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Purification of fire-derived markers for μg scale isotope analysis ($\delta^{13}C$, $\Delta^{14}C$) using high-performance liquid chromatography (HPLC)

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ABSTRACT

Black carbon (BC) is the residue of incomplete biomass combustion. It is ubiquitous in nature and, due to its relative persistence, is an important factor in Earth's slow-cycling carbon pool. This resistant nature makes pure BC one of the most used materials for ^{14}C dating to elucidate its formation date or residence time in the environment. However, most BC samples cannot be physically separated from their matrices, precluding accurate ^{14}C values. Here we present a method for radiocarbon dating of the oxidation products of BC, benzene polycarboxylic acids, thereby circumventing interference from extraneous carbon. Individual compounds were isolated using high performance liquid chromatography (HPLC) and converted to CO_2 via wet chemical oxidation for ^{13}C and ^{14}C isotope analysis. A detailed assessment was performed to identify and quantify sources of extraneous carbon contamination with two process standards of distinct isotopic signatures. The average blank was 1.6 ± 0.7 μg C and had an average radiocarbon content of 0.90 ± 0.50 $F^{14}C$. We successfully analyzed the ^{14}C content of individual benzene polycarboxylic acids with a sample size as small as 20-30 μg C after correcting for the presence of the average blank. The combination of $\delta^{13}C$ and $F^{14}C$ analysis helps interpret the results and enables monitoring of extraneous carbon contribution in a fast and cost efficient way. Such a molecular approach to radiocarbon dating of BC residues enables the expansion of isotopic BC studies to samples that have either been too small or strongly affected by non-fire derived carbon.

Keywords: Compound-specific radiocarbon and isotope analyses; CSRA; benzene polycarboxylic acids; HPLC

1. Introduction

The solid residues of incomplete biomass combustion are generally summarized under the term black carbon (BC). It is ubiquitous in nature and can be found in the atmosphere, sediments, water and ice (Goldberg, 1985) and includes a continuum of combustion products ranging from slightly charred biomass to char and charcoal to highly condensed refractory soot (Hedges et al., 2000; Masiello, 2004). Fire derived components are of interest for the investigation of the global C cycle due to their relative persistence in the environment. It is widely accepted that BC contributes significantly to the Earth's slow-cycling C pool (Skjemstad et al., 1996, 2001; Schmidt et al., 2000; Preston and Schmidt, 2006; Knicker et al., 2008) and in models of soil organic matter (OM) turnover it is defined as a C pool with relatively high resistance to degradation (Skjemstad et al., 2004). It is also utilized in the reconstruction of fire history from geological records (e.g. Glaser et al., 2000; Carcaillet et al., 2002; Tinner et al., 2005). Similarly, pure charcoal is of importance for archeological research; in addition, the presence of BC at excavation sites allows precise determination of the age of the finds from ^{14}C analysis. Consequently, pieces of pure charcoal are one of the most targeted materials for ^{14}C dating in archaeological or geological research (Bird et al., 1999).

Nevertheless, the physicochemical properties and the biological stability of BC are poorly understood and even quantification is inherently difficult. One promising approach towards a better understanding and quantification of BC is a molecular method, the so-called 'BPCA method' introduced by Glaser et al. (1998). Benzene polycarboxylic acids (BPCAs) result from the digestion of BC with HNO_3 under high pressure and temperature, and can be analyzed using either gas chromatography (GC; Glaser et al., 1998) or high performance liquid chromatography (HPLC; Dittmar, 2008; Wiedemeier et al., 2013). The BPCAs derive unambiguously from BC and provide insight into the original BC at a molecular scale. In addition to being a quantifiable molecular proxy for the total amount of BC in a complex matrix, the relative distribution of individual BPCAs can provide further information. For example, a relatively high amount of highly carboxylated BPCAs such as mellitic acid (B6CA) and benzene pentacarboxylic acid (B5CA) is indicative of a high degree of condensation (Dittmar, 2008; Schneider et al., 2011). The numbers in the notation indicate the number of substituted carboxylic acid groups per benzene ring.

The radiocarbon signature of BPCAs has potential for elucidating the fate and source of BC in nature, as the concentration of ^{14}C in a BC sample can be directly related to its age or mean residence time. For recent BC samples, ^{14}C analysis allows source apportioning of the BC between fossil fuel derived charcoal that is depleted in ^{14}C ($F^{14}C$ 0) and burned modern

biomass that reflects atmospheric radiocarbon content ($F^{14}C \geq 1$). On the whole, there is an essential advantage in determining the ^{14}C content of these specific biomarkers vs. dating of bulk BC samples (e.g. Ziolkowski and Druffel, 2009; Zimmerman, 2010; Yarnes et al., 2011). In particular, analysis of bulk samples frequently suffers from large uncertainty due to small sample size and the challenge in physically separating pure BC from interfering OM. For example, OM in soils and sediments is a complex mixture of compounds, which can range from recently produced compounds to very old material (Hedges et al., 2000). The same is true for buried archaeological samples. The matrix of pottery can contain organic carbon-bearing clay closely associated with the charred residue, or organic carbon can be taken up from the burial environment, causing further interference.

Eglinton et al. (1996) introduced the concept of compound-specific radiocarbon analysis (CSRA) applied to certain solvent extractable lipids. The first application using the BPCA method to date BC on a molecular scale was by Ziolkowski and Druffel (2009a), who separated individual BPCAs using preparative GC, and achieved reliable results with reasonably low and constant blanks. Nevertheless, the method has several drawbacks. BPCAs must be treated to form GC-amenable derivatives, requiring the addition of external C. The authors applied trimethylsilyl-diazomethane as derivatization agent. Even though it has been reported to be more efficient than other derivatization protocols (Ziolkowski and Druffel, 2009b), it is known that losses can occur. This is also true for the most common derivatization technique, silylation with BSTFA (Schneider et al., 2011). Finally, a GC column has limited capacity, necessitating a number of injections to collect sufficient material for dating. Many of the problems can be circumvented by separating the BPCAs using HPLC (Dittmar, 2008; Wiedemeier et al., 2013).

In general, ^{14}C analysis is sensitive to any contribution from extraneous carbon (C_{ex}) added to the sample during the laboratory protocol. This is particularly true for ultra small scale samples containing < 30 μg C. Purification procedures for CSRA must therefore be designed to minimize and accurately quantify C_{ex} addition (Pearson et al., 1998; Shah and Pearson, 2007; Ziolkowski and Druffel, 2009a; Birkholz et al., 2013; Lang et al., 2013).

In this study we present a new approach for molecular scale ^{14}C analysis of fire-derived compounds on the basis of the separation of BPCAs using liquid chromatography (Wiedemeier et al., 2013) combined with a recent method of wet oxidation suitable for combined ^{13}C and ^{14}C isotope analysis (Lang et al., 2012, 2013). The method allows sample oxidation despite the presence of concentrated H_3PO_4, which is essential for achieving separation of the BPCAs with HPLC. The direct conversion of the BPCAs to CO_2 within a gas tight vial allows the sample gas to be subsampled for $\delta^{13}C$ analysis prior to injection for accelerator mass spectrometry (AMS).

104 We describe the successful purification of individual B5CAs and B6CAs followed by $\delta^{13}C$
105 and $F^{14}C$ analysis. A detailed blank assessment was carried out using direct and indirect
106 approaches to assess the amount and the isotopic signature of C_{ex}. Two different types of
107 charcoal were selected as standards. The first was an archaeological charcoal with a ^{14}C age >
108 50000 BP ($\leq$ 0.02 $F^{14}C$) and the second a modern charcoal ($\geq$ 1 $F^{14}C$) prepared from a
109 recently cut tree. Together they represented the end members of ^{14}C analysis and thereby
110 allowed a good evaluation of this new method for BC dating.

111 **2. Experimental**

112 The data were produced from two successive series of experiments. Both included HPLC
113 isolation followed by wet oxidation, GC-isotope ratio mass spectrometry (GC-IRMS) and
114 AMS measurements. All glassware was pre-heated to 450 °C for 5 h prior to use to remove
115 organic contaminants. Ultra pure water was supplied from a MilliQ Advantage A10 system
116 (Millipore, USA) and all chemicals were of the highest available grade and were tested for
117 impurities before use.

