Purification of fire derived markers for µg scale isotope analysis (δ13C, Δ14C) using high performance liquid chromatography (HPLC)

Gierga, Merle ; Schneider, Maximilian P W ; Wiedemeier, Daniel B ; Lang, Susan Q ; Smittenberg, Rienk H ; Hajdas, Irka ; Bernasconi, Stefano M ; Schmidt, Michael W I

DOI: https://doi.org/10.1016/j.orggeochem.2014.02.008

Organic Geochemistry 70 (2014) 1–9

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

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

Merle Gierga [a,*], Maximilian P.W. Schneider [b], Daniel B. Wiedemeier [b], Susan Q. Lang [a,1], Rienk H. Smittenberg [c], Irka Hajdas [d], Stefano M. Bernasconi [a], Michael W.I. Schmidt [b]

[a] Department of Earth Sciences, ETH Zürich, Sonneggstrasse 5, 8092 Zürich, Switzerland
[b] Department of Geography, University of Zürich, 8057 Zürich, Switzerland
[c] Geological Sciences, Stockholm University, 10691 Stockholm, Sweden
[d] Laboratory of Ion Beam Physics, ETH Zürich, 8093 Zürich, Switzerland

ARTICLE INFO

Article history:
Received 1 November 2013
Received in revised form 30 January 2014
Accepted 10 February 2014
Available online 25 February 2014

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 using two process standards with 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.

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 and Noack, 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,

* Corresponding author. Tel.: +41 446328715.
E-mail address: merle.gierga@erdw.ethz.ch (M. Gierga).
[1] Present address: Department of Earth and Ocean Sciences, University of South Carolina, Columbia, SC 29208, USA.

http://dx.doi.org/10.1016/j.orggeochem.2014.02.008
0146-6380/

2008; Wiedemeier et al., 2013). They 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 ($F^{14}C \geqslant 1$). 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, 2009a; 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 these 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).

We describe the successful purification of individual B5CAs and B6CAs followed by $\delta^{13}C$ and $F^{14}C$ analysis. A detailed blank assessment was carried out using direct and indirect approaches to assess the amount and the isotopic signature of C_{ex}. Two different types of charcoal were selected as standards. The first was an archaeological charcoal with a ^{14}C age > 50,000 BP ($\leqslant$0.02 $F^{14}C$) and the second a modern charcoal ($\geqslant$1 $F^{14}C$) prepared from a recently cut tree. Together they represented the end members of ^{14}C analysis and thereby allowed a good evaluation of this new method for BC dating.

2. Experimental

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

2.1. Process standards

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

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

2.2. Sample extraction and purification

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

2.3. HPLC purification

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

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

2.4. Wet oxidation

The compound specific isotopic signature ($\delta^{13}C$, $F^{14}C$) of isolated BPCAs was determined with the methods described by Lang et al. (2012, 2013). Specifically, isolated and acidified samples were transferred to 12 ml gas tight vials, diluted with Milli-Q water to a total volume of 4 ml, and spiked with 0.75 ml supersaturated oxidizing solution (100 ml H_2O + 2.0 g $K_2S_2O_8$ + 200 μl 85% H_3PO_4). Vials were sealed using a standard cap with a butyl rubber septum and flushed with high purity He (grade 5.0, 99.999%) for 8 min at 125 ml/min to 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, $F^{14}C \leqslant 0.0025$ (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 with 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 gas tight 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., 2013a,b). 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 the CO_2 was released by heating the zeolite to 450 °C. The amount of CO_2 in the syringe was detected pneumatically to allow dilution of the sample gas with He to 5%, v/v CO_2 in He. This gas mixture was then pushed continuously out of the syringe into the ion source. Oxalic acid I (OX-1) gas was used as a modern standard (Stuiver and Polach, 1977) for normalization and fossil CO_2 gas served as a blank.

