University of Groningen

Molecular characterization of charcoal to identify adsorbed SOM and assess the effectiveness of common SOM-removing pretreatments prior to radiocarbon dating

Wagner, T.V.; Mouter, A.K.; Parsons, John R.; Sevink, Jan; van der Plicht, Johannes; Jansen, Boris

Published in:
Quaternary Geochronology

DOI:
[10.1016/j.quageo.2017.10.006](https://doi.org/10.1016/j.quageo.2017.10.006)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Wagner, T. V., Mouter, A. K., Parsons, J. R., Sevink, J., van der Plicht, J., & Jansen, B. (2018). Molecular characterization of charcoal to identify adsorbed SOM and assess the effectiveness of common SOM-removing pretreatments prior to radiocarbon dating. *Quaternary Geochronology*, 45, 74-84.
<https://doi.org/10.1016/j.quageo.2017.10.006>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Molecular characterization of charcoal to identify adsorbed SOM and assess the effectiveness of common SOM-removing pretreatments prior to radiocarbon dating

Thomas V. Wagner^{a,*}, Anne K. Mouter^a, John R. Parsons^a, Jan Sevink^a,
Johannes van der Plicht^{b,c}, Boris Jansen^a

^a Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

^b Centre for Isotope Research, Groningen University, Nijenborgh 4, 9747 AG Groningen, The Netherlands

^c Faculty of Archaeology, Leiden University, P.O. Box 9515, 2300 RA Leiden, The Netherlands

ARTICLE INFO

Keywords:

Charcoal
Radiocarbon dating
Molecular characterization
Pyrolysis-GC-MS
ABA
ABOx

ABSTRACT

A significant part of our knowledge on Holocene landscape development and associated human history in northwestern Europe is based on radiocarbon dating of charcoal originating from podzols. However, these soils are characterized by leaching of soil organic matter (SOM) that may adsorb to older charcoal, resulting in incorrect charcoal radiocarbon ages. Several pretreatments, such as an acid-base-acid (ABA) sequence for Holocene-aged samples and a harsher acid-base-oxidation (ABOx) sequence for older samples, have been developed to remove the adsorbed fractions. While commonly used, evidence for the effectiveness of these pretreatments thus far has been circumstantial, by assessing the impact of these pretreatments on the radiocarbon age.

In this study, we performed a direct molecular assessment of the presence, adsorption, and removal of SOM-compounds by pyrolysis-GC-MS based characterization of charcoal fragments and SOM obtained from a podzol located in the Laarder Wasmeren area, the Netherlands. In addition, the removal efficiency of adsorbed SOM by four different pretreatments was analysed. Molecular evidence for significant adsorption of SOM was found and the four pretreatments showed different efficiencies in removal thereof as evidenced by the residual charcoal composition. ABOx resulted in the most simplified Py-GC-MS chromatogram and thus comes closest to the isolation of recalcitrant carbon. The most recalcitrant organic fraction is likely to have the best interpretable age, since the highest decontamination can be expected for this fraction. Hence, it is advisable to use ABOx as standard pretreatment for the removal of adsorbed SOM fractions from charcoal, provided that the sample size is large enough.

1. Introduction

A significant part of our current knowledge on the Holocene landscape development and associated human history of northwestern Europe is based on the radiocarbon dating of charcoal originating from podzols in sandy deposits (Groenendijk, 1987; Reiß et al., 2006). However, such podzols are characterized by leaching of mobile soil organic matter (SOM) (IUSS Working Group, 2014). SOM consists of various functional pools of organic material, each with its own origin, age, turnover rate and mobility (von Lützow et al., 2007). Though charcoal is basically well-suited for radiocarbon dating because of its relative resistance to chemical and physical alteration after formation, it is known to adsorb organic compounds present in the soil (Sander and

Pignatello, 2005). This may either be the adsorption of SOM formed in situ such as microbial residues in the form of proteins and lipids (Kögel-Knabner, 2002; Ludwig et al., 2015) or root decay material consisting of lignins and tannins (Kögel-Knabner, 2002; Baumann et al., 2013). In addition, mobile fractions of dissolved organic matter (DOM) such as organic acids, amino acids and sugars are transported downwards through the soil profile (Kalblitz et al., 2000; Gabor et al., 2014), potentially reaching charcoal in deeper soil layers.

The adsorption of organic material with a significantly younger age than the charcoal may lead to incorrect radiocarbon ages (Higham, 2011). This is especially the case for older charcoal samples that are close to the radiocarbon dating limit of $\pm 50,000$ ^{14}C yrs BP (Reimer et al., 2013): A contamination by only one percent of younger organic

* Corresponding author.

E-mail address: t.v.wagner@uva.nl (T.V. Wagner).

Fig. 1. Schematic overview of the soil profile including layer 'X' from which the charcoal was obtained and its location in the Netherlands (N 52 13.65, E 5 12.61).

material in a sample with an 'infinite age' can lead to an apparent age of 37,000 ^{14}C yrs BP (Higham et al., 2009). To prevent incorrect radiocarbon ages as a result of adsorption of younger SOM, an acid-base-acid (ABA) pretreatment was developed to remove such contamination (e.g. Olsson, 2009). In general, this method has proven to work well for many thousands of samples analysed worldwide. Nonetheless, the ABA pretreatment does not always lead to a correct age estimation, especially for samples older than 20,000 ^{14}C yrs BP (Gillespie et al., 1992; Haesaerts et al., 2013). As a result, harsher oxidation treatments were designed to remove labile young carbon fractions. This led to the development of the acid-base-oxidation (ABOx) method with stepped combustion (Bird et al., 1999). Several studies have shown that this pretreatment results in more accurate radiocarbon ages for older samples (Wood et al., 2012; Haesaerts et al., 2013; Bird et al., 2014). A disadvantage of the ABOx pretreatment is that it is very rigorous and can cause significant loss of sample material. Therefore, it is unsuited for samples that are available in small quantities only or of delicate nature (Ascough et al., 2010).

