 differences between NO-1 and CR-1 by NMR are smaller than observed by Py-GC/MS.
569 This indicates that, as Py-GC/MS data seem to be best supported, NMR results must be
570 taken carefully.

571 The spectrum of CR-2 showed the highest intensity in the chemical shift region of
572 aromatic C (45%). Considering the absence of methoxyl C signal, the width of the
573 signal band (from 90 to 140 ppm) and the composition of the pyrolysis fingerprint, this
574 aromatic signal originates from BC (see also Skjemstad et al. 1996; Knicker et al.
575 2005). Moreover, the contribution of alkyl C was strongly reduced upon $\text{K}_2\text{Cr}_2\text{O}_7$
576 oxidation (10% in CR-2).

577 In summary, in NO samples, ^{13}C NMR spectroscopy shows a relative decrease
578 with depth of aliphatic C, carbohydrates and lignin moieties and a relative increase with
579 depth of a non-lignin aromatic fraction (probably BC) and carboxyl/amide C (possibly
580 oxidized BC in combination with N-rich microbial SOM), which is in agreement with
581 Py-GC/MS data. With regard to the effects of chemical oxidation, both Py-GC/MS and

582 ^{13}C NMR spectroscopy showed a decrease of easily degradable SOM (mainly composed
583 of fresh lignin and polysaccharides) and an increase of the aromatic fraction. Besides,
584 lignin was slightly oxidized by KMnO_4 contrary to that observed by previous studies
585 (van Soest and Wine 1986; Tyrol-Padre and Ladha 2004; Skjemstad et al. 2006) where
586 lignin was strongly degraded. The increase of aliphatic moieties upon chemical
587 oxidation, as suggested by Py-GC/MS, was also observed by NMR spectroscopy in the
588 superficial sample S1 although it strongly decreased in S2. However here one has to
589 bear in mind that the alkyl C region does not only contain intensity of lipids but have
590 considerable contributions of peptide structures or short alkyl side-chains (such as C_3 -
591 side chains in lignin). Those moieties may be expected to be relatively susceptible to
592 oxidation resulting in a relative depletion of the signal intensity in the alkyl C region
593 even though longer chain aliphatic components may have experienced a relative
594 enrichment.

595

596 4. Conclusions

597 The molecular study of SOM fractions of three horizons of a colluvial soil
598 representing ages of 100, 5,000 and 9,700 yr, before and after treatment with KMnO_4
599 and $\text{K}_2\text{Cr}_2\text{O}_7$, provided detailed information on SOM composition (with regard to
600 source and degradation/preservation state) and the behaviour of different SOM fractions
601 towards these oxidation agents. *Eucalyptus*-derived terpenes and sesquiterpenes were
602 only present in the youngest sample and resisted KMnO_4 but not $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation.
603 Microbial/degraded SOM, mostly composed of carbohydrates and chitin, was especially
604 abundant in the deeper layers of the soil and appeared highly susceptible to both
605 KMnO_4 and $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation. As such, KMnO_4 could be used as an indication of the
606 abundance of microbial biomass. Both oxidants, $\text{K}_2\text{Cr}_2\text{O}_7$ in particular, concentrated

607 two other SOM fractions abundant in this soil: aliphatic and pyrogenic material (BC),
608 the latter having a significant amount of N-containing functional groups (BN). These
609 fractions probably survived $K_2Cr_2O_7$ oxidation because of the chemical stability of
610 polyaromatic moieties (BC) and resistant C-C bonds in methylene chains and/or
611 hydrophobicity of the aliphatic fraction (which is probably root-derived). It appeared
612 that especially $K_2Cr_2O_7$ oxidation efficiently concentrates BC and oxidation-resistant
613 aliphatic structures from other SOM sources, and that in combination with Py-GC/MS it
614 is possible to distinguish between these sources (yet not quantitatively) while ^{13}C NMR
615 may assist in obtaining estimations of their relative proportions. Finally, BC isolation by
616 dichromate oxidation and posterior quantification through total digestion (Knicker et al.,
617 2007) is discouraged as a significant aliphatic fraction resists dichromate producing an
618 overestimation of its contents.

619

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625

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855 Figure captions

856 Fig. 1. Example of pyrograms from permanganate- and dichromate-oxidation resistant
857 soil organic matter, and that from non-oxidized samples.

858 Fig. 2. Projection of the factor loadings of the pyrolysis products and sample scores in
859 F1-F2 space. The corresponding pyrolysis product codes are given in Appendix A.