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

3. Results and discussion

3.1. Isolation of individual BPCAs with HPLC

The first goal in method development was the definition of appropriate chromatographic conditions for providing sufficient amounts of the pure target compounds. BPCA concentration in the total digests was determined following Wiedemeier et al. (2013). In both samples B6CA and B5CA represented ca. 80% of the total quantified BPCA C. For CSRA these two compounds provided the best conditions for successfully isolating and dating them. As a second step we injected as much as 1 μg C from each of the two and were still able to define robust retention times of the baseline-separated peaks. Even though the column was slightly overloaded, no tailing of the target peaks to other fractions was observed (Fig. 1A) by re-injection of the eluent collected just before and after collection of the sample peak. The time window for fraction collection was set as narrow as possible to minimize the amount of potential co-eluting extraneous compound and, especially, column bleed. Next to the quantified BPCAs a suite of other peaks were present (Fig. 1A). These were other byproducts of the digestion and were most likely nitrated BPCAs (Ziolkowski and Druffel, 2009b). We isolated B5CA and B6CA fractions from both process standards, the fossil and modern char, in 3 replicates, respectively. As a preliminary assessment of C_{ex}, aliquots of the collected fractions were re-injected; this did not show any UV-detectable contaminants (Fig. 1B). Fractions were typically collected and combined from 20 to 30 repeated injections. Sample recovery varied between 50% and 84% (Table 1). Losses might have occurred

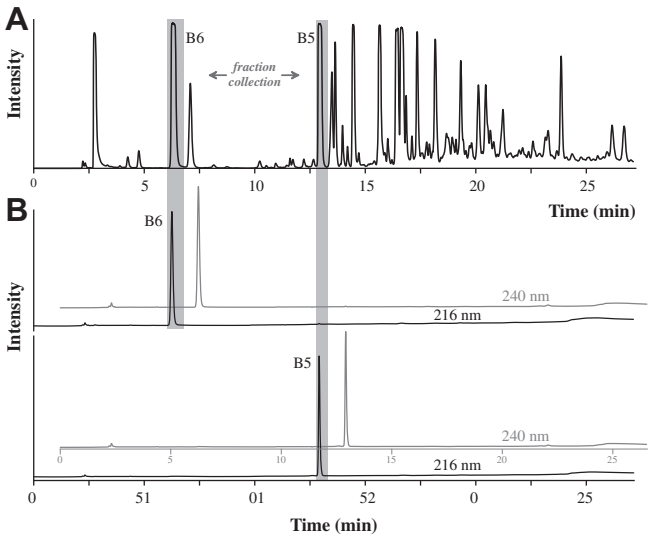

Fig. 1. HPLC-DAD chromatogram from a 5 µl injection of a BPCA extract of modern char for fraction collection (A) and HPLC-DAD chromatograms of aliquots of purified B6CA and B5CA fractions detected at 216 and 240 nm (B). Time windows for single peak collection of B6CA (B6) and B5CA (B5) are highlighted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

during fraction collection or during transfer and concentration of the individual fractions. We did not detect significant amounts of the target compounds in fractions collected subsequently after the time window for B6CA or B5CA, which suggests that no significant tailing occurred after the detector was passed and before the fraction collector. As we considered it more important to avoid the collection of other peaks eluting shortly after the target compounds, we did not try to optimize the recovery by widening the collection window as soon as recovery exceeded 50%. Isotopic fractionation effects over the chromatographic peak were not expected for the ^{14}C content (Zencak et al., 2007). This can be explained by the fact that $F^{14}C$ values are corrected for isotopic fractionation, which is expected to occur during AMS analysis. It is also possible that losses occurred during the transfer and concentration of the collected sample volumes. Up to 15 ml of that aqueous solution had to be reduced to a final volume of 2 ml, resulting in a high concentration of H_3PO_4 as only water and MeCN were volatilized under N_2 flow.