A detailed understanding of the effects of the different pretreatment techniques remains lacking. Most evidence for the effectiveness of the different pretreatment methods produced thus far is circumstantial, and obtained by assessing their impact on the measured radiocarbon age. In addition, Raman spectroscopy has been employed to ensure sufficient

removal of contaminants (Alon et al., 2002; Yizhaq et al., 2005). However, Raman spectroscopy does not provide detailed information on the composition of the contamination at a molecular level. Tomiyama et al. (2016) performed Fourier-transform infrared spectroscopy (FTIR) and elemental analysis to elucidate changes in the chemical structure after ABA and ABOx treatment. They found that i) the base (B) treatment removed adsorbed organic compounds from the charcoal and ii) that the oxidation took place at the edges of the charcoal fragment. Stepped combustion resulted in a further reduction of adsorbed organics. However, for a complete understanding of contamination by adsorption of young SOM, and the effectiveness of different pretreatments to remove such contamination, a molecular characterization is needed to unravel what exactly is removed by the various methods.

Only a few molecular characterization studies have been performed to study the fractions of SOM that are initially adsorbed and subsequently removed by the pretreatment methods. To our knowledge, the only attempts at a molecular characterization in this context are by Meredith et al. (2013) and Zhang et al. (2015) who studied the molecular composition of the recovered hydropyrolysis 'non-charcoal' product. However, they did not include the treated charcoal fragments themselves in this molecular research, nor did they study the earlier mentioned podzols where charcoal is particularly prone to

contamination by younger mobile SOM.

Our present study aims to address this knowledge gap by performing a detailed characterization of the molecular composition of charcoal from a podzol from the Netherlands that was subjected to different pretreatments to i) establish whether it was possible to determine adsorbed fractions of SOM; ii) provide a molecular insight into the removal efficiency of different charcoal pretreatment methods. Therefore, the molecular composition of differently treated charcoal fragments and SOM from a podzol was determined applying pyrolysis gas chromatography (Py-GC-MS).

2. Materials and methods

2.1. Study area

Charcoal samples were obtained from the Laarder Wasmeren area, a small nature reserve near Hilversum, The Netherlands (N 52 13.65, E 5 12.61). The Laarder Wasmeren area holds a complex sequence of Pleistocene and Holocene aeolian sands and paleosols (Sevink et al., 2013). The latter includes an incipient podzol, which contains abundant charcoal fragments. It consists of a dark humic Ah horizon, and weakly developed E and Bs horizons, and is covered by about 1 m aeolian sand (Fig. 1). In the latter, a prominent podzol occurs with dark coloured organic fibres consisting of organic material extending through the incipient podzol into the underlying aeolian sand. The whole is covered by recent drift sand. The rationale for choosing this particular charcoal was the ambiguity in the age of layer X (Fig. 1). It was earlier assumed that this layer represented the Usselo layer (Kaiser et al., 2009; Sevink et al., 2013) which formed during the Allerød interstadial (12.650–13.900 cal. ^{14}C yrs BP) (Kaiser et al., 2009). However, preliminary Optical Stimulated Luminescence (OSL) and radiocarbon dating of the charcoal present in this layer yielded ages of 8130 ± 34 yrs BP and 8445–9240 cal. ^{14}C yrs BP, respectively, which were significantly younger than its presumed age. Evidence of bioturbation was found in situ, which was held responsible for the unexpected low OSL age. In addition, it was hypothesized that the low radiocarbon age could be the result of the overlying podzol including the fibres (Fig. 1), that would continuously supply young organic material downwards through the soil profile. The standard ABA pretreatment that was used prior to radiocarbon dating of these charcoal samples might be unable to remove all of this potentially adsorbed contamination.

2.2. Sampling

More than 100 kg of the charcoal rich 'X' horizon (Fig. 1) was taken, which was subsequently dried overnight at 60 °C and sieved at 1 mm. Charcoal fragments > 1 mm were handpicked from the residue. Samples of the thick SOM containing podzol B horizon were dried according to the same procedure. Microscopic inspection of the charcoal showed that the charcoal fragments originated from *Pinus sylvestris*, while a reflectance study according to the method of Braadbaart and Poole (2008) indicated that the formation temperature of the charcoal in the Laarder Wasmeren area was approximately 450–500 °C (Braadbaart, pers. comm.). For further analysis, mixed charcoal fragments were used. Charcoal samples were not ground before pretreatment to allow for an assessment of the removal of adsorbed contaminants from the inside of the charcoal fragments.

2.3. Pretreatments

Four different treatments were performed on both the Laarder Wasmeer and the reference charcoal. An untreated control was used to determine the fraction of adsorbed SOM. The mass recovery after each treatment determines the amount of sample needed prior to pretreatment in order to perform successful radiocarbon dating. The mass

recovery was determined by weighing the dried sample pre- and post-treatment. The amount of sample and the ratios of solid/liquid were such that the expected quantities of residue would be sufficient for ^{14}C AMS dating, based on recommendations by Brock et al. (2010).

In addition to the common ABA and ABOx pretreatment, the influence of two alternative contaminant removal strategies, Accelerated Solvent Extraction (ASE) and Thermal Oxidation (TO), were studied. ASE is an extraction technique that employs a solvent, high temperature and high pressure to weaken the Van der Waals interaction and hydrogen bonds between organic compounds in soil or sediment (Richter et al., 1996). ASE has for instance been used for the extraction of straight chain lipids (Jansen et al., 2006) or polycyclic aromatic hydrocarbons (PAHs) (Bandowe et al., 2010) from the soil matrix. For this study, no specific target compound had been defined. Thermal oxidation at 375 °C has successfully been used to isolate and quantify sedimentary black carbon from the soil matrix (Elmquist et al., 2004; Zencak et al., 2007). The original method developed by Gustafsson et al. (1996) consisted of a thermal oxidation step aimed at removing the organic carbon followed by acidification to remove inorganic carbonates. In this study, only the impact of the thermal oxidation at 375 °C on the composition of the charcoal was assessed.

2.3.1. Untreated control

Charcoal aliquots of 50–60 mg were used. The untreated charcoal fragments were rinsed $3 \times$ with ultrapure Milli-Q water to remove remaining soil fragments and dried at 40 °C.