118 *2.1. Process standards*

119 The archaeological charcoal sample ('fossil char') was from in situ charred trees sampled
120 from paroxysmal flow deposits in the Maninjau caldera in West-Central Sumatra (Alloway et
121 al., 2004). Its precise dating using conventional AMS demonstrated that it lacked ^{14}C and had
122 an age of ca. 50 ka BP (Alloway et al., 2004; Ascough et al., 2009). The modern analog
123 ('modern char') was produced from chestnut wood (*Castanea sativa*) from a single tree cut in
124 a forest in Southern Switzerland. The wood was charred at 450 °C for 5 h under a N_2
125 atmosphere (Hammes et al., 2006).

126 The samples were of almost pure charcoal, one was recovered in-situ and the other was
127 produced in the laboratory under controlled conditions, allowing the assumption that they
128 were not significantly affected by interfering C-bearing material. Therefore the radiocarbon
129 contents of the bulk samples were expected to be the same as that of the isolated BPCAs.
130 Bulk subsamples were analyzed for ^{14}C content as solid targets at the Laboratory of Ion Beam
131 Physics of the ETH Zürich, Switzerland after being sequentially extracted with acid and base
132 reagents to remove contaminants from the surfaces. The so-called acid-base-acid (ABA)
133 treatment is a standard cleaning procedure prior to ^{14}C analysis (Hajdas et al., 2004). The ^{14}C
134 content of the fossil char was found to be 0.003 ± 0.001 $F^{14}C$ (ETH-50456). The modern char
135 was produced from unaltered dried wood that had a ^{14}C content of 1.142 ± 0.004 $F^{14}C$ (ETH-
136 50458) and its charred residue was almost identical at 1.149 ± 0.004 $F^{14}C$ (ETH-50457).
137 These values were used as reference values.

138 *2.2. Sample extraction and purification*

139 Extraction and purification of BPCAs was carried out according to the protocol of
140 Wiedemeier et al. (2013) with modifications to make it amenable for CSRA. In brief, 15-25
141 mg of the dried and milled sample was directly digested in a quartz tube with 2 ml HNO_3 (65
142 wt.% or 14.4 mol/l) at 170 °C for 8 h. After cooling, the aqueous solution was filtered over
143 pre-rinsed quartz fiber filters. The extract was eluted over a cation exchange resin and freeze
144 dried to remove water and acid. The dried residue was dissolved in MeOH/water (1:1, v/v)
145 and applied to a pre-conditioned C_{18} solid phase extraction cartridge to remove apolar
146 components. Finally, the eluate was dried again using an Eppendorf concentrator system.

147 *2.3. HPLC purification*

148 Individual BPCAs were isolated using an Agilent 1290 Infinity UPLC instrument (Santa
149 Clara, USA). Separation was achieved with an Agilent Poroshell 120-SB C_{18} column (4.6 ×
150 100 mm, 2.7 μm pore size) using a gradient of diluted ortho H_3PO_4 buffered to pH 1.2 with
151 NaH_2PO_4 (mobile phase A) and pure MeCN (mobile phase B). Compounds were detected
152 with an Agilent 1290 Infinity diode array detector at 216 and 240 nm.

153 Extracted samples ('total digest') were diluted in ultra pure water to achieve a concentration
154 of B5CA and B6CA of ca. 200 ± 50 ng C/ μl for HPLC injection. A small (1 μl) initial
155 injection was made to quantify the peaks and assign retention times. Larger (5 μl) injections
156 (10-30 in total) were then made for fraction collection. The mobile phase was collected during
157 the time windows corresponding to the elution of the B5CA and B6CA compounds into pre-
158 combusted glass vials with a time-programmed analytical fraction collector (Agilent 1260
159 AS-FC). The collected fractions were transferred to screw cap vials with borosilicate pipettes
160 and dried under a stream of N_2 to remove all mobile phases with the exception of non-volatile
161 H_3PO_4. Before drying, a small aliquot was re-injected on HPLC to assess the recovery and
162 purity of the isolated compounds. Quantification was carried out with an external standard
163 series that contained a mixture of commercially available BPCAs (Wiedemeier et al., 2013).

164 *2.4. Wet oxidation*

165 The compound-specific isotopic signature ($\delta^{13}C$, $F^{14}C$) of isolated BPCAs was determined
166 with the methods described by Lang et al. (2012, 2013). Specifically, isolated and acidified
167 samples were transferred to 12 ml gas tight vials, diluted with Milli-Q water to a total volume
168 of 4 ml, and spiked with 0.75 ml supersaturated oxidizing solution (100 ml H_2O + 2.0 g
169 $K_2S_2O_8$ + 200 μl 85% H_3PO_4). Vials were sealed using a standard cap with a butyl rubber
170 septum and flushed with high purity He (grade 5.0, 99.999%) for 8 min at 125 ml/min to
171 remove atmospheric CO_2 from the headspace. The output gas stream passed through a water

trap to prevent backflow of atmospheric CO_2. Then, the vials were heated to 100 °C for 60 min to oxidize the BPCAs to CO_2. Samples were allowed to cool to room temperature overnight. Modern sucrose [Sigma Aldrich, P/N S7903, lot 090M02112V, $F^{14}C$ 1.053 ± 0.03 (ETH-47293)] and ^{14}C-free phthalic acid [Sigma Aldrich, P/N 80010, lot 1431342V, $F^{14}C \leq$ 0.0025 (ETH-42443)] were used to assess the addition of external carbonaceous material during oxidation and transfer. Both were oxidized and analyzed for $\delta^{13}C$ and $F^{14}C$.

2.5. Stable carbon isotope analysis ($\delta^{13}C$)

The stable carbon isotopic composition of the headspace CO_2 was measured with two approaches. For initial tests, we analyzed the C content of various blank samples to determine background values, while the isotopic composition was of secondary interest. These samples were analyzed on a GasBench II on-line gas preparation and introduction system (Thermo Fisher Scientific, Bremen, Germany) coupled to a ConFlo IV interface and a Delta V Plus mass spectrometer (both Thermo Fisher Scientific), allowing the accurate detection of C content and ^{13}C of samples as small as 5 μg C. As $\delta^{13}C$ analysis with the GasBench uses the majority of the CO_2, samples were also analyzed with a second method designed to preserve the majority of the CO_2 for ^{14}C analysis. In this approach, 100 μl of headspace gas was removed from the vials with a gastight syringe (Hamilton). The gas was injected into a gas chromatograph (Agilent 6890) with a split/splitless inlet and which was directly connected to a Delta V Plus via a ConFlo IV interface (both Thermo Fisher Scientific, Bremen, German). CO_2 was separated from interfering gases with a CP Poraplot Q column (27.5 m × 0.32 μm; 10 μm; Varian) maintained at 100 °C and a He flow rate of 2.0 ml/min.

The raw $\delta^{13}C$ values of each series of samples were corrected for fractionation effects between headspace and dissolved CO_2, as well for blank values and instrumental drift using standards of known composition (Lang et al., 2012). The amount of C was calculated by comparison with a dilution series of phthalic acid.

2.6. Radiocarbon analysis ($F^{14}C$)

Radiocarbon analysis was carried out at the Laboratory for Ion Beam Physics of ETH Zürich, Switzerland using the MICADAS (mini carbon dating system) equipped with a gas ion source (Ruff et al., 2007; Synal et al., 2007) that allows direct introduction of CO_2 from the headspace into the gas ion source. Detailed descriptions about the instrumentation can be found elsewhere (Lang et al., 2013; Wacker et al., 2013). In brief, sample CO_2 was removed from the vials by flushing with He and diverting the output over a magnesium perchlorate water trap to a trap containing X13 zeolite molecular sieve, which adsorbs CO_2 at room temperature. After trapping, a valve was toggled to connect the trap to a gas tight syringe, and

206 the CO_2 was released by heating the zeolite to 450 °C. The amount of CO_2 in the syringe was
207 detected pneumatically to allow dilution of the sample gas with He to 5 %, v/v CO_2 in He.
208 This gas mixture was then pushed continuously out of the syringe into the ion source. Oxalic
209 acid I (OX-1) gas was used as a modern standard (Stuiver and Polach, 1977) for
210 normalization and fossil CO_2 gas served as a blank.

211 The raw data output was processed with the BATS software (Wacker et al., 2010) so that the
212 results are reported as fraction modern ($F^{14}C$; Reimer et al., 2004) being corrected for
213 instrumental background, standard normalization and evaluated for uncertainty. Further
214 corrections for wet oxidation and purification of BPCAs are discussed below.