860 Fig. 3. Solid- state ^{13}C NMR spectra of the NaOH-extracts of untreated samples (NO-1,
861 NO-2 and NO-3) and the NaOH-extracts of potassium permanganate and dichromate
862 oxidized residues of S1 sample (MN-1 and CR-1) and the NaOH-extract of the
863 potassium dichromate oxidized residues of S2 sample (CR-2).

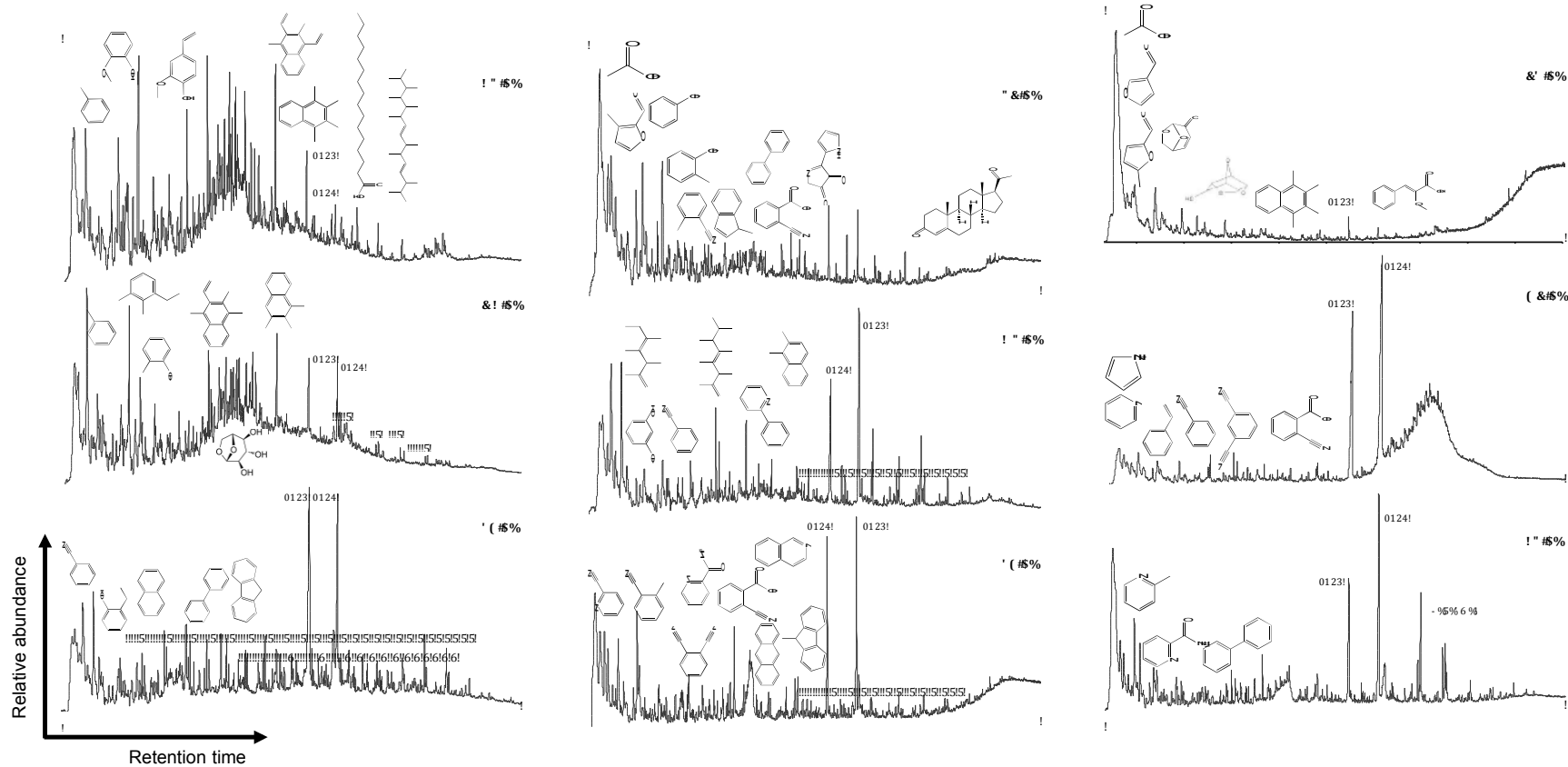


Fig. 1

NO-1: toluene, guaiacol, 4-vinyl guaiacol, C_{4:1} naphthalene compound, C₄-naphthalene, hexadecanoic acid (C₁₆ *n*-fatty acid) and octadecanoic acid (C₁₈ *n*-fatty acid), and branched alkene. MN-1: toluene, methyl phenol, 2-ethyl 3,4-dimethyl phenol, C_{4:1} naphthalene compound, levoglucosan, C₃-naphthalene, C₁₆ *n*-fatty acid and C₁₈ *n*-fatty acid, and series of pairs of *n*-alkanes/alkenes (*). CR-1: benzonitrile, ethyl phenol, naphthalene, biphenyl, fluorene and regular series of pairs of *n*-alkanes/alkenes (*) and *n*-fatty acids (†). NO-2: acetic acid, methyl furaldehyde, phenol, methyl phenol, methyl benzonitrile, methyl indene, biphenyl, cyanobenzoic acid, diketodipyrrole and sterol compound (preg-4-ene 3,20-dione compound). MN-2: branched alkenes, resorcinol, benzonitrile, phenyl pyridine, C₂ naphthalene, C₁₆ *n*-fatty acid and C₁₈ *n*-fatty acid and regular series of pairs of *n*-alkanes/alkenes (*). CR-2: pyridine carbonitrile, methyl benzonitrile, benzenedicarbodinitrile, picolinamide, anthracene, methyl fluorene, cyanobenzoic acid, isoquinoline, C₁₆ *n*-fatty acid and C₁₈ *n*-fatty acid and regular series of pairs of *n*-alkanes/alkenes (*). NO-3: acetic acid, furaldehyde, methyl furaldehyde, levoglucosenone, dianhydro- α , D-glucopyranose, C₄-naphthalene and C₁₆ *n*-fatty acid. MN-3: pyridine, pyrrole, styrene, benzonitrile, benzenedicarbodinitrile, cyanobenzoic acid and C₁₆ *n*-fatty acid and C₁₈ *n*-fatty acid. CR-3: methyl pyridine, picolinamide, biphenyl, C₁₆ *n*-fatty acid and C₁₈ *n*-fatty acid and a set of unidentified compounds.