2.3.2. ABA

The ABA treatment was based on the method described by Hatté et al. (2001). 5 ml of 1 M HCl was added to 50–60 mg charcoal aliquots and the solution was shaken at 40 rpm for 2 h at 60 °C to remove adsorbed carbonates (Higham et al., 2009). Afterwards, the sample was rinsed $5 \times$ with Milli-Q-water. 11 ml of 0.1 M NaOH solution was added and the solution was shaken overnight at 40 rpm and 60 °C to remove humic complexes (Higham et al., 2009). After rinsing $5 \times$ with Milli-Q-water until the supernatant was clear, a final acid step was performed with 9 ml of 1 M HCl, shaken at 40 rpm and 60 °C for 2 h to remove possible young carbon incorporated during the base step (Hatté et al., 2001) and subsequently rinsed $5 \times$ with Milli-Q water and dried at 40 °C overnight.

2.3.3. ABOx

The ABOx treatment was based on the method of Bird et al. (1999). The stepped combustion procedure was not incorporated in this study to allow direct comparison with the ABA results. Aliquots of 60–70 mg charcoal were used. The ABOx treatment followed the first two steps of the ABA treatment, followed by oxidation. Note that these two steps in this study use 6 and 10 times less concentrated acid and base treatment, respectively, than used in the study by Bird et al. (1999), consistent with the ABOx treatment in Hatté et al. (2001). Oxidation was performed with 9 ml of 0.1 M $\text{K}_2\text{Cr}_2\text{O}_7$ (potassium dichromate) in 2 M H_2SO_4 solution. Samples were shaken for 48 h at 40 rpm and 60 °C and subsequently rinsed $8 \times$ with Milli-Q water, and dried at 40 °C overnight.

2.3.4. ASE

Charcoal aliquots of approximately 100 mg were used. ASE extraction was performed with a Dionex ASE 200 with 40 ml extraction cells. Acetonitrile was used as extractant, as it has been shown to be able to desorb possibly adsorbed fractions of SOM (Jansen et al., 2006). Extractions were carried out under a pressure of 1500 psi and a temperature of 200 °C. The extraction method consisted of two cycles of 2 min pre-heating time, 110% flush and 90 s flush time.

2.3.5. TO-375

Charcoal aliquots of 50–60 mg were used. The samples were placed

Table 1

Carbon and nitrogen content and mass recovery of charcoal samples subjected to different pretreatments.

Pretreatment	Mass recovery (%)	%C	%N
NT	–	46.2	0.3
ABA	45.9	63.6	0.3
ASE	96.2	46.6	0.7
ABOx	15.8	53.6	0.2
TO375	23.9	7.3	0.2

in a Carbolite Furnaces CSF 1100 oven for 18 h at a temperature of 375 °C and a constant input of air. Afterwards, samples were cooled in a desiccator.

2.4. Soil organic matter extraction

To extract the SOM from the mineral B horizon (Fig. 1), an adapted version of the method applied by Nierop and Buurman (1998, 1999) was used: dried soil samples were sieved to pass 1 mm. 1 M NaOH was added to isolate the organic matter and the suspensions were shaken for 2 h. After shaking, the solution was centrifuged at 2000 rpm for 10 min. The supernatant with the organic matter was poured into an Erlenmeyer flask and the procedure was repeated with Milli-Q water with a shaking-time of 5 min until the solution was clear. The supernatants were combined and the solution was acidified to pH 2–2.5 with HCl/HF (1:3 v/v) and shaken for 24 h. The solution was then dialysed against demineralized water and freeze-dried. The freeze-dried samples consisted of sheets of organic material, which were ground with mortar and pestle before further analysis.

2.5. CHN analysis

The elemental composition of the charcoal was analysed in triplicate to determine the residual carbon (C) and nitrogen (N) content after the various pretreatments. The C and N content give an indication of the pretreatment efficiencies and potentially identify samples with too little carbon for ¹⁴C analysis. CHN analysis was performed using an Elementar Vario EL-cube. Samples were dried at 70 °C and ground with mortar and pestle prior to the analysis. Sulfanilic acid was used as control compound.

2.6. Pyrolysis GC–MS

2.6.1. Pretreatment

Py-GC–MS is capable of separating and identifying the various organic fractions present in charcoal (Fabbri and Helleur, 1999; Kaal et al., 2008b; Kaal and Rumpel, 2009; de la Rosa Arranz et al., 2009) and SOM (Huang et al., 1998; Nierop and Buurman, 1999; Kisand et al., 2013). Prior to pyrolysis GC–MS, dried charcoal and SOM samples were ground with pestle and mortar. 3 mg of sample was mixed with 3 µl of internal standard androstane in a hexane solution (0.1 µg/ml). TMAH (tetramethylammonium hydroxide) was used as derivatizing agent before Pyrolysis-GC–MS. Derivatization with TMAH results in enhanced chromatographic behaviour of polar compounds (Kaal et al., 2008a). Several studies have shown that TMAH pretreatment improves the identification of organic compounds when using Pyrolysis GC–MS (Clifford et al., 1995; Zang et al., 2001; Kaal et al., 2008a). TMAH results in conversion of all alcohols and acids into their respective methylated ethers and esters.

20 µl of 25% TMAH was added and mixed by hand for 1 min. The solution was placed on a pyrolysis wire and dried under a lamp for 1 min. Afterwards, the pyrolysis wire was placed in a glass capillary tube and pyrolyzed immediately afterwards.

2.6.2. GC programming

Pyrolysis GC–MS was performed in triplicate on a Horizon Curie-point pyrolyzer coupled to a Thermo Trace GC. Pyrolysis occurred at 600 °C. A Phenomenex Zebron ZB-1MS column (30 m, 0.25 mm internal thickness, 0.50 µm film thickness) was used for separation of the pyrolysis products. Helium was used as carrier gas. The initial oven temperature was set at 50 °C, maintained for 1 min. The temperature was raised at 7 °C/min to a final temperature of 320 °C and maintained at this level for 10 min. The GC was coupled to a Finnigan Trace MS with a detector voltage of 350 V, operated in full scan mode with a scan range of *m/z* 47–500 and an acquisition delay of 6 min.

2.6.3. Identification

Identification of the pyrolysis products was performed using Xcalibur software (Thermo Scientific) and associated Xcalibur and NIST libraries, and was based on the dominant mass fragments. Py-GC–MS is less suitable for absolute quantification (Galletti and Bocchini, 1995). Therefore, the content of the charcoal and SOM is not absolutely quantified but the relative contribution of different compound groups found is compared and their origin is determined. The contribution of the different compound groups is displayed as the percentage of the total quantified peak area of the total ion chromatogram.