215 **3. Results and Discussion**

216 *3.1. Isolation of individual BPCAs with HPLC*

217 The first goal in method development was the definition of appropriate chromatographic
218 conditions for providing sufficient amounts of the pure target compounds. BPCA
219 concentrations in the total digests where determined following Wiedemeier et al. (2013). In
220 both samples B6CA and B5CA represented ca. 80 % of the total quantified BPCA-C. For
221 CSRA these two compounds provided the best conditions for successfully isolating and
222 dating them. As a second step we injected as much as 1 μg C from each of the two target
223 compounds and were still able to define robust retention times of the baseline separated
224 peaks. Even though the column was slightly overloaded, no tailing of the target peaks to other
225 fractions was observed (Fig. 1A) by re-injection of the eluent collected just before and after
226 collection of the sample peak. The time window for fraction collection was set as narrow as
227 possible to minimize the amount of potential co-eluting extraneous compounds and,
228 especially, column bleed. Next to the quantified BPCAs a suite of other peaks are present (Fig
229 1A). These were other by-products of the digestion and were most likely nitrated BPCAs
230 (Ziolkowski and Druffel, 2009b). We isolated B5CA and B6CA fractions from both process
231 standards, the fossil and modern char, in 3 replicates, respectively. As a preliminary
232 assessment of C_{ex}, aliquots of the collected fractions were re-injected; this did not show any
233 UV-detectable contaminants (Fig. 1B). Fractions were typically collected and combined from
234 20 to 30 repeated injections. Sample recovery varied between 50 and 84% (Table 1). Losses
235 might have occurred during fraction collection or during transfer and concentration of the
236 individual fractions. We did not detect significant amounts of the target compounds in
237 fractions collected subsequently after the time window for B6CA or B5CA, which suggests
238 that no significant tailing occurred after passing the detector and before the fraction collector.
239 As we considered it more important to avoid the collection of other peaks eluting shortly after

240 the target compounds, we did not try to optimize the recovery widening the collection
241 window, as soon as recovery exceeded 50%. Isotopic fractionation effects over the
242 chromatographic peak were not expected for the ^{14}C content (Zencak et al., 2007). This can be
243 explained by the fact that $F^{14}C$ values are corrected for isotopic fractionation, which is
244 expected to occur during AMS analysis. It is also possible that losses occurred during the
245 transfer and concentration of the collected sample volumes. Up to 15 ml of that aqueous
246 solution had to be reduced to a final volume of 2 ml, resulting in a high concentration of
247 H_3PO_4 as only water and MeCN were volatilized under N_2 flow.

248 *3.2. Wet oxidation and $\delta^{13}C$ values*

249 The wet oxidation method was originally designed to oxidize organic acids (Lang et al. 2012;
250 2013) but proved to be also suitable for BPCAs. The oxidation efficiency was tested by
251 oxidizing a known amount of a benzene pentacarboxylic acid standard, with recovery always
252 > 90%. Furthermore, no isotopic fractionation was observed when the $\delta^{13}C$ values of the
253 oxidized standard material were compared with the reference values obtained with total
254 combustion of the bulk sample powder using elemental analysis (EA)-IRMS. Attempts to
255 further optimize the oxidation parameters by varying temperature and reaction time did not
256 result in improvement of recovery. Wet oxidation of a mellitic acid standard gave consistent
257 results compared with the B5CA standard.

258 The final $\delta^{13}C$ values of each isolated B5CA or B6CA sample, as well of the entire digestion
259 extracts are listed in Table 1. The values are corrected for fractionation effects between the
260 liquid and gas phase and for process and instrumental background of the wet oxidation
261 procedure itself, as described by Lang et al. (2012). In brief, the process blank was
262 determined using oxidized ultra pure water with similar volumes to the samples. The peak
263 area of these blanks averaged 0.55 ± 0.09 V·s (n = 3), which corresponds to a value near the
264 limit of detection of ≤ 0.2 μg C. As these peaks were too small for reliable $\delta^{13}C$ values, the
265 isotopic composition of the process blank was estimated indirectly by comparing the values
266 for the oxidized phthalic acid standard samples with the known reference value. The $\delta^{13}C$ of
267 the blanks is very sensitive, so it was individually calculated for each prepared series. The
268 blanks were calculated to be -14.1 ± 1.1‰ for the first and -9.1 ± 1.1‰ for the second series.
269 At first sight, there is a significant difference between the two blanks. It should be taken into
270 account, however, that small differences detected with such small signals could lead to large
271 differences in the $\delta^{13}C$ values for the blanks. The isotopic shift of ca. 5‰ for the blank results
272 probably resulted from slightly changes in the quality of the chemicals in use, for instance the
273 oxidizing reagent or the ultra pure water. This assumption is supported by the fact that the
274 $\delta^{13}C$ values for the fire-derived compounds from the fossil and modern char showed no

275 significant difference after being corrected for the blank value for the wet oxidation procedure
276 (Table 1). The BPCAs had slightly lower $\delta^{13}C$ values than the total digests (Fig. 2), while
277 B5CA was more negative than B6CA. These small differences may be the result of
278 inhomogeneity of the parent material. Another explanation might be an incomplete collection
279 of the chromatographic peak, as $\delta^{13}C$ values are much more sensitive to fractionation effects
280 than $F^{14}C$ values (Zencak et al., 2007). The overall reproducibility (1σ) was $\leq 0.7‰$. Even if
281 this is slightly higher than the reported precision for the chemical oxidation method (≤ 0.4 ‰,
282 Lang et al., 2012), the values are still satisfyingly accurate. With this technique, only a very
283 small part of the isolated samples was used for stable isotope analysis in order to assure large
284 enough samples for the AMS analysis. More accurate results could potentially be achieved by
285 isolating a separate sample dedicated only to $\delta^{13}C$ using a GasBench device.

286 For the modern charcoal, we compared the isotopic composition of total digests and
287 compound-specific $\delta^{13}C$ values with data from Yarnes et al. (2011), who successfully
288 performed continuous flow ^{13}C analysis after separation of BPCAs using a laborious 2 h ion
289 exchange chromatography method. The values of Yarnes et al. (2011) are shown in Fig. 2 for
290 direct comparison with our results. Their results for B5CA and B6CA from the modern char
291 were within the error of our results. Furthermore, we obtained values for the BPCA extract of
292 the modern char comparable to their value obtained using EA-IRMS analysis with the bulk
293 sample. Compared with our distinct results the reported values by Yarnes et al. (2011) were
294 systematically shifted by a value as small as -0.5‰, which is still in the range of the precision
295 of both studies. This comparison demonstrates that our method for analyzing the $\delta^{13}C$ values
296 of BPCAs is of high quality and can be performed on a very small aliquot of a sample whose
297 main part is needed for CSRA.

298 *3.3. Assessment of extraneous carbon*

299 As mentioned above, radiocarbon analysis is more sensitive to the addition of extraneous C
300 than $\delta^{13}C$ analysis (e.g. Shah and Pearson, 2007; Ziolkowski and Druffel, 2009a). It is
301 therefore mandatory to minimize and precisely determine the contribution from C_{ex} to carry
302 out an appropriate blank correction and obtain reliable radiocarbon values. Generally
303 speaking, a measured $F^{14}C$ value is composed of a contribution from both the compound of
304 interest and from C_{ex}. This can be expressed with the following mass balance equation:

305 $$F_T \cdot C_T = F_S \cdot C_S + F_{ex} \cdot C_{ex} \quad (1)$$

306 Where F is the $F^{14}C$ value and C the amount of carbon in μg. The subscript T refers to total as
307 measured, S to sample and ex to extraneous. In order to solve the equation for F_S, the amount
308 and radiocarbon content of C_{ex} need to be determined beforehand, given that $C_S = C_T - C_{ex}$.

There are several possible sources of contaminating C added to the sample. Considering the entire laboratory protocol, it might be taken up during the extraction and cleaning procedure ($C_{chemistry}$), the HPLC isolation (C_{HPLC}), the wet oxidation procedure (C_{ox}) and finally during the AMS analysis itself (C_{AMS}). Accordingly C_{ex} can be expressed as a sum of the following components: $C_{ex} = C_{chemistry} + C_{HPLC} + C_{ox} + C_{AMS}$. The most straightforward way to trace back to C_{ex} is to start at the end of the laboratory protocol, going back to the first steps.