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

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

The final $\delta^{13}C$ values of each isolated B5CA or B6CA sample, as well of the entire digestion extracts are listed in Table 1. The values are corrected for fractionation effects between the liquid and gas phase and for process and instrumental background of the wet oxidation procedure itself, as described by Lang et al. (2012). In brief, the process blank was determined using oxidized ultra pure water with similar volumes to the samples. The peak area of these blanks averaged 0.55 ± 0.09 V s (n = 3), which corresponds to a value near the limit of detection of ⩽0.2 µg C. As these peaks were too small for reliable $\delta^{13}C$ values, the isotopic composition of the process blank was estimated indirectly by comparing the values for the oxidized phthalic acid standard samples with the known reference value. The $\delta^{13}C$ of the blanks is very sensitive, so it was individually calculated for each prepared series. The blanks were calculated to be −14.1 ± 1.1‰ for the first and −9.1 ± 1.1‰ for the second series. At first sight, there is a significant difference between the two blanks. It should be taken into account, however, that small differences detected with such small signals could lead to large differences in the $\delta^{13}C$ values for the blanks. The isotopic shift of ca. 5‰ for the blank results probably resulted from slight changes in the quality of the chemicals in use, for instance the oxidizing

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	Compound	HPLC			GC-IRMS		Yarnes et al. (2011)
		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 × 5 µl	22.3	76%	29.3	−25.5 ± 0.3	
	B6CA	30 × 5 µl	35.2	80%	39.7	−24.5 ± 0.1	
	Avg. B6CA					−25.0 ± 0.7	
	B5CA	20 × 5 µl	16.3	55%	17.2	−26.9 ± 0.3	
	B5CA	25 × 5 µl	20.4	55%	21.7	−26.4 ± 0.3	
	B5CA	30 × 5 µl	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 × 5 µl	19.9	74%	23.3	−27.9 ± 0.3	
	B6CA	25 × 5 µl	28.6	84%	32.5	−28.1 ± 0.1	
	B6CA	25 × 5 µl	25.9	76%	31.1	−27.1 ± 0.3	
	Avg. B6CA					−27.7 ± 0.5	−28.24 ± 0.36
	B5CA	20 × 5 µl	19.7	50%	25.8	−28.4 ± 0.3	
	B5CA	25 × 5 µl	30.6	62%	29.5	−27.7 ± 0.1	
	B5CA	25 × 5 µl	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.

reagent or the ultra pure water. This assumption is supported by the fact that the $\delta^{13}C$ values for the fire-derived compounds from the fossil and modern char showed no significant difference after being corrected for the blank value for the wet oxidation procedure (Table 1). The BPCAs had slightly lower $\delta^{13}C$ values than the total digests (Fig. 2), while B5CA was more negative than B6CA. These small differences may be the result of inhomogeneity in the parent material. Another explanation might be an incomplete collection of the chromatographic peak, as $\delta^{13}C$ values are much more sensitive to fractionation effects than $F^{14}C$ values (Zencak et al., 2007). The overall reproducibility (1σ) was $\leqslant$0.7‰. Even if this is slightly higher than the reported precision for the chemical oxidation method ($\leqslant$ 0.4‰, Lang et al., 2012), the values are still satisfyingly accurate. With this technique, only a very small part of the isolated samples was used for stable isotope analysis in order to assure large enough samples for AMS analysis. More accurate results could potentially be achieved by isolating a separate sample dedicated only to $\delta^{13}C$ using a GasBench device.

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

3.3. Assessment of extraneous carbon

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

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

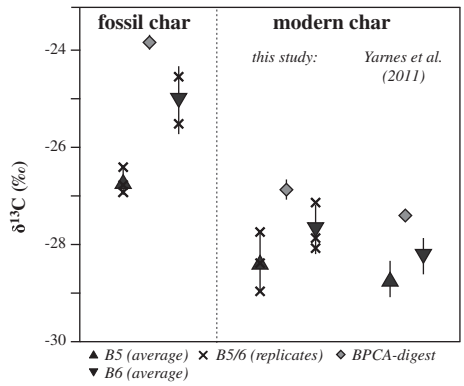

Fig. 2. $\delta^{13}C$ values of individual B5CA and B6CA and of the whole BPCA digest of fossil (left) modern char (right) measured in this study and as published by Yarnes et al. (2011) for the modern char.

where F is the $F^{14}C$ value and C the amount of carbon in µg. The subscript T refers to total as measured, S to sample and ex to extraneous. In order to solve the equation for F_S, the amount 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 2× 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, with only the HPLC step, the extraction procedure being omitted. 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 (n = 2), whereas C_{HPLC}

Table 2
Direct assessment of C_{HPLC} (n.a., not analyzed).