3. Results and discussion

3.1. Mass recovery

The mass recovery after pretreatment provides a first indication the removal efficiency of a treatment method. ABA treatment resulted in a mass recovery of 45.9% (Table 1), which is in the same order of magnitude as the ABA treated charcoal in Rebollo et al. (2011). It was shown by Rebollo et al. (2008) and Ascough et al. (2011a) that the base step in the ABA treatment was responsible for the most significant weight loss, as result of the breakdown of covalent bonds by the hydroxyl group in the base step (Rebollo et al., 2008). ABOx treatment resulted in a significantly lower mass recovery of 15.8% (Table 1), demonstrating the harshness of oxidation with K₂Cr₂O₇. Weight loss after ABOx treatment was approximately 95% in Rebollo et al. (2011). However, in that study, the stepped combustion procedure was incorporated, possibly resulting in the additional mass removal. Thermal oxidation resulted in 23.9% recovery of the initial charcoal sample (Table 1), denoting the treatments ability to remove certain fractions. In contrast, ASE treatment did result in 96.2% recovery of the initial charcoal sample (Table 1).

3.2. Charcoal C and N content

The untreated LW-charcoal had an average content of 46.2% C and 0.3% N (Table 1). Both ABA and ABOx treatment resulted in an increase in average C-content, which can be explained by the removal of adsorbed compounds with lower C/x ratio than the original charcoal fractions (Table 1). Mook and Streurman (1983) state that a C content of 68 ± 5% for charcoal is indicative of the complete removal of contamination by the ABA method. The ABA treated charcoal contained 63.6% C, falling well within this range (Table 1). However, the C content of charcoal after treatment depends heavily on its formation temperature, with lower C contents for charcoal formed at lower temperatures (Ascough et al., 2011a). The higher increase in ABA C% than of ABOx C% is comparable with Tomiyama et al. (2016). ABOx treatment resulted in a lower C content than the ABA treated charcoal (Table 1), likely as a result of the final oxidation step (Knicker et al., 2007). This is in contrast with the ABA and ABOx treated charcoal in Wood et al. (2012), where a higher C content was found for ABOx treated charcoal. This could be a result of the formation temperature, which was > 500 °C (Wood et al., 2012). In addition, it could be a result of the missing stepped combustion in this study, which was

Table 2

Compounds with highest peak intensity in Py-GC-MS chromatograms for all charcoal treatments. M.e. = methyl ester.

#	Compound	Compound group	Diagnostic mass fragments	Retention time
1	1H-pyrrole, 2,3,4,5-tetramethyl	N-compound	122 + 108 + 79	10.30
2	benzoic acid, m.e.	Methoxy	136 + 105 + 77	10.33
3	pyrrolidine, 1(1-cyclopenten-1-yl)	N-compound	136 + 122	12.87
4	benzoic acid, 4-methoxy, m.e.	Methoxy	166 + 135 + 77	15.90
5	1,2-benzenedicarboxylic acid, dim.e.	BPCA	163 + 77	17.23
6	1H-indole, 1,2,3-trimethyl	N-compound	159 + 144 + 115	18.84
7	1,2-benzenedicarboxylic acid, diethylester	BPCA	222 + 177 + 149	19.75
8	octylether	Aliphatic	242 + 113 + 84	21.44
9	1,2,4-benzenetricarboxylic acid, trim.e.	BPCA	252 + 222 + 221	23.60
10	3,6,6-trimethyl-1-O-tolyl-1,5,6,7-tetrahydro-indozol-4-one	N-compound	268 + 253 + 197	23.65
11	1,2-benzenedicarboxylic acid, dibutylester	BPCA	278 + 149	24.14
12	hexadecanoic acid, m.e.	Aliphatic	284 + 143 + 87	25.32
13	androstane	Internal standard		26.83
14	benzene, 1,3-dimethoxy	Methoxy	138 + 109 + 78	11.82
15	benzoic acid, 2-methyl, m.e.	Methoxy	150 + 119 + 91	12.15
16	benzoic acid, 3-methyl, m.e.	Methoxy	150 + 119 + 91	12.68
17	benzaldehyde, 2,4-dimethyl	Methoxy	134 + 105	12.80
18	Unidentified Methoxy	Methoxy	150 + 119 + 91	12.88
19	benzoic acid, 2-methoxy, m.e.	Methoxy	166 + 135 + 77	15.08
20	benzoic acid, 3-methoxy, m.e.	Methoxy	166 + 135 + 107	15.24
21	1H-isoindole-1,3(2H)-dione, 2-methyl	N-compound	161 + 117 + 104	16.60
22	1,4-benzenedicarboxylic acid, dim.e.	BPCA	194 + 163 + 135	18.23
23	1,3-benzenedicarboxylic acid, dim.e.	BPCA	194 + 163 + 135	18.40
24	1,2-benzenedicarboxylic acid, 4-methyl, dim.e.	BPCA	208 + 177	19.23
25	benzoic acid, 3,5-dimethoxy, m.e.	Methoxy	196 + 165 + 138	19.43
26	benzoic acid, 3,4-dimethoxy, m.e.	Methoxy	196 + 165	19.62
27	benzoic acid, 2,4-dimethoxy, m.e.	Methoxy	196 + 165	19.87
28	1,3-benzenedicarboxylic acid, 4-methyl, dim.e.	BPCA	208 + 177 + 149	20.00
29	pyrazole, 5-methyl-3-(5-nitro-2-furyl)	N-compound	193 + 163 + 119	21.15
30	benzoic acid, 4-hydroxy-2-methoxy-3,5,6-trimethyl, m.e.	Methoxy	224 + 193 + 177	21.64
31	1,2,3-benzenetricarboxylic acid, trim.e.	BPCA	252 + 221	23.19
32	1,3,5-benzenetricarboxylic acid, trim.e.	BPCA	252 + 221	24.66
33	Unidentified BPCA	BPCA	252 + 221	25.53
34	2,6-naphthalenecarboxylic acid, dim.e.	PAH	244 + 213 + 185	25.72
35	1,2,3,4-benzenetetracarboxylic acid, tetram.e.	BPCA	279	27.39
36	1,2,4,5-benzenetetracarboxylic acid, tetram.e.	BPCA	279	27.83
37	octadecanoic acid, m.e.	Aliphatic	298 + 255 + 199	28.07
38	1,2,3,5-benzenetetracarboxylic acid, tetram.e.	BPCA	279	28.29
39	benzene, pentamethyl	Methoxy	148 + 133 + 105	14.50
40	benzene, hexamethyl	Methoxy	162 + 147	17.79
41	4-methoxycarbonylmethoxybenzoic acid, m.e.	Methoxy	224 + 193 + 165	22.23
42	1,2,4-benzenetricarboxylic acid, trim.e.	BPCA	252 + 221	23.64
43	2-cyclopenten-1-one,2,3,4-trimethyl	Methoxy	124 + 109 + 81	9.59
44	phenol, 2,5-dimethyl	Methoxy	122 + 107 + 77	10.58
45	phenol, 2,3,6-trimethyl	Methoxy	136 + 121 + 91	13.10
46	2,5-dimethyl-1-propylpyrrole	N-compound	137 + 108 + 94	13.20
47	phenol, 3-ethylamine-4-methyl	N-compound	151 + 136 + 121	13.88
48	naphthalene, 1,2-dihydro-4-methyl	PAH	144 + 129 + 115	14.19
49	phenol, 2,3,4,6 tetramethyl	Methoxy	150 + 135 + 105	15.84
50	benzenemethanol, 4-(1,1dimethylethyl)	Methoxy	164 + 149 + 91	18.95
51	1,2,3,7-tetramethylindole	N-compound	173 + 172 + 158	20.63
52	naphthalene, 1,2-dihydro-4-methyl	PAH	128 + 64	12.36
53	dibenzofuran	PAH	168 + 139 + 84	18.74
54	fluorene	PAH	166 + 165 + 82	19.88
55	2-naphthalenecarboxylic acid, m.e.	PAH	186 + 155 + 127	20.44
56	9H-fluoren-9-one	PAH	180 + 152 + 126	22.22