3.3.1. C_{AMS}

The AMS instrumental background is routinely determined during each measurement campaign. As mentioned above, reported values are normalized using the results of small scale Ox-I standards and are corrected for small scale AMS blanks using the BATS software (Wacker et al., 2010). Because of this, C_{AMS} is not discussed further here. Accordingly, the subscript T ($F^{14}C_T$, C_T) indicates in the following that the raw values had already been corrected with the BATS software.

3.3.2. C_{ox}

The contribution of C_{ex} added during wet oxidation was calculated indirectly using two standards of known radiocarbon content (^{14}C-free phthalic acid and modern sucrose). With this method it is possible to assess separately two contaminant fractions. A detailed description of the method is given by Lang et al. (2013). As we adopted the approach for the assessment of $C_{chemistry}$ and C_{HPLC}, further information is given in the following section.

For the samples measured during the first campaign, we determined a small influence from a modern C_{ox} source, corresponding to 0.13 ± 0.04 μg C (n = 5), whereas the contribution from radiocarbon-dead C_{ox} corresponded to 0.85 ± 0.44 μg C (n = 5). In combination, this resulted in a total blank of 0.97 ± 0.44 μg C with a $F^{14}C$ value of 0.13 ± 0.07. For the second measurement campaign, we were able to reduce the amount of C_{ox} via 0.07 ± 0.03 μg C modern C_{ox} and 0.45 ± 0.43 μg C of radiocarbon-dead material, i.e. a total of 0.52 ± 0.44 μg C_{ox} with 0.14 ± 0.13 $F^{14}C$. Evaluation of the first data set pointed to some sources of C_{ox} that could easily be reduced, especially for the reagent used for the wet oxidation. The reagent was recrystallized 2x in water before use during the second campaign. This resulted in a reduction of radiocarbon-dead C_{ox} of ca. 0.5 μg C per sample, while the average $F^{14}C$ of C_{ox} remained comparable with the F_{ox} determined for the first campaign. The correction for C_{ox} was applied to all samples before determining $C_{chemistry}$ and C_{HPLC}. The corrected values are indicated as $C_{T'}$ and $F^{14}C_{T'}$ in the following.

3.3.3. $C_{chemistry}$ and C_{HPLC}

Initially, we directly collected and analyzed $C_{chemistry}$ and C_{HPLC}. Blank samples were run through the entire laboratory protocol except for radiocarbon dating. Another set of blanks was produced, performing only the HPLC step, omitting the extraction procedure. Because the total amount of extraneous C was very low, the number of injections and the duration of the fraction collection window were increased vs. regular sample fraction collection. The amount of C and its $\delta^{13}C$ values were determined by analyzing all material with the GasBench device (Table 2), which gives better accuracy than the GC option described above. For one sample, it was not possible to obtain a reliable $\delta^{13}C$ value, as the sample size was too small. The average blank that passed both extraction and HPLC contained 0.23 ± 0.12 µg $C_{chemistry+HPLC}$ ml^{-1} (n = 2), whereas C_{HPLC} showed an average of 0.22 ± 0.04 µg ml^{-1} (n = 5). This showed that the chemical extraction process ($C_{chemistry}$) did not significantly contribute to the amount of the sum of C_{ex} and could be assumed to be zero. Most likely, if any $C_{chemistry}$ were present, it would again be removed during the HPLC purification step to a level below the detection limit. The isotopic signatures of the C_{HPLC} replicates showed comparable values, within a relatively larger error due to the small sample sizes. The $\delta^{13}C$ value of C_{HPLC} averaged -29.5 ± 1.3‰ (Table 2), implying that the source of C_{ex} remained constant and that no unexpected and uncontrolled addition of C_{ex} occurred. Separate analysis of the aqueous eluent before usage showed that this was most probably the main source of C_{HPLC}, as it already contained 0.3 ± 0.1 µg C/ml. Future efforts to reduce C_{HPLC} should therefore focus on the mobile phase in the HPLC step.

A strong relationship (R^2 0.91) existed between the collected volumes of C_{HPLC} and their C content (Fig. 3). The intercept of near zero suggests that any constant background was absent, while the slope (0.22 µg C ml^{-1}) of the linear regression represented the amount of C_{HPLC} eluting per ml eluent. Therefore, multiplying this value by the volume collected for a specific sample should give a preliminary estimate of C_{HPLC} for an individual sample, enabling calculation of F_{HPLC} for each sample. However, we discovered that this estimate was not accurate enough: calculated values of C_{HPLC} for individual samples ranged between 0.9 and 2.1 µg C, resulting in reasonable estimates for F_{HPLC} for some samples (Supplementary material), but also in non-natural values of $F_{HPLC} > 2$ for others. Interestingly, the averaged values for F_{HPLC} for each process standard were at the same level of a blank with a modern radiocarbon value ($F^{14}C \approx 1$).

It is also possible to determine the blank contribution (C_{HPLC}) and its radiocarbon signature (F_{HPLC}) indirectly. For this approach, C_{ex} needs to be assumed as a constant amount of carbon being added to each sample during sample preparation. It is based on the theoretical assumption that the F_{ex} would be composed of two pools characterized by opposite ^{14}C content (i.e. modern and ^{14}C-free). The combination of the results from two standards with

that with opposite ^{14}C content then allows a mathematical solution for both unknowns, here C_{HPLC} and F_{HPLC}. It is a common approach to determine a theoretical contribution from modern C_{ex} ($F^{14}C = 1$) by use of a radiocarbon-dead ($F^{14}C = 0$) process standard and vice versa (Shah and Pearson, 2007; Ziolkowski and Druffel, 2009a; Lang et al., 2013). For this, Eq. 1 was re-arranged and modified:

$$C_{HPLC} = (F_{T'} \cdot C_{T'} - F_S \cdot C_S) / (F_{HPLC} - F_S) \quad (2)$$

Accordingly, a C_{HPLC} value for each replicate of fossil char was calculated assuming $F_{HPLC} = 1$ and similarly for the modern char, assuming $F_{HPLC} = 0$ (Table 3). To solve the equation the reference values from the bulk powder were used as F_S. The average modern C_{HPLC} based on the individual B5CAs and B6CAs from the fossil charcoal, was 1.4 ± 0.5 μg C (n = 5; Table 3). In contrast, the BPCAs isolated from the modern char sample were hardly affected (Fig. 3), resulting in an average contribution of ^{14}C-free C_{HPLC} of 0.2 ± 0.4 μg C.

While the subdivision of the amount of blank C into two pools is a convenient mathematical concept, in nature it is more likely that there is a single C pool with a distinct ^{14}C signature displaying a mean value of all various compounds. In order to determine more realistic values, C_{ex} and F_{ex} can be combined by addition of the two theoretical C_{ex} values and by calculating the weighted average of the two F_{ex} values:

$$F_{ex} = (F_{modern} \cdot C_{modern} + F_{dead} \cdot C_{dead}) / (C_{modern} + C_{dead}) \quad (3)$$

While the subscript *ex* can be substituted with the subscript describing the respective part of the sample preparation. Here, it resulted in C_{HPLC} = 1.6 ± 0.7 μg C with an average radiocarbon content F_{HPLC} of 0.90 ± 0.50 $F^{14}C$. This amount was satisfyingly low for samples with > 15 μg C. Consequently, a fossil source for C_{HPLC} can be excluded; nevertheless, it is hardly possible to directly identify the origin of C_{HPLC}. A significant change of F_{HPLC} during later measurements is though a clear indication for additional contribution of C_{HPLC}, in general.