Sample	HPLC collected ml	µg C[a]	GC-IRMS	
			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] $n = 2$.

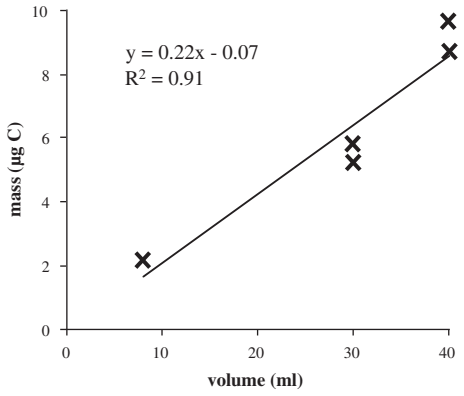

Fig. 3. Mass of HPLC blank vs. volume of collected eluent.

showed an average of 0.22 ± 0.04 µg/ml ($n = 5$). 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) 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. 4), 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

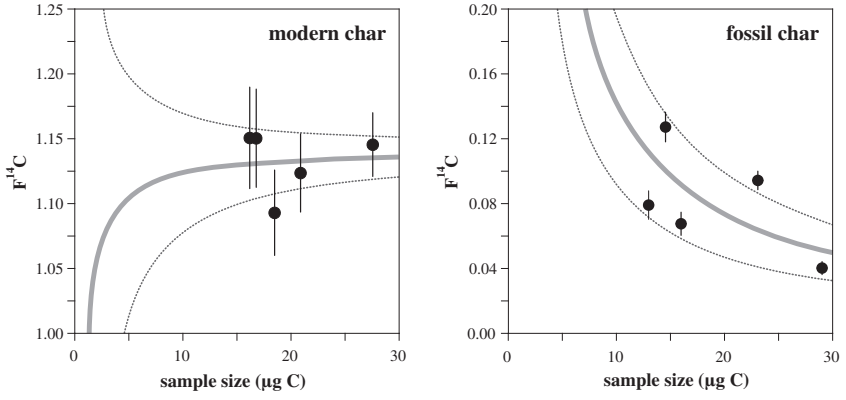

Fig. 4. Radiocarbon values for B5CA and B6CA isolated from the modern (left) and fossil char (right). The given error is composed of corrections for instrumental AMS background and the blank for wet oxidation. The solid gray line represents an idealized line for the mixture of the real $F^{14}C$ value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

Table 3
Amount and radiocarbon content of isolated process standards B5CA and B6CA, calculated amount of external C (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 (avg. C_{ex}):				1.4 ± 0.5		
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 (avg. C_{ex}):				0.2 ± 0.4		

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

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 in F_{HPLC} during later measurements is however a clear indication for an additional contribution of C_{HPLC}, in general.