performed by Wood et al. (2012). The C content of ASE treated charcoal was similar to that of the untreated charcoal, implying a limited removal of contamination with a low C/x ratio. The C content of the TO-375 treated charcoals of 7.3% and 16.0% for the LW and REF charcoal, respectively (Table 1), is significantly lower than that of the C content of the other pretreated samples. The high C loss is likely the result of the combustion of charcoal at 375 °C, resulting in a high sample mass loss as result of thermochemical oxidation (Nguyen et al., 2004; Roth et al., 2012).

3.3. Charcoal composition

3.3.1. Benzene polycarboxylic acids (BPCA)

Various groups of compounds could be distinguished after Py-GC-

MS of the charcoal. BPCAs (Table 2, compound 5,7,9,11,22,23,24,28,31,32,33,35,36 and 38) form as a result of the oxidative degradation of black carbon after its formation, leading to an increase in carboxylic groups (Glaser et al., 1998; Schneider et al., 2010). However, although carboxylation of the aromatic structures is indicative of environmentally altered charcoal, its rate is strongly determined by production- and environmental conditions, such as formation temperature, redox conditions and pH (Braadbaart et al., 2009; Ascough et al., 2011b). The presence of BPCAs in the soil matrix is a strong indicator of the presence of charcoal in the soil since it was shown that these compounds were mainly originating from charcoal (Glaser et al., 1998). However, more recent publications have shown that BPCAs are also released from other environmental samples like degraded wood (Dickens et al., 2007). Nevertheless, being plausible

Fig. 2. Pyrolysis GC-MS chromatogram of differently pretreated charcoals.

Fig. 3. Composition of charcoal subjected to different pretreatments and SOM.

markers for charcoal, a significant presence of these compounds was not attributed to the adsorption of younger organic material and was considered to be part of the original charcoal fraction. Nevertheless, as BPCAs are the result of oxidative degradation of aromatic structures, it is possible that a fraction of the BPCAs is the result of oxidative degradation of potentially adsorbed aromatic contaminants during the ABOx pretreatment.

3.3.2. Polycyclic aromatic hydrocarbons (PAHs)

PAHs (Table 2, compound 34) are generally considered to be compounds formed during the combustion of organic material to charcoal (Rumpel et al., 2007). However, Song & Peng (2010) found only 0.94% of ≥ 3 ring PAHs in wood charcoal after pyrolysis-GC/MS. PAHs have been shown to adsorb to charcoal after its formation (Cornelissen et al., 2005). The main PAHs found in this study were (methyl)naphthalenes (Table 2). The origin of these methyl naphthalenes is debatable, since Sáiz-Jiménez (1994) found that methyl naphthalenes were formed after pyrolysis of unsaturated fatty acids, compounds that are potentially adsorbed (see paragraph 3.3.4). In this study, the presence of the methyl naphthalenes was attributed to the pyrolysis of adsorbed compounds, while the other PAHs were attributed to the original charcoal fraction itself.

3.3.3. Methoxybenzoic acids, methoxybenzenes and phenols (methoxy fraction)

Methoxybenzenes and methoxybenzoic acids (Table 2, compound 2,4,14,15,16,17,18,19,20,25,26,27,30,39 and 40) are derived from lignin (Challinor, 1995). The presence of phenols in the studied samples indicates the incomplete methylation of methoxybenzenes and methoxybenzoic acids after TMAH derivatization (Kaal et al., 2008a). Methoxy compounds represent possible contamination by young SOM, although caution should be taken in attributing the whole fraction of these compounds to adsorption, since i) a part of it might be the result of the incomplete charring of wood during the charcoal formation process, leaving the wooden interior intact (Kaal et al., 2008a); and ii) experimentally charred wood and peat samples have been shown to contain methoxy-benzenes and benzoic acids (Calvelo Pereira et al.,

2011; Kaal et al., 2012).

3.3.4. Fatty acids (FAs), alkanes and alkenes (aliphatics)

Fatty acids (Table 2, compound 8,12 and 37) are most likely derived from suberin, a cell wall component that together with cutin forms the cuticle of a plant (Kögel-Knabner, 2002). The presence of the FAs might therefore be an indication for the adsorption of SOM. However, Suárez-Abelenda et al. (2014) found significant enrichment of C_{16} and C_{18} fatty acids after oxidation of SOM with $K_2Cr_2O_7$. These fatty acids are the most abundant fatty acids in all the charcoal samples studied in this study, so this might not only be an artefact of the ABOx treatment but an artefact of the pyrolysis.