Fig. 3 illustrates the size-dependent relationship of the radiocarbon content of the samples not corrected for C_{HPLC} vs. the theoretically modeled F_{HPLC} deduced from the mixture of a C_S with varying sample size and the constant C_{HPLC}. Note that the given data set does not show a significant dependence between the amounts of repeated injections (i.e. the amount of collected mobile phase) and C_{HPLC}. However, the opposite was concluded after direct analysis of C_{HPLC} (Fig. 3). This apparent contradiction can be attributed to the fact that the process samples were only collected over 20 to 30 injections, with a total of 4-12 ml (but mostly 6-8 ml), as opposed to the volume range of 8 up to 40 ml collected for the C_{HPLC} tests. In short, this volume range was too small for detection of a significant size dependence of C_{HPLC}. The

412 correct radiocarbon content (F_S) values from the individual samples are listed in Table 3.
413 There was no evidence for a significant difference in the $F^{14}C$ values between the B5CAs and
414 B6CAs. Hence, replicate analyses of B5CAs and B6CAs from the same process standard
415 were taken as equal. The 5 replicates of the modern charcoal sample give a mean $F^{14}C$ value
416 of 1.142, with a standard deviation of 0.025, i.e. a precision of 2.2%. This value mirrors both
417 the $F^{14}C$ of the digest (ETH-49860.1.1) and the reference analysis of the bulk material (ETH-
418 50458). The individual samples exhibited values with a slightly greater uncertainty that in
419 turn depended on the sample size and lower counting statistics. The largest sample containing
420 27 μg C had a precision of 2.9% and the smallest (16 μg C) a precision of 4.5%. Likewise, the
421 F_S for the largest sample (ETH-49868.1.1) was also the one closest to the reference value. The
422 same was true for the fossil charcoal standard being depleted in ^{14}C, even if no size dependent
423 increase in precision is given for these duplicate values. The average F_S from 5 individual
424 measurements was 0.001 ± 0.032. Samples with < 15 μg C were not analyzed, as
425 demonstrated by Birkholz et al. (2013) that samples designated for CSRA and < 10 μg C are
426 usually affected by C_{ex} to such an extent that no reliable results can be obtained. However, the
427 data here demonstrate that sample amounts of 25 to 35 μg C are suitable for high precision
428 analysis. Additional replicate analyses are still recommended so that possible outliers can be
429 easily identified. In summary, the given uncertainty in an individual measurement is
430 satisfactory for C turnover studies or C source apportion. For precise dating purposes a higher
431 precision is usually required. Indeed, there is room for further minimization of C_{ex}, especially
432 during the HPLC isolation procedure. Comparing the background level of organic C in the
433 ultra-pure water used (i.e. in the aqueous HPLC solvent) with published references indicates
434 that the system used for this study could be improved with better maintenance. Lang et al.
435 (2012) reported that not more than 0.04 μg C/ml were detected in the aquatic mobile phase. In
436 contrast, a concentration of up to 0.3 μg C/ml was measured for the eluents used here.

437 **4. Conclusions**

438 We present a method to purify individual BPCAs as compound-specific biomarkers for BC,
439 followed by the determination of $\delta^{13}C$ and $F^{14}C$. The combination of two measurements on
440 the same sample reduces the efforts specified by an isolation protocol for the particular
441 analysis. Furthermore, knowing both the $\delta^{13}C$ and $F^{14}C$ values for a sample helps interpret the
442 results with respect to the impact of contamination that might be difficult to detect, especially
443 when they have a different $\delta^{13}C$ value from the sample of interest. Another benefit is the
444 possibility of monitoring the development of the general C_{ex} background in an easy and cost
445 effective way. C content and its $\delta^{13}C$ value should give enough information and help avoid

446 expensive radiocarbon analysis of contaminated samples. Finally the wet oxidation method
447 avoids the problems encountered in the combustion of H_3PO_4-rich sample residues.

448 A constant addition of extraneous C to the isolated samples was identified. Nevertheless, a
449 size dependent component C_{ex} cannot be excluded. This is especially true for much larger
450 samples (> 50 μg C) that need to be isolated with a greater extent of injections (e.g. 100-150
451 injections to yield ca. 100 μg BPCA C). Contamination of 1.6 ± 0.2 μg C, with $F^{14}C$ 0.90 ±
452 0.14, was calculated. We have shown that the precision of individual measurements of
453 samples with > 15 μg C is adequate for studies aimed at determining C turnover or source
454 apportioning in soils and sediments. In addition, our data show that there is potential for also
455 applying the method for dating purposes. Samples isolated in replicates each containing > 25
456 μg C should give values precise enough for an age determination of, for example, combustion
457 residues on pottery or other samples with very fine charcoal that cannot be analyzed directly
458 or BC that is mixed with other OM. Based on the experience from study, we recommend that
459 process standards and blanks are determined regularly, as it is possible that C concentration in
460 chemicals and/or solvent changes through time.

461 The method can also be applied to other marker compounds (e.g. B4CA or B3CA), although
462 it might require minor tuning of the HPLC method to obtain a clean chromatographic
463 separation of the target compounds. Additional improvements could include the use of a
464 HPLC column with more capacity to reduce the number of injections required for isolation of
465 sufficient material. This might lead to less C_{ex}, although the flow rate would need to be
466 increased.

467 **5. Author contributions**

468 The study was proposed by M.G., M.P.W.S., M.W.I.S.,R.H.S. and S.M.B.; M.G. and
469 M.P.W.S. carried out the experiments and data analysis. D.B.W., S.Q.L. and R.H.S. provided
470 technical expertise for method development. I.H. carried out AMS analysis with solid
471 graphite targets.

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481 **References**

482 Alloway, B.V., Pribadi, A., Westgate, J.A., Bird, M., Fifield, L.K., Hogg, A., Smith, I., 2004.
483 Correspondence between glass-FT and ^{14}C ages of silicic pyroclastic flow deposits sourced
484 from Maninjau caldera, west-central Sumatra. Earth and Planetary Science Letters 227, 121–
485 133.

486 Ascough, P.L., Bird, M.I., Brock, F., Higham, T.F.G., Meredith, W., Snape, C.E., Vane, C.H.,
487 2009. Hydropyrolysis as a new tool for radiocarbon pre-treatment and the quantification of
488 black carbon. Quaternary Geochronology 4, 140–147.

489 Bird, M.I., Ayliffe, L.K., Fifield, L.K., Turney, C.S.M., Cresswell, R.G., Barows, T.T.,
490 David, B., 1999. Radiocarbon dating of "old" charcoal using a wet oxidation, stepped-
491 combustion procedure. Radiocarbon 41, 127–140.

492 Birkholz, A., Smittenberg, R.H., Hajdas, I., Wacker, L., Bernasconi, S.M., 2013. Isolation and
493 compound specific radiocarbon dating of terrigenous branched glycerol dialkyl glycerol
494 tetraethers (brGDGTs). Organic Geochemistry 60, 9-19.

495 Carcaillet, C., Almquist, H., Asnong, H., Bradshaw, R.H.W., Carrión, J.S., Gaillard, M.-J.,
496 Gajewski, K., Haas, J.N., Haberle, S.G., Hadorn, P., Müller, S.D., Richard, P.J.H., Richoz, I.,
497 Rösch, M., Sánchez Goñi, M.F.S., Von Stedingk, H., Stevenson, A.C., Talon, B., Tardy, C.,
498 Tinner, W., Tryterud, E., Wick, L., Willis, K.J., 2002. Holocene biomass burning and global
499 dynamics of the carbon cycle. Chemosphere 49, 845–863.

500 Dittmar, T., 2008. The molecular level determination of black carbon in marine dissolved
501 organic matter. Organic Geochemistry 39, 396–407.

502 Eglinton, T.I., Aluwihare, L.I., Bauer, J.E., Druffel, E.R.M., McNichol, A.P., 1996. Gas
503 chromatographic isolation of individual compounds from complex matrices for radiocarbon
504 dating. Analytical Chemistry 68, 904–912.

505 Glaser, B., Balashov, E., Haumaier, L., Guggenberger, G., Zech, W., 2000. Black carbon in
506 density fractions of anthropogenic soils of the Brazilian Amazon region. Organic
507 Geochemistry 31, 669–678.

508 Glaser, B., Haumaier, L., Guggenberger, G., Zech, W., 1998. Black carbon in soils: the use of
509 benzenecarboxylic acids as specifc markers. Organic Geochemistry 29, 811–819.

510 Goldberg, E.D., 1985. Black Carbon in the Environment. Properties and Distribution. Wiley,
511 Chichester, UK.

512 Hajdas, I., Bonani, G., Thut, H., Leone, G., Pfenninger, R., Maden, C., 2004. A report on
513 sample preparation at the ETH/PSI AMS facility in Zurich. Nuclear Instruments & Methods
514 in Physics Research Section B-Beam Interactions with Materials and Atoms 223-224, 267–
515 271.

516 Hammes, K., Smernik, R.J., Skjemstad, J.O., Herzog, A., Vogt, U.F., Schmidt, M.W.I., 2006.
517 Synthesis and characterisation of laboratory-charred grass straw (*Oryza sativa*) and chestnut
518 wood (*Castanea sativa*) as reference materials for black carbon quantification. Organic
519 Geochemistry 37, 1629–1633.