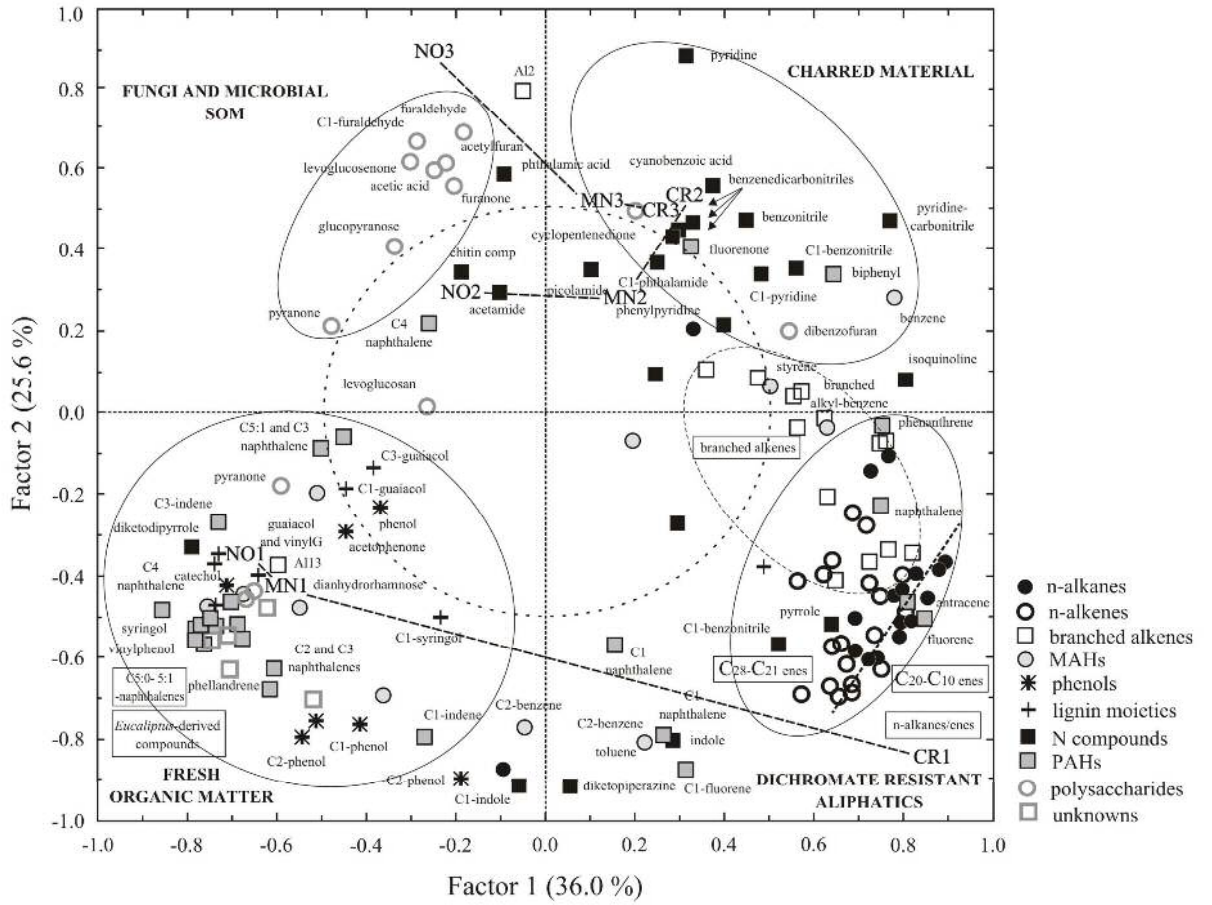


Fig.2

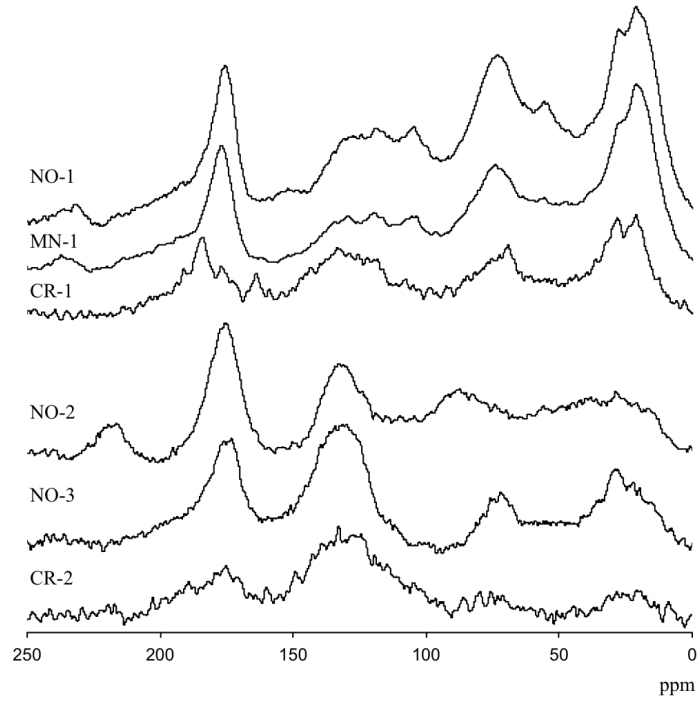


Fig.3

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Table 1. General information of samples studied from soil PRD-4. OC_{per} = permanganate-oxidizable organic C, OC_{dichro} = dichromate-oxidizable C.

| Sample | S1 | S2 | S3 |
|---------------------------------------|-------------------------------|-----------------|-----------------|
| depth | 5-10 cm | 95-100 cm | 190-195 cm |
| conventional ^{14}C age BP | 104.3 ± 0.4 pMC (present) | 4090 ± 30 | 9760 ± 50 |
| radiocarbon sample code | Ua-34719 | β -299230 | β -240963 |
| C [$mg\ g^{-1}$ soil] | 62.3 ± 0.6 | 36.7 ± 0.3 | 42.9 ± 0.4 |
| OC_{per} [$mg\ g^{-1}$ soil] | 2.3 ± 0.1 | 1.3 ± 0.1 | 1.7 ± 0.1 |
| OC_{dichro} [$mg\ g^{-1}$ soil] | 27.5 ± 0.6 | 12.3 ± 0.3 | 14.3 ± 0.1 |
| C/N (atomic) [-] | 15.9 | 24.4 | 23.9 |
| pH- H_2O [-] | 4.6 | 5.0 | 5.2 |
| charcoal >2 mm [$mg\ g^{-1}$ soil] | 0.03 | 1.97 | 0.07 |

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Table 2. Relative contributions of pyrolysis product groups and benzene/alkylbenzenes and PAH/alkyl-PAHs ratios of total quantified peak area (% TQPA).