Repeating sets of alkenes and alkanes with increasing chain lengths were found at regular intervals in the pyrolysates of the SOM. These non-polar compounds originate from the leaf and needle surface tissues of plants where they form the cuticle together with polyesters as cutin and suberin (Kögel-Knabner, 2002).

3.3.5. N-compounds

Nitrogen containing aromatic compounds (Table 2, compound 1,3,6,10,21 and 29) were found in small quantities in the pyrolysates of all charcoal samples. Compounds containing nitrogen are possibly derived from adsorbed proteinaceous plant material (Kaal et al., 2008a,b; Xiaoli et al., 2008), that might have been transported through the soil (Kalblitz et al., 2000). However, N-containing compounds were also produced in situ in weakly charred plant material (Calvelo Pereira et al., 2011). The abundant pyrroles, indoles and pyridines in all charcoal samples (Table 2) are likely to be indicative of N-containing groups produced in black carbon (Suárez-Abelenda et al., 2014). The whole fraction of N compounds thus cannot be attributed to either the charcoal fraction or adsorbed SOM; however, a high contribution of these compounds might be an indication for the adsorption of younger organic material after the formation of the charcoal.

3.3.6. C₄/C₅-diacids

The C₄/C₅-diacids consist of different compounds present in both charcoal and soil samples. Charcoal samples contained only C₄-diacids

as butanedioic acid and 2-butenedioic acid, while the SOM also contained C₅-diacids as pentanedioic acid. Similarly to Kaal et al. (2008a), the C₄/C₅-diacids are not considered part of the charcoal fraction and most likely are adsorbed fractions of mobile younger SOM (Kalblitz et al., 2000).

3.4. Pretreatment removal efficiencies

3.4.1. No treatment (control)

The untreated (NT) charcoal showed a large amount of individual compounds (Fig. 2). The relative abundance of possibly adsorbed FA's, N compounds and methoxy fractions reached values of 19.9%, 20.0% and 23.4% respectively (Fig. 3). BPCAs and PAHs accounted for 19.0% and 10.7% of the total composition. The PAHs consisted mainly of methylnaphthalenes. As mentioned in paragraph 3.2.1., it has been shown that methylnaphthalenes can form after the pyrolysis of unsaturated fatty acids. Hence, the fraction of PAHs is not necessarily completely derived from the charcoal itself. The cumulative fraction of possibly adsorbed compounds exceeds the 'charcoal' fraction, indicating massive adsorption. However, the large number of compounds resulted in convolution of several peaks in the Py-GC-MS chromatogram, leading to uncertainties in the identification. An example of this is shown in Fig. 2, where compounds 1 and 2, markers for lignin and charcoal, respectively (Table 2), are represented by the same convoluted peak.

3.4.2. ABA

The ABA treated charcoal contained less compounds than the untreated charcoal (Fig. 2), which can be attributed to the removal of adsorbed compounds by the ABA procedure. Acid treatment has shown to desorb potentially adsorbed material, while the base treatment results in the breakdown of covalent bonds as result of the hydroxyl group (Rebolto et al., 2008). Removal of compounds from the charcoal sample resulted in less convolution of different peaks in the Py-GC-MS chromatogram and less uncertainty in identification. Thus, the ABA pretreatment resulted in an improved analysability of the Py-GC-MS chromatogram. A simplified Py-GC-MS chromatogram is indicative of a purer charcoal composition that should have a better interpretable age.

The ABA treatment resulted in a higher relative contribution of BPCAs, indicative of the charcoal fraction, than in the untreated charcoal (Fig. 3). In addition, ABA treatment resulted in a lower contribution of PAHs than in the untreated charcoal (Fig. 3), probably as result of the removal of adsorbed fatty acids preventing the pyrolysis artefact mentioned in paragraph 3.3.4. Relative contributions of N compounds, FAs and C₄/C₅-diacids were lower than for the untreated charcoal, indicating removal of these potentially adsorbed compounds (Fig. 3).

3.4.3. ABOx

The composition of the ABOx treated charcoal is heavily dominated by BPCAs, representing the 'initial charcoal' fraction (Fig. 3). However, oxidation with K₂Cr₂O₇ is not a straightforward process resulting in the direct removal of labile fraction of SOM. This oxidation step in the ABOx treatment possibly resulted in the formation of BPCA compounds from adsorbed (polycyclic) aromatic hydrocarbons including methoxy compounds (Glaser et al., 1998; Knicker et al., 2007). This would result in an overestimation of the BPCA-content and thus the 'charcoal' fraction. However, Ascough et al. (2011b) have shown that oxidation by K₂Cr₂O₇ results in the removal of small potentially adsorbed aromatic structures, while a minor fraction of highly aromatic and resistant carbon remains. Oxidation with K₂Cr₂O₇ has also shown to remove fractions associated with the 'initial charcoal' (Knicker et al., 2007). Furthermore, the large mass loss of ABOx treated charcoal (Table 1) indicates loss of material rather than transformation of contaminants into a 'charcoal fraction'. In addition, ABOx treatment showed the highest overall 'contaminant' removal, with significantly less aliphatics, methoxy and N-compounds present than after the ABA treatment

(Fig. 3). Furthermore, the ABOx treated charcoal contained more PAHs that are more likely to be part of the actual charcoal instead of methylnaphthalenes (Table 2). Hence, it can be concluded that ABOx treatment results in a higher contaminant removal than ABA treatment.

3.4.4. ASE

The abundance of BPCAs in the ASE treated charcoal was similar to the untreated charcoal (Fig. 3). On the other hand, the relative contribution of PAHs was significantly higher. These PAHs consisted mainly of methylnaphthalenes, indicating a low removal of aliphatic compounds. The large fraction of phenolic compounds (Fig. 3) denotes the occurrence of incomplete methylation of the charcoal sample (Kaal et al., 2008a). This might obscure the presence of charcoal marker BPCA in the ASE treated sample, that is only analysable in its methylated state. In conclusion, ASE was unable to remove FAs and other possibly adsorbed fractions from charcoal. Hence, the 'charcoal' fraction is the lowest of all pretreatments.