520 Hedges, J.I., Eglinton, G., Hatcher, P.G., Kirchman, D.L., Arnosti, C., Derenne, S., Evershed,
521 R.P., Kögel-Knabner, I., de Leeuw, J.W., Littke, R., Michaelis, W., Rullkötter, J., 2000. The
522 molecularly-uncharacterized component of nonliving organic matter in natural environments.
523 Organic Geochemistry 31, 945–958.

524 Knicker, H., Hilscher, A., González-Vila, F.J., Almendros, G., 2008. A new conceptual model
525 for the structural properties of char produced during vegetation fires. Organic Geochemistry
526 39, 935–939.

527 Lang, S.Q., Bernasconi, S.M., Früh-Green, G.L., 2012. Stable isotope analysis of organic
528 carbon in small (μg C) samples and dissolved organic matter using a GasBench preparation
529 device. Rapid Communications in Mass Spectrometry 26, 9–16.

530 Lang, S.Q., Früh-Green, G.L., Bernasconi, S.M., Wacker, L., 2013. Isotopic ($\delta^{13}C$, $\Delta^{14}C$)
531 analysis of organic acids in marine samples using wet chemical oxidation. Limnology and
532 Oceanography: Methods 11, 161–175.

533 Masiello, C.A., 2004. New directions in black carbon organic geochemistry. Marine
534 Chemistry 92, 201–213.

535 Pearson, A., McNichol, A.P., Schneider, R.J., von Reden, K.F., 1998. Microscale AMS ^{14}C
536 measurement at NOSAMS. Radiocarbon 40, 61–75.

537 Preston, C.M., Schmidt, M.W.I., 2006. Black (pyrogenic) carbon: a synthesis of current
538 knowledge and uncertainties with special consideration of boreal regions. Biogeosciences 3,
539 397–420.

540 Reimer, P.J., Brown, T.A., Reimer, R.W., 2004. Discussion: Reporting and calibration of
541 post-bomb ^{14}C data. Radiocarbon 46, 1299–1304.

Ruff, M., Wacker, L., Gäggeler, H.W., Suter, M., Synal, H.A., Szidat, S., 2007. A gas ion source for radiocarbon measurements at 200 kV. Radiocarbon 49, 307–314.

Schmidt, M.W.I., Noack, A.G., 2000. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. Global Biogeochemical Cycles 14, 777–793.

Schneider, M.P.W., Smittenberg, R.H., Dittmar, T., Schmidt, M.W.I., 2011. Comparison of gas with liquid chromatography for the determination of benzenepolycarboxylic acids as molecular tracers of black carbon. Organic Geochemistry 42, 275–282.

Shah, S.R., Pearson, A., 2007. Ultra-microscale (5–25 μg C) analysis of individual lipids by ^{14}C AMS: Assessment and correction for sample processing blanks. Radiocarbon 49, 69–82.

Skjemstad, J.O., Clarke, P., Taylor, J.A., Oades, J.M., McClure, S.G., 1996. The chemistry and nature of protected carbon in soil. Australian Journal of Soil Research 34, 251–271.

Skjemstad, J.O., Dalal, R.C., Janik, L.J., McGowan, J.A., 2001. Changes in chemical nature of soil organic carbon in Vertisols under wheat in south-eastern Queensland. Australian Journal of Soil Research 39, 343–359.

Skjemstad, J.O., Spouncer, L.R., Cowie, B., Swift, R.S., 2004. Calibration of the Rothamsted organic carbon turnover model (RothC ver. 26.3), using measurable soil organic carbon pools. Australian Journal of Soil Research 42, 79–88.

Stuiver, M., Polach, H.A., 1977. Discussion: Reporting of ^{14}C data. Radiocarbon 19, 355–363.

Synal, H.A., Stocker, M., Suter, M., 2007. MICADAS: A new compact radiocarbon AMS system. Nuclear Instruments & Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 259, 7–13.

Tinner, W., Conedera, M., Ammann, B., Lotter, A.F., 2005. Fire ecology north and south of the Alps since the last ice age. The Holocene 15, 1214–1226.

Wacker, L., Christl, M., Synal, H.A., 2010. Bats: A new tool for AMS data reduction. Nuclear Instruments Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 268, 976–979.

Wacker, L., Fahrni, S.M., Hajdas, I., Molnar, M., Synal, H.-A., Szidat, S., Zhang, Y.L., 2013. A versatile gas interface for routine radiocarbon analysis with a gas ion source. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 294, 315–319.

Wacker, L., Lippold, J., Molnár, M., Schulz, H., 2013. Towards radiocarbon dating of single foraminifera with a gas ion source. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 294, 307–310.

Wiedemeier, D.B., Hilf, M.D., Smittenberg, R.H., Haberle, S.G., Schmidt, M.W.I., 2013. Improved assessment of pyrogenic carbon quantity and quality in environmental samples by high-performance liquid chromatography. Journal of Chromatography A 1304, 246–250.

Yarnes, C., Santos, F., Singh, N., Abiven, S., Schmidt, M.W.I., Bird, J.A., 2011. Stable isotopic analysis of pyrogenic organic matter in soils by liquid chromatography-isotope-ratio mass spectrometry of benzene polycarboxylic acids. Rapid Communications in Mass Spectrometry 25, 3723–3731.

Zencak, Z., Reddy, C.M., Teuten, E.L., Xu, L., McNichol, A.P., Gustafsson, Ö., 2007. Evaluation of gas chromatographic isotope fractionation and process contamination by carbon in compound-specific radiocarbon analysis. Analytical Chemistry 79, 2042–2049.

Zimmerman, A.R., 2010. Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). Environmental Science & Technology 44, 1295–1301.

Ziolkowski, L.A., Druffel, E.R.M., 2009a. Quantification of extraneous carbon during compound specific radiocarbon analysis of black carbon. Analytical Chemistry 81, 10156–10161.

Ziolkowski, L.A., Druffel, E.R.M., 2009b. The feasibility of isolation and detection of fullerenes and carbon nanotubes using the benzene polycarboxylic acid method. Marine Pollution Bulletin 59, 213–218.

595 **Figure captions**

596 **Fig. 1.** HPLC-DAD chromatogram from a 5 µl injection of a BPCA extract of modern char
597 for fraction collection (A), and HPLC-DAD chromatograms of aliquots of purified B6CA and
598 B5CA fractions detected at 216 and 240nm (B). Time windows for single peak collection of
599 B6CA (B6) and B5CA (B5) are highlighted in the gray boxes.

600 **Fig. 2.** $\delta^{13}C$ values of individual B5CA and B6CA and of the whole BPCA digest of fossil
601 (left) and modern char (right) measured in this study, and as published by Yarnes et al. (2011)
602 for the modern char.

603 **Fig. 3.** Mass of HPLC blank vs. volume of collected eluent.

604 **Fig. 4.** Radiocarbon values for B5CA and B6CA isolated from the modern (left) and fossil
605 char (right). The given error is composed of corrections for instrumental AMS background
606 and the blank for wet oxidation. The solid gray line represents an idealized line for the
607 mixture of the real $F^{14}C$ value of sample and the determined mean external contamination.

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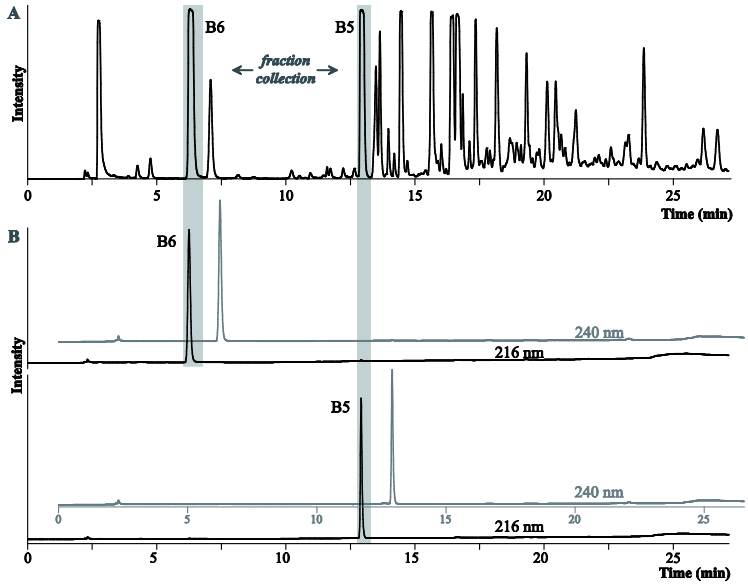