| | | total n-enes | n- enes >C ₁₈ | n-enes C ₁₈ -C ₁₀ | total n- anes | n-anes >C ₁₈ | n-anes C ₁₈ - C ₁₀ | other aliph ⁺ | phs | C ₂ - ph | total Lg | total MAHs | alkyl- B | total PAHs | ITPB- PAHs | BC- PAHs | total N | BN | total Ps | well pres Ps | degr Ps | U | benz/ alkyl- benz | PAH/ alkyl- PAHs |
|------|-----------|-----------------|--------------------------------|--|---------------------|----------------------------|--|-----------------------------|-------------|------------------------|-------------|---------------|-------------|---------------|---------------|-------------|-------------|------|-------------|--------------------|------------|-----|-------------------------|------------------------|
| NO-1 | TQPA % | 1.7 | 0.9 | 0.8 | 1.9 | 0.8 | 1.1 | 1.4 | 16.6 | 1.8 | 7.4 | 16.4 | 6.6 | 10.6 | 10.1 | 0.5 | 10.7 | 3.6 | 30.4 | 17.8 | 12.4 | 2.5 | 0.2 | 0.02 |
| | %* | | 51.4 | 48.6 | | 40.1 | 59.9 | | | | | | 40.0 | 95.6 | 4.4 | | 33.2 | | 58.4 | 40.7 | | | | |
| MN-1 | TQPA % | 1.6 | 0.5 | 1.1 | 2.0 | 0.3 | 1.7 | 4.1 | 14.1 | 1.9 | 3.0 | 30.0 | 13.4 | 12.3 | 11.4 | 1.0 | 11.7 | 4.8 | 18.4 | 10.4 | 7.7 | 2.7 | 0.2 | 0.04 |
| | %* | | 33.6 | 66.4 | | 13.4 | 86.6 | | | | | | 44.7 | 92.2 | 7.8 | | 40.7 | | 56.6 | 41.7 | | | | |
| CR-1 | TQPA % | 9.3 | 3.0 | 6.3 | 10.2 | 3.3 | 6.9 | 14.6 | 10.9 | 1.4 | 1.0 | 26.3 | 4.6 | 4.8 | 2.2 | 2.6 | 15.8 | 7.4 | 6.4 | 0.8 | 5.4 | 0.7 | 1.2 | 0.60 |
| | %* | | 32.2 | 67.8 | | 32.2 | 67.8 | | | | | | 17.5 | 45.7 | 54.3 | | 47.0 | | 12.4 | 84.7 | | | | |
| NO-2 | TQPA % | 1.8 | 0.6 | 1.2 | 1.8 | 0.6 | 1.2 | 1.7 | 13.7 | 0.8 | 1.8 | 20.4 | 4.9 | 5.7 | 4.2 | 1.5 | 20.9 | 11.9 | 31.5 | 4.0 | 27.1 | 0.7 | 0.9 | 0.21 |
| | %* | | 34.2 | 65.8 | | 34.1 | 65.9 | | | | | | 23.8 | 73.0 | 27.0 | | 57.1 | | 12.6 | 86.3 | | | | |
| MN-2 | TQPA % | 2.5 | 0.7 | 1.9 | 3.2 | 0.5 | 2.7 | 17.4 | 11.6 | 0.9 | 1.4 | 20.4 | 3.6 | 3.3 | 2.2 | 1.2 | 22.2 | 13.9 | 17.4 | 3.3 | 13.6 | 0.5 | 1.3 | 0.26 |
| | %* | | 26.0 | 74.0 | | 16.7 | 83.3 | | | | | | 17.5 | 65.0 | 35.0 | | 62.6 | | 18.7 | 77.9 | | | | |
| CR-2 | TQPA % | 4.6 | 1.6 | 3.0 | 2.5 | 0.9 | 1.5 | 7.0 | 4.8 | 0.5 | 0.8 | 16.9 | 2.0 | 3.4 | 0.9 | 2.5 | 32.2 | 23.8 | 27.6 | 15.7 | 11.5 | 0.2 | 3.9 | 1.31 |
| | %* | | 35.3 | 64.7 | | 37.7 | 62.3 | | | | | | 11.6 | 25.3 | 74.7 | | 74.1 | | 57.0 | 41.8 | | | | |
| NO-3 | TQPA % | 0.9 | 0.3 | 0.5 | 1.0 | 0.5 | 0.5 | 1.5 | 6.6 | 0.0 | 0.7 | 10.3 | 2.5 | 1.4 | 0.9 | 0.5 | 15.0 | 9.0 | 62.2 | 2.7 | 59.2 | 0.3 | 1.3 | 0.35 |
| | %* | | 36.6 | 63.4 | | 47.3 | 52.7 | | | | | | 24.0 | 65.0 | 35.0 | | 60.2 | | 4.4 | 95.2 | | | | |
| MN-3 | TQPA % | 2.7 | 0.8 | 1.9 | 2.9 | 1.2 | 1.7 | 9.2 | 7.7 | 0.0 | 1.2 | 17.5 | 4.1 | 2.4 | 1.4 | 1.0 | 38.3 | 28.9 | 17.7 | 1.1 | 16.0 | 0.4 | 0.8 | 0.34 |
| | %* | | 30.2 | 69.8 | | 42.0 | 58.0 | | | | | | 23.2 | 57.9 | 42.1 | | 75.5 | | 6.5 | 90.7 | | | | |
| CR-3 | TQPA % | 3.5 | 1.2 | 2.3 | 2.6 | 1.1 | 1.5 | 10.7 | 5.0 | 0.0 | 2.2 | 22.3 | 2.7 | 2.5 | 0.7 | 1.7 | 22.1 | 16.5 | 28.8 | 16.5 | 11.4 | 0.3 | 2.7 | 0.89 |
| | %* | | 35.0 | 65.0 | | 41.1 | 58.9 | | | | | | 11.9 | 30.0 | 70.0 | | 74.8 | | 57.5 | 39.6 | | | | |