Fig. 4 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 20–30 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 correct radiocarbon content (F_S) values from the individual samples are listed in Table 3. There was no evidence for a significant difference in the $F^{14}C$ values between the B5CAs and B6CAs. Hence, replicate analyses of B5CAs and B6CAs from the same process standard were taken as equal. The 5 replicates of the modern charcoal sample gave a mean $F^{14}C$ value of 1.142, with a standard deviation of 0.025, i.e. a precision of 2.2%. This value mirrors both the $F^{14}C$ of the digest (ETH-49860) and the reference analysis of the bulk material (ETH-50458). The individual samples exhibited values with a slightly greater uncertainty that in turn depended on the sample size and lower counting statistics. The largest sample containing 27 μg C had a precision of 2.9% and the smallest (16 μg C) a precision of 4.5%. Likewise, the F_S for the largest sample (ETH-49868.1.1) was also the one closest to the reference value. The same was true for the fossil charcoal standard being depleted in ^{14}C, even if no size dependent increase in precision is given for these duplicate values. The average F_S from 5 individual measurements was 0.001 ± 0.032. Samples with < 15 μg C were not analyzed, as demonstrated by Birkholz et al. (2013) that samples designated for CSRA and < 10 μg C are usually affected by C_{ex} to such an extent that no reliable results can be obtained. However, the data here demonstrate that sample amounts of 25–35 μg C are suitable for high precision analysis. Additional replicate analyses are still recommended so that possible outliers can be easily identified. In summary, the given uncertainty in an individual measurement is satisfactory for C turnover studies or C source apportion. For precise dating purposes a higher precision is usually required. Indeed, there is room for further minimization of C_{ex}, especially during the HPLC isolation procedure. Comparing the background level of organic C in the ultra-pure water used (i.e. in the aqueous HPLC solvent) with published references indicates that the system used for this study could be improved with better maintenance. Lang et al. (2012) reported that not more than 0.04 μg C/ml were detected in the aquatic mobile phase. In contrast, a concentration of up to 0.3 μg C/ml was measured for the eluents used here.

4. Conclusions

We present a method to purify individual BPCAs as compound specific biomarkers for BC, followed by the determination of $\delta^{13}C$ and $F^{14}C$. The combination of two measurements on the same sample reduces the efforts specified by an isolation protocol for the particular analysis. Furthermore, knowing both the $\delta^{13}C$ and $F^{14}C$ values for a sample helps interpret the results with respect to the impact of contamination that might be difficult to detect, especially when they have a different $\delta^{13}C$ value from the sample of interest. Another benefit is the possibility of monitoring the development of the general C_{ex} background in an easy and cost effective way. C content and its $\delta^{13}C$ value should give enough information and help avoid expensive radiocarbon analysis of contaminated samples. Finally the wet oxidation method avoids the problems encountered in the combustion of H_3PO_4-rich sample residues.

A constant addition of extraneous C to the isolated samples was identified. Nevertheless, a size dependent component C_{ex} cannot be excluded. This is especially true for much larger samples (> 50 μg C) that need to be isolated with a greater extent of injections

(e.g. 100–150 injections to yield ca. 100 μg BPCA C). Contamination of 1.6 ± 0.2 μg C, with $F^{14}C$ 0.90 ± 0.14, was calculated. We have shown that the precision of individual measurements of samples with > 15 μg C is adequate for studies aimed at determining C turnover or source apportioning in soils and sediments. In addition, our data show that there is also potential for applying the method for dating purposes. Samples isolated in replicate, each containing > 25 μg C, should give values precise enough for an age determination of, for example, combustion residues on pottery or other samples with very fine charcoal that cannot be analyzed directly, or BC that is mixed with other OM. Based on the experience from this study, we recommend that process standards and blanks are determined regularly, as it is possible that C concentration in chemicals and/or solvent changes through time.

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

5. Author contributions

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 M.P.W.S. carried out the experiments and data analysis. D.B.W., S.Q.L. and R.H.S. provided technical expertise for method development. I.H. carried out AMS analysis with solid graphite targets.

Acknowledgements

We would like to thank two anonymous reviewers for their constructive comments. Funding support from Swiss National Science Foundation (SNF) project Nr 200021-119950/200020-13484 and Nr 200020-131922, and also University of Zurich Forschungskredit Nr 57061004 Fellowship is gratefully acknowledged. We thank M. Hilf, C. McIntyre and L. Wacker for assistance with HPLC and AMS measurements. T. Eglinton generously donated funding for the AMS measurements. We also thank P. Ascough and M. Bird for donating the archaeological charcoal.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orggeochem.2014.02.008.

Associate Editor—**M.J. Simpson**

References

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Reimer, P.J., Brown, T.A., Reimer, R.W., 2004. Discussion: reporting and calibration of 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., 2013a. 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., 2013b. 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.