3.4.5. TO375

TO-375 treatment resulted in charcoal composed of significantly less compounds compared to the ABA and ASE treatments, but similar to ABOx treatment (Fig. 2). The TO-375 treated charcoal Py-GC-MS chromatogram is dominated by two compound groups, namely BPCAs and methoxy compounds (Fig. 3). The large contribution of BPCAs indicates a large charcoal fraction. The large methoxy fraction might be the result of the inability of the method to remove these compounds. However, incomplete charring of wood during the charcoal formation could also explain the high lignin-derived methoxy fractions. In this case, combustion at 375 °C would result in the removal of the outer part of the charcoal fragment, leaving the residual inner lignin-dominated part, which is not adsorbed and obscures the actual pretreatment removal efficiency. In addition, the TO-375 thermal oxidation could have resulted in the formation of new BPCAs and methoxy compounds, similar to the ABOx method.

The low fractions of FAs, N-compounds and C₄/C₅-diacids indicate a strong removal of these possibly adsorbed compound groups by the TO-375 method. The PAHs, however, are still dominated by methylnaphthalenes.

3.5. SOM composition

The SOM showed a high relative abundance of sets of aliphatic compounds that were not present in the charcoal samples and thus have not been adsorbed (Fig. 3). This is likely the result of their low mobility in the soil. In contrast, methoxy compounds, markers for lignin, abound. Both fractions indicate a high input of plant decay material. The fraction of BPCAs, markers for black carbon in the soil matrix, is extremely low (Fig. 3). Consequently, the conclusion is justified that the abundance of such compounds in the analysed charcoal can indeed be attributed as originating from the charcoal itself. Several specific compounds like di- and trimethoxybenzenes and methoxybenzoic acids (Table 2), possibly adsorbed to the (untreated) charcoal samples, were present in SOM. This indicates that these soil layers are indeed a possible source of adsorbed younger organic material that may compromise reliable radiocarbon dating.

Fig. 4 illustrates i) the presence of similar compounds in the SOM and the charcoal; ii) the removal efficiency of the pretreatments. Fig. 4a–d shows part of the Py-GC-MS chromatograms of the (treated) charcoals and SOM. 3,4-Dimethoxybenzoic acid (Table 2, compound 26), a putative lignin derived compound (blue arrow), is clearly present in the SOM (Fig. 4d). The untreated charcoal showed a low contribution of 1,3-benzenedicarboxylic acid (Table 2, compound 23), a putative charcoal marker (red arrow), while the possibly adsorbed lignin compound was, amongst others, also present (Fig. 4a). ABA treatment (Fig. 4b) resulted in the removal of adsorbed compounds, leading to a higher relative contribution of the putative charcoal marker.

Fig. 4. Part of Py-GC-MS total ion chromatogram of a) untreated charcoal; b) ABA treated charcoal; c) ABOx treated charcoal; d) SOM. The red arrow indicates 1,3-benzendicarboxylic acid, a marker for charcoal. The blue arrow indicates 3,4-dimethoxybenzoic acid, a lignin derived compound, which is potentially adsorbed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Nevertheless, the lignin marker was still present. ABOx treatment (Fig. 4c) resulted in a high relative contribution of the charcoal marker, while the lignin compound was no longer present.

4. Conclusions

Molecular analysis of charcoal from a podzol by pyrolysis-GC-MS showed that it is possible to distinguish potentially adsorbed fractions of younger SOM that can influence radiocarbon dating from the original charcoal fraction. Furthermore, we were able to link these specific adsorbed fractions to the SOM composition of the sampled soil fractions.

The charcoal showed significant differences in composition between differently pretreated samples. ABA treatment resulted in significant removal of potentially adsorbed fractions compared to the non-treated charcoal. However, the ABOx pretreatment showed the simplest Py-GC-MS chromatogram, indicating a more pure charcoal that should have a better interpretable age. Nevertheless, caution should be taken in ascribing the complete simplification of the Py-GC-MS chromatogram to the removal of contaminants, since the oxidation step has been shown to be responsible for the transformation of non-charcoal material into what appears to be ‘charcoal-material’.

To overcome future problems with incorrect radiocarbon ages of charcoal fragments coming from podzols, proper pretreatment is required. Because of the strong adsorption of mobile younger SOM, the harsh ABOx pretreatment is likely to be the best pretreatment method for the removal of SOM from charcoal fragments originating from these

soils, since this harsher treatment is more likely to come closer to isolating recalcitrant charcoal carbon.

When linking differences in molecular composition to radiocarbon age differences in future studies, it would be beneficial to perform the different treatments on one single charcoal fragment with a known age. We also recommend testing the effectiveness of the ABOx pretreatment for charcoal samples with an age known to be significantly lower than the current ABA/ABOx boundary of $\pm 20,000$ ^{14}C yrs BP, since ABOx might provide stronger decontamination resulting in more reliable radiocarbon ages for this samples, compared to treatment by ABA. Additionally, ages of the different fractions that are being removed by the various treatment steps could be analysed in order to gain a more detailed understanding of what fraction is removed by which treatment step. This way, the different treatment steps could be optimized for the removal of specific fractions of contaminants. Pyrolysis GC–MS analysis of charcoal fragments also allows to test the effectiveness of newly proposed charcoal pretreatment methods.

Acknowledgements

The authors thank J. Westerveld, C. Cerli, R. Helmus and L. Hoitinga for their laboratory assistance. E. van Hees is thanked for microscopic inspection of the charcoal. F. Braadbaart is thanked for performing the reflectance analysis. J. van Arkel is thanked for help with the graphical illustrations. GNR is thanked for their permission to perform research in the Laarder Wasmeren area. Three reviewers are thanked for their constructive comments. This study was funded by the University of

Amsterdam.