*relative proportions within main group (*n*-alkanes/ enes, fatty acids, MAHs, PAHs, nitrogen compounds and polysaccharides). In bold the main groups.

⁺the aliphatic compound with mass 83+280 (likely associated to fresh OM) was not added because is not indicative of the charring effect.

Total *n*-enes: total *n*-alkenes; *n*-enes >C₁₈: long-chain *n*-alkenes (>C₁₈); *n*-enes C₁₈-C₁₀: short-chain *n*-alkenes (C₁₈-C₁₀); total *n*-anes: total *n*-alkanes; *n*-anes >C₁₈: long-chain *n*-alkanes (>C₁₈); *n*-anes C₁₈-C₁₀: short-chain *n*-alkanes (C₁₈-C₁₀); other aliph: other aliphatic compounds (predominantly branched alkenes); phs: phenols; total Lg: total lignin markers; total MAHs: total Monocyclic Aromatic Hydrocarbons; alkyl-B: alkyl-benzenes; total PAHs: total Polycyclic Aromatic Hydrocarbons; ITPB-PAHs: intact terpene-like plant biomass Polycyclic Aromatic Hydrocarbons; BC-PAHs: black carbon derived Polycyclic Aromatic Hydrocarbons; total N: total nitrogen compounds; BN: black carbon derived nitrogen compounds; total Ps: total polysaccharides; well pres Ps: well preserved polysaccharides; degr Ps: degraded polysaccharides; U: unidentified compounds; benz/alkyl-benz: benzene/alkyl-benzenes ratio and PAHs/alkyl-PAHs: total Polycyclic Aromatic Hydrocarbons/ alkylated Polycyclic Aromatic Hydrocarbons ratio.

Table 3. Chemical shift region distribution (relative proportions, %) obtained from solid-state ^{13}C NMR.

| | Alkyl C (0-45 ppm) | N-alkyl C, methoxyl C (45-60 ppm) | O-alkyl C (60-110 ppm) | C-H Aromatic C (110-140 ppm) | COR Aromatic C (140-160 ppm) | Carboxyl C, amide C (160-220 ppm) | Ketone C, aldehyde C (220-245 ppm) |
|------|-----------------------|---|---------------------------|---------------------------------|---------------------------------|---|--|
| NO-1 | 29 | 9 | 29 | 14 | 3 | 15 | 1 |
| MN-1 | 34 | 8 | 26 | 11 | 2 | 17 | 1 |
| CR-1 | 29 | 4 | 22 | 19 | 6 | 20 | 0 |
| NO-2 | 23 | 5 | 26 | 19 | 1 | 21 | 4 |
| CR-2 | 10 | 2 | 16 | 42 | 3 | 23 | 4 |
| NO-3 | 19 | 5 | 11 | 31 | 5 | 26 | 3 |

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Appendix A. Pyrolysis product list, molecular mass (M^+), fragment ions used for quantification and retention times relative to guaiacol (RT).