609

610 **Figure 1**

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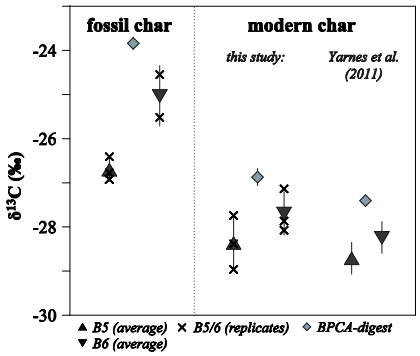

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613 **Figure 2**

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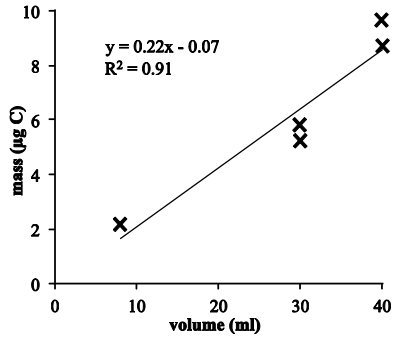

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616 **Figure 3**

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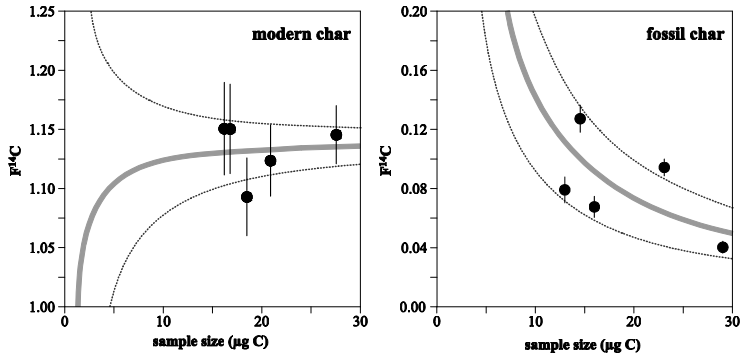
modern char
fossil char
F14C
sample size (µg C)
1.25
1.20
1.15
1.10
1.05
1.00
0.20
0.16
0.12
0.08
0.04
0.00
0
10
20
30

.

Figure 4

Tables

Table 1

Isolation of B5CA and B6CA from two process standards with HPLC, recovered mass as analyzed with HPLC and GC-IRMS and corresponding $\delta^{13}C$ values.

Sample		HPLC			GC-IRMS		Yarnes et al., 2011
	Compound	Injection	µg C[a]	Recovery	µg C[b]	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)[c]
Fossil char	Digest				43.4	-23.8 ± 0.1	
	B6CA	20×5µl	22.3	76%	29.3	-25.5 ± 0.3	
	B6CA	30×5µl	35.2	80%	39.7	-24.5 ± 0.1	
	Avg. B6CA					-25.0 ± 0.7	
	B5CA	20×5µl	16.3	55%	17.2	-26.9 ± 0.3	
	B5CA	25×5µl	20.4	55%	21.7	-26.4 ± 0.3	
	B5CA	30×5µl	21.8	49%	22.9	-26.8 ± 0.2	
	Avg. B5CA					-26.7 ± 0.4	
Modern char	Digest				17.7	-26.9 ± 0.2	-27.4[d]
	B6CA	20×5µl	19.9	74%	23.3	-27.9 ± 0.3	
	B6CA	25×5µl	28.6	84%	32.5	-28.1 ± 0.1	
	B6CA	25×5µl	25.9	76%	31.1	-27.1 ± 0.3	
	Avg. B6CA					-27.7 ± 0.5	-28.24 ± 0.36
	B5CA	20×5µl	19.7	50%	25.8	-28.4 ± 0.3	
	B5CA	25×5µl	30.6	62%	29.5	-27.7 ± 0.1	
	B5CA	25×5µl	25.3	52%	28.1	-29.0 ± 0.3	
	Avg. B5CA					-28.4 ± 0.6	-28.71 ± 0.36

[a] Determined by comparing sample peak areas with those from a dilution series of BPCA standards with known concentration;
[b] determined on amount of CO_2 generated during oxidation in the headspace of the vials, by comparing peak areas with those of a series of standards of known concentration;
[c] determined with ion chromatography IRMS;
[d] bulk sample analyzed with EA-IRMS.

Table 2

Direct assessment of C_{HPLC} (n.a.: not analyzed).

	HPLC	GC-IRMS		
Sample	Collected ml	µg C[a]	µg /ml	$\delta^{13}C$ (‰)
$C_{chem.+HPLC}$[b]	20		0.23 ± 0.12	n.a.
C_{HPLC}	40	9.7	0.24	-28.6 ± 0.1
C_{HPLC}	40	8.7	0.22	-28.5 ± 0.1
C_{HPLC}	8	2.2	0.28	n.a.
C_{HPLC}	30	5.2	0.17	-29.8 ± 0.1
C_{HPLC}	30	5.8	0.19	-31.4 ± 0.1
Avg. C_{HPLC}			0.22 ± 0.04	-29.5 ± 1.3

[a] Determined on amount of CO_2 generated during oxidation in the headspace of the vials, by comparing peak areas with those of a series of standards of known concentration;
[b] n = 2.

638 **Table 3**

639 Amount and radiocarbon content of isolated process standards B5CA and B6CA, calculated amount of external C
640 (C_{ex}) added to the related sample and residual $F^{14}C$ values after correction for the blank (F_S).

Sample		$F_{T'}$ ($F^{14}C$) [a]	$C_{T'}$ (µg C)	*Calculated* C_{ex} (µg C)	*Corrected values* F_S ($F^{14}C$)[b]	Lab code
Fossil char	Bulk[c]				0.003 ± 0.001	ETH-50456
	Digest				0.010 ± 0.002	ETH-49849
	B6CA	0.094 ± 0.004	23.1 ± 0.5	2.1 ± 0.1	0.036 ± 0.025	ETH-50461
	B6CA	0.040 ± 0.003	29.0 ± 0.5	1.1 ± 0.1	-0.009 ± 0.019	ETH-49854
	B5CA	0.079 ± 0.006	13.0 ± 0.5	1.0 ± 0.1	-0.033 ± 0.045	ETH-50459
	B5CA	0.068 ± 0.005	16.0 ± 0.5	1.0 ± 0.1	-0.022 ± 0.036	ETH-50460
	B5CA	0.127 ± 0.007	14.5 ± 0.5	1.8 ± 0.1	0.034 ± 0.040	ETH-49859
Modern extraneous C ($F^{14}C$ = 1) addition:				1.4 ± 0.5	Avg. C_{ex}	
Modern char	Bulk[c]				1.142 ± 0.004	ETH-50458
	Digest				1.143 ± 0.020	ETH-49860
	B6CA	1.151 ± 0.025	16.2 ± 0.5	-0.1 ± 0.5	1.162 ± 0.074	ETH-50462
	B6CA	1.093 ± 0.021	18.5 ± 0.5	0.8 ± 0.5	1.102 ± 0.056	ETH-49864
	B6CA	1.124 ± 0.020	20.9 ± 0.5	0.3 ± 0.5	1.132 ± 0.056	ETH-50463
	B5CA	1.145 ± 0.019	27.6 ± 0.5	-0.1 ± 0.6	1.152 ± 0.034	ETH-49868
	B5CA	1.150 ± 0.024	16.8 ± 0.5	-0.1 ± 0.5	1.161 ± 0.071	ETH-50465
Radiocarbon dead extraneous C ($F^{14}C$ = 1) addition:				0.2 ± 0.4	Avg. C_{ex}	

641 [a] Subscript *T'* indicates that values were corrected for instrumental background using the BATS program
642 (Wacker, 2010) and for the wet oxidation procedure (Lang et al., 2013);
643 [b] subscript *S* indicated that values were corrected for mean modern or radiocarbon dead extraneous carbon
644 addition occurring during the entire laboratory protocol. Please note that the fractions of 'bulk' and 'extract'
645 required less corrections than those isolated by HPLC.
646 [c] ^{14}C values from bulk sample material were corrected for instrumental background using BATS software.
647

Supplementary

A. Correction for wet oxidation procedure

A. Tables

Table A 1: Amounts and radiocarbon contents of the isolated process standards of the wet oxidation – phthalic acid and sucrose with the corresponding calculated amount of external C (C_{ex}) added to the standard, and the residual $F^{14}C$ values after correction for the blank (F_S).