References

- Alon, D., Mintz, G., Cohen, I., Weiner, S., Boaretto, E., 2002. The use of Raman spectroscopy to monitor the removal of humic substances from charcoal: quality control for ^{14}C dating of charcoal. *Radiocarbon* 44, 1–11.
- Ascough, P., Bird, M., Meredith, W., Wood, R., Snape, C., Brock, F., Higham, T., Large, D., Apperley, D., 2010. Hydropyrolysis: implications for radiocarbon pretreatment and characterization of black carbon. *Radiocarbon* 52, 1336–1350.
- Ascough, P.L., Bird, M.I., Francis, S.M., Lebl, T., 2011a. Alkali extraction of archaeological and geological charcoal: evidence for diagenetic degradation and formation of humic acids. *J. Archaeol. Sci.* 38, 69–78.
- Ascough, P.L., Bird, M.I., Francis, S.M., Thornton, B., Midwood, A.J., Scott, A.C., Apperley, D., 2011b. Variability in oxidative degradation of charcoal: influence of production conditions and environmental exposure. *Geochimica Cosmochimica Acta* 75, 2361–2378.
- Bandowe, B.A.M., Shukurov, N., Kersten, M., Wilcke, W., 2010. Polycyclic aromatic hydrocarbons (PAHs) and their oxygen-containing derivatives (OPAHs) in soils from the Angren industrial area. *Uzb. Environ. Pollut.* 158, 2888–2899.
- Baumann, K., Sanaullah, M., Chabbi, A., Dignac, M., Bardoux, G., Steffens, M., Kögel-Knabner, I., Rumpel, C., 2013. Changes in litter chemistry and soil lignin signature during decomposition and stabilisation of ^{13}C labelled wheat roots in three subsoil horizons. *Soil Biol. Biochem.* 67, 55–61.
- Bird, M., Ayliffe, L., Fifield, L., Turney, C., Cresswell, R., Barrows, T., David, B., 1999. Radiocarbon dating of "old" charcoal using a wet oxidation, stepped-combustion procedure. *Radiocarbon* 41, 127–140.
- Bird, M.I., Levchenko, V., Ascough, P.L., Meredith, W., Wurster, C.M., Williams, A., Tilston, E.L., Snape, C.E., Apperley, D.C., 2014. The efficiency of charcoal decontamination for radiocarbon dating by three pretreatments—ABOX, ABA and hypy. *Quat. Geochronol.* 22, 25–32.
- Braadbaart, F., Poole, I., 2008. Morphological, chemical and physical changes during charcoalification of wood and its relevance to archaeological contexts. *J. Archaeol. Sci.* 35, 2434–2445.
- Braadbaart, F., Poole, I., van Brussel, A.A., 2009. Preservation potential of charcoal in alkaline environments: an experimental approach and implication for the archaeological record. *J. Archaeological Sci.* 36, 1672–1679.
- Brock, F., Higham, T., Ditchfield, P., Bronk Ramsey, C., 2010. Current pretreatment methods for AMS radiocarbon dating at the oxford radiocarbon accelerator unit (ORAU). *Radiocarbon* 52, 103–112.
- Calvelo Pereira, R., Kaal, J., Arbestain, M., Lorenzo, R., Aitkenhead, W., Hedley, M., Macías, F., Hindmarsh, J., Maciá-Agulló, J., 2011. Contribution to characterisation of biochar to estimate the labile fraction of carbon. *Org. Geochem* 42, 1331–1342.
- Challinor, J., 1995. Characterisation of wood by pyrolysis derivatisation—gas chromatography/mass spectrometry. *J. Anal. Appl. Pyrolysis* 35, 93–107.
- Clifford, D.J., Carson, D.M., McKinney, D.E., Bortiatynski, J.M., Hatcher, P.G., 1995. A new rapid technique for the characterization of lignin in vascular plants: thermochemolysis with tetramethylammonium hydroxide (TMAH). *Org. Geochem* 23, 169–175.
- Cornelissen, G., Gustafsson, Ö., Bucheli, T.D., Jonker, M.T., Koelmans, A.A., van Noort, P.C., 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environ. Sci. Technol.* 39, 6881–6895.
- de la Rosa Arranz, J., González-Vila, F.J., Lopez-Capel, E., Manning, D., Knicker, H., González-Pérez, J., 2009. Structural properties of non-combustion-derived refractory organic matter which interfere with BC quantification. *J. Anal. Appl. Pyrolysis* 85, 399–407.
- Dickens, A.F., Gudeman, J.A., Gélinas, Y., Baldock, J.A., Tinner, W., Hu, F.S., Hedges, J.I., 2007. Sources and distribution of CuO-derived benzene carboxylic acids in soils and sediments. *Org. Geochem.* 38, 1256–1276.
- Elmqvist, M., Gustafsson, Ö., Andersson, P., 2004. Quantification of sedimentary black carbon using the chemothermal oxidation method: an evaluation of ex situ pretreatments and standard additions approaches. *Limnol. Oceanogr. Methods* 2, 417–427.
- Fabbri, D., Helleur, R., 1999. Characterization of the tetramethylammonium hydroxide thermochemolysis products of carbohydrates. *J. Anal. Appl. Pyrolysis* 49, 277–293.
- Gabor, R.S., Eilers, K., McKnight, D.M., Fierer, N., Anderson, S.P., 2014. From the litter layer to the saprolite: chemical changes in water-soluble soil organic matter and their correlation to microbial community composition. *Soil Biol. Biochem.* 68, 166–176.
- Galletti, G.C., Bocchini, P., 1995. Pyrolysis/gas chromatography/mass spectrometry of lignocellulose. *Rapid Commun. Mass Spectrom.* 9, 815–826.
- Gillespie, R., Hammond, A.P., Goh, K.M., Tonkin, P.J., Lowe, D.C., Sparka, R.J., Wallace, G., 1992. AMS dating of a late Quaternary tephra at Graham's Terrace, New Zealand. *Radiocarbon* 34, 21–27.
- Glaser, B., Haumaier, L., Guggenberger, G., Zech, W., 1998. Black carbon in soils: the use of benzenecarboxylic acids as specific markers. *Org. Geochem* 29, 811–819.
- Groenendijk, H.A., 1987. Mesolithic hearth-pits in the Veenkoloniën (prov. Groningen, The Netherlands), defining a specific use of fire in the mesolithic. *Palaeohistoria* 29, 85–102.
- Gustafsson, Ö., Haghseta, F., Chan, C., MacFarlane, J., Gschwend, P.M., 1996. Quantification of the dilute sedimentary soot phase: implications for PAH speciation and bioavailability. *Environ. Sci. Technol.* 31, 203–209.
- Haesaerts, P., Damblon, F., Nigst, P.R., Hublin, J., 2013. ABA and ABOx radiocarbon cross-dating on charcoal from middle pleniglacial loess deposits in Austria, moravia, and western Ukraine. *Radiocarbon* 55, 641–647.
- Hatté, C., Morvan, J., Noury, C., Paterne, M., 2001. Is classical acid-alkali-acid treatment responsible for contamination? An alternative proposition. *