| code | Name | M^+ | mass | RT/guaiacol |
|-------------|---|-----------|----------|---------------|
| 10:1 - 28:1 | C10-28 alkene | 140 - 392 | 55+69 | 0.832 - 3.965 |
| 10:0 - 28:0 | C10-28 alkane | 142 - 394 | 57+71 | 0.857 - 3.969 |
| A11 | aliphatic compound | n.d. | 55+70 | 0.549 |
| A12 | alkane/anal or methylated alkanol | n.d. | 57+69+70 | 1.157 |
| A13 | branched alkene | n.d. | 55+69 | 1.160 |
| A14 | branched alkene | n.d. | 55+69 | 1.573 |
| A15 | branched alkene | n.d. | 55+69 | 1.590 |
| A16 | branched alkene | n.d. | 55+69 | 1.610 |
| A17 | alkene | n.d. | 55+69 | 1.901 |
| A18 | alkene | n.d. | 55+69 | 2.301 |
| A19 | branched alkene | n.d. | 55+69 | 2.428 |
| A110 | branched alkene | n.d. | 55+69 | 2.941 |
| A111 | alkene | n.d. | 55+69 | 3.069 |
| A112 | branched alkene | n.d. | 55+69 | 3.272 |
| A113 | aliphatic compound | 83+280 | 83+280 | 3.342 |
| A114 | branched alkene | n.d. | 55+69 | 3.565 |
| A115 | branched alkene | n.d. | 55+69 | 3.799 |
| Ph1 | phenol | 94 | 66+94 | 0.782 |
| Ph2 | acetophenone | 120 | 77+105 | 0.937 |
| Ph3 | C1-phenol | 108 | 107+108 | 0.941 |
| Ph4 | C1-phenol | 108 | 107+108 | 0.991 |
| Ph5 | C2-phenol | 122 | 107+122 | 1.158 |
| Ph6 | C2-phenol | 122 | 107+122 | 1.202 |
| Ph7 | catechol | 110 | 110+64 | 1.348 |
| Lg1 | guaiacol | 124 | 109+124 | 1.000 |
| Lg2 | 4-methylguaiacol | 138 | 123+138 | 1.245 |
| Lg3 | 4-vinylphenol | 120 | 120+91 | 1.326 |
| Lg4 | 4-vinylguaiacol | 150 | 135+150 | 1.519 |
| Lg5 | syringol | 154 | 154+139 | 1.585 |
| Lg6 | 4-methylsyringol | 168 | 153+168 | 1.798 |
| Lg7 | C3-guaiacol | 196 | 149+164 | 1.815 |
| Lg8 | Propenoic acid, 3-(4-methoxyphenol) | 178 | 161+178 | 3.296 |
| Ar1 | benzene | 78 | 78 | 0.347 |
| Ar2 | toluene | 92 | 91+92 | 0.431 |
| Ar3 | C2-benzene ethyl benzene | 106 | 91+106 | 0.559 |
| Ar4 | C2-benzene dimethyl benzene | 106 | 91+106 | 0.574 |
| Ar5 | styrene | 104 | 78+104 | 0.605 |
| Ar6 | C2-benzene dimethyl benzene | 106 | 91+106 | 0.612 |
| Ar7 | C3-benzene | 120 | 105+120 | 0.815 |
| Ar8 | C4-benzene | 134 | 91+119 | 0.887 |
| Ar9 | C5-benzene (dimethyl-methylethyl) | 148 | 133 | 1.160 |
| Ar10 | C4:1-benzene (α -dimethylstyrene) | 132 | 132+117 | 1.026 |
| Ar11 | C7-benzene | 176 | 91+92 | 1.662 |
| Ar12 | branched alkyl-benzene | 148 | 91+119 | 1.194 |