Standard	*BATS output*		*Calculated*	*Corrected values*	**Lab code**
	F_T ($F^{14}C$) [a]	C_T (µg C)	C_{ex} (µg C)	F_S ($F^{14}C$)	
1st campaign					
Phthalic acid [b]	0.0140 ± 0.002	12.4 ± 0.5	0.14 ± 0.03	0.004 ± 0.004	ETH-49846.11.1
	0.0165 ± 0.003	13.4 ± 0.5	0.19 ± 0.04	0.007 ± 0.004	ETH-49846.12.1
	0.0078 ± 0.002	15.3 ± 0.5	0.08 ± 0.03	-0.001 ± 0.003	ETH-49846.17.1
	0.0083 ± 0.002	17.8 ± 0.5	0.10 ± 0.04	0.001 ± 0.003	ETH-49846.7.1
	0.0084 ± 0.002	21.0 ± 0.5	0.12 ± 0.03	0.002 ± 0.003	ETH-49846.13.1
Average addition of C_{ex} with $F^{14}C$ = 1:			**0.13 ± 0.04**		
Sucrose [c]	1.018 ± 0.012	17.4 ± 0.5	0.58 ± 0.88	1.070 ± 0.050	ETH-49845.2.1
	1.004 ± 0.011	21.5 ± 0.5	1.01 ± 0.95	1.045 ± 0.040	ETH-49845.1.1
	1.012 ± 0.012	21.7 ± 0.5	0.85 ± 0.96	1.053 ± 0.040	ETH-49845.3.1
	1.004 ± 0.012	23.4 ± 0.5	1.09 ± 1.00	1.042 ± 0.037	ETH-49845.4.1
	1.026 ± 0.012	27.3 ± 0.5	0.70 ± 1.09	1.059 ± 0.033	ETH-49845.12.1
Average addition of C_{ex} with $F^{14}C$ = 0:			**0.85 ± 0.44**		
2nd campaign					
Phthalic acid	0.0243 ± 0.007	3.7 ± 0.5	0.08 ± 0.03	0.005 ± 0.012	ETH-49846.23.1
	0.0049 ± 0.003	9.1 ± 0.5	0.02 ± 0.03	-0.003 ± 0.005	ETH-49846.31.1
	0.0080 ± 0.003	11.5 ± 0.5	0.06 ± 0.04	0.002 ± 0.004	ETH-49846.25.1
	0.0086 ± 0.003	13.8 ± 0.5	0.08 ± 0.04	0.003 ± 0.004	ETH-49846.26.1
	0.0080 ± 0.003	20.0 ± 0.5	0.11 ± 0.07	0.004 ± 0.004	ETH-49846.30.1
Average addition of C_{ex} with $F^{14}C$ = 1:			**0.07 ± 0.03**		
Sucrose	1.0016 ± 0.012	11.6 ± 0.5	0.57 ± 0.78	1.042 ± 0.066	ETH-49845.17.1
	1.0161 ± 0.013	12.4 ± 0.5	0.43 ± 0.79	1.054 ± 0.063	ETH-49845.18.1
	1.0359 ± 0.011	17.9 ± 0.5	0.29 ± 0.89	1.062 ± 0.044	ETH-49845.22.1
	1.0315 ± 0.010	24.0 ± 0.5	0.49 ± 1.00	1.051 ± 0.033	ETH-49845.20.1
Average addition of C_{ex} with $F^{14}C$ = 0:			**0.45 ± 0.43**		

[a] Subscript *T* indicates that values have been corrected for instrumental background using the program BATS (Wacker, 2010) and for the wet oxidation procedure (Lang et al., 2013), if required.

[b] The radiocarbon content of 1.053 ± 0.03 $F^{14}C$ was determined on the powdered phthalic acid standard by conventional AMS methods.

[c] The radiocarbon content of ≤ 0.0025 $F^{14}C$ was determined on the powdered sucrose standard by conventional AMS methods.

A. Figure caption

Figure A 1: Radiocarbon values of phthalic acid (above) and sucrose (below) after the wet oxidation procedure of both campaigns (I left, and II right). The given error bars derive only from corrections of instrumental AMS background and counting statistics. The solid grey line represents an idealized line of the mixture of the $F^{14}C$ value of the process standard and the mean external contamination of modern ($F^{14}C$ = 1, Phthalic acid) or radiocarbon dead ($F^{14}C$ = 0, Sucrose) carbon.

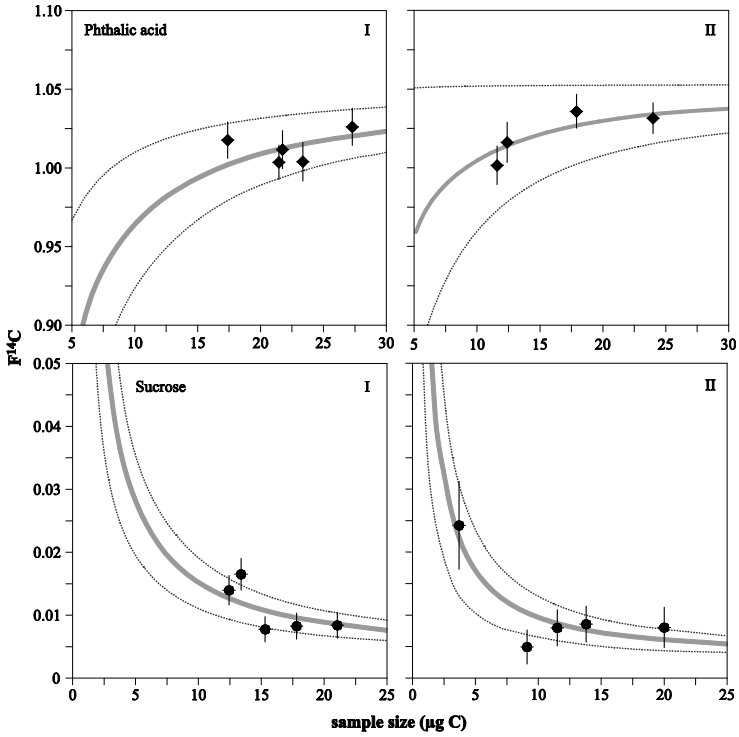

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Figure A1

B. Direct assessment of $C_{chemistry}/C_{HPLC}$

B. Table

Table B 1: Amounts and radiocarbon contents of isolated process standards B5CA and B6CA, the volume of collected eluent of each sample and the calculated amount of external C (C_{ex}) and its radiocarbon content (F_{ex}), assuming that a C_{ex} of 0.22 ± 0.04 μg ml^{-1} elutes with the mobile phase.

Sample		F_T ($F^{14}C$) [a]	C_T (μg C)	*Collected* V_{el} (ml)	*Calculated* C_{ex} (μg C)	*Calculated* F_{ex} ($F^{14}C$)	Lab code
Fossil char	B6CA	0.094 ± 0.004	23.1 ± 0.2	4	0.9	2.4	ETH-50461.1.1
	B6CA	0.040 ± 0.003	29.0 ± 0.3	9	2.0	0.5	ETH-49854.1.1
	B5CA	0.079 ± 0.006	13.0 ± 0.2	4	0.9	1.1	ETH-50459.1.1
	B5CA	0.068 ± 0.005	16.0 ± 0.2	5	1.1	0.9	ETH-50460.1.1
	B5CA	0.127 ± 0.007	14.5 ± 0.3	12	2.6	0.7	ETH-49859.1.1
					Average F_{ex}	**1.1**	
Modern char	B6CA	1.151 ± 0.025	16.2 ± 0.2	6	1.3	1.2	ETH-50462.1.1
	B6CA	1.093 ± 0.021	18.5 ± 0.2	8	1.8	1.6	ETH-49864.1.1
	B6CA	1.124 ± 0.020	20.9 ± 0.2	8	1.8	0.8	ETH-50463.1.1
	B5CA	1.183 ± 0.023	22.0 ± 0.3	5	1.1	0.2	ETH-50464.1.1
	B5CA	1.145 ± 0.019	27.6 ± 0.3	5	1.1	1.1	ETH-49868.1.1
	B5CA	1.150 ± 0.024	16.8 ± 0.2	6	1.3	1.2	ETH-50465.1.1
					Average F_{ex}	**1.0**	

[a] Subscript *T* indicates that values have been corrected for instrumental background using the program BATS (Wacker, 2010) and for the wet oxidation procedure (Lang et al., 2013).