| | | | | |
|------|--------------------------------|-----|---------|-------|
| Pa1 | C1-indene | 130 | 130+115 | 1.146 |
| Pa2 | naphthalene | 128 | 128 | 1.231 |
| Pa3 | C1-naphthalene | 142 | 141+142 | 1.491 |
| Pa4 | C1-naphthalene | 142 | 141+142 | 1.523 |
| Pa5 | biphenyl | 154 | 154 | 1.668 |
| Pa6 | C3-indene | 158 | 143+158 | 1.723 |
| Pa7 | C2-naphthalene | 156 | 141+156 | 1.772 |
| Pa8 | C3-naphthalene | 170 | 155+170 | 2.052 |
| Pa9 | C3-naphthalene | 170 | 155+170 | 2.056 |
| Pa10 | Fluorene | 166 | 165+166 | 2.090 |
| Pa11 | C3-naphthalene | 170 | 155+170 | 2.103 |
| Pa12 | C1-Fluorene | 180 | 165+180 | 2.314 |
| Pa13 | 9H-Fluoren-9-one | 180 | 152+180 | 2.353 |
| Pa14 | C4-naphthalene | 184 | 169+184 | 2.382 |
| Pa15 | phenanthrene | 178 | 178 | 2.442 |
| Pa16 | anthracene | 178 | 178 | 2.461 |
| Pa17 | C5-naphthalene | 198 | 183+198 | 2.470 |
| Pa18 | C5-naphthalene (or C2-azulene) | 198 | 183+198 | 2.643 |
| Pa19 | C5:1-naphthalene | 202 | 159+145 | 1.649 |
| Pa20 | C5-naphthalene | 204 | 147+162 | 1.739 |
| Pa21 | C5:1-naphthalene | 202 | 159+202 | 1.767 |
| Pa22 | C5-naphthalene | 204 | 91+105 | 1.801 |
| Pa23 | C5-naphthalene | 204 | 105+133 | 1.850 |
| Pa24 | C5-naphthalene | 204 | 91+105 | 1.853 |
| Pa25 | C5-naphthalene | 204 | 91+105 | 1.928 |
| Pa26 | C5-naphthalene | 204 | 91+105 | 1.974 |
| Pa27 | C5-naphthalene | 204 | 173+189 | 1.987 |
| Pa28 | C4-naphthalene | 186 | 143+171 | 2.000 |
| Pa29 | C5:1-naphthalene | 202 | 159+180 | 2.037 |
| Pa30 | C5:1-naphthalene | 202 | 146+133 | 2.143 |
| Pa31 | C5:1-naphthalene | 202 | 159+202 | 2.211 |
| N1 | pyridine | 79 | 52+79 | 0.396 |
| N2 | pyrrole | 67 | 67 | 0.402 |
| N3 | acetamide | n.d | 59 | 0.421 |
| N4 | C1-pyrrole | 81 | 80+81 | 0.501 |
| N5 | C1-pyrrole | 81 | 80+81 | 0.521 |
| N6 | C1-pyridine | 93 | 66+93 | 0.543 |
| N7 | benzonitrile | 103 | 76+103 | 0.749 |
| N8 | pyridinecarbonitrile | 104 | 104+77 | 0.887 |
| N9 | C1-benzonitrile | 117 | 90+117 | 1.011 |
| N10 | C1-benzonitrile | 117 | 90+117 | 1.067 |
| N11 | 1,3-benzenedicarbonitrile | 128 | 101+128 | 1.302 |
| N12 | picolinamide | 122 | 79+122 | 1.320 |
| N13 | 1,3-benzenedicarbonitrile | 128 | 101+128 | 1.322 |
| N14 | isoquinoline | 129 | 129 | 1.334 |
| N15 | indole | 117 | 90+117 | 1.457 |
| N16 | phthalamic acid | 104 | 104+76 | 1.457 |
| N17 | 1,3-benzenedicarbonitrile | 128 | 101+128 | 1.463 |
| N18 | C1-indole | 131 | 130+131 | 1.664 |
| N19 | C1-phthalimide | 161 | 161+76 | 1.719 |

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|------|--------------------------------------|-----|-----------|-------|
| N20 | cyanobenzoic acid | 147 | 76+147 | 1.827 |
| N21 | phenylpyridine | 155 | 154+155 | 1.833 |
| N22 | chitin-derived compound | 167 | 125+167 | 1.883 |
| N23 | diketodipyrrole | 186 | 93+186 | 2.280 |
| N24 | diketopiperazine compound | 194 | 70+194 | 2.646 |
| Ps1 | acetic acid | 60 | 60 | 0.203 |
| Ps2 | furanone compound | 84 | 54+84 | 0.434 |
| Ps3 | 3/2-furaldehyde | 96 | 95+96 | 0.483 |
| Ps4 | acetyl furan | 110 | 95+110 | 0.613 |
| Ps5 | 5-methyl-2-furaldehyde | 110 | 109+110 | 0.686 |
| Ps6 | 4-hydroxy-5,6-dihydro-(2H)-pyranone | 114 | 58+114 | 0.755 |
| Ps7 | dianhydrorhamnose | 128 | 113+128 | 0.861 |
| Ps8 | cyclopentenedione compound | 112 | 69+112 | 0.869 |
| Ps9 | levoglucosenone | 126 | 68+98 | 0.989 |
| Ps10 | 3-hydroxy-2-methyl-4H-pyran-4-one | 126 | 71+126 | 1.046 |
| Ps11 | dianhydro- α ,D-glucopyranose | 144 | 57+69 | 1.263 |
| Ps12 | dibenzofuran | 168 | 168+139 | 1.953 |
| Ps13 | levoglucosan | 162 | 60+73 | 2.123 |
| U1 | alpha phellandrene | 136 | 91+93+136 | 0.827 |
| U2 | U2 (possibly methylcyclohexane) | 96 | 67+96 | 0.710 |
| U3 | U3 | 157 | 117+157 | 1.871 |
| U4 | U4 | 200 | 185+200 | 2.186 |
| U5 | U5 | 212 | 197+202 | 2.247 |
| U6 | U6 | 203 | 203 | 3.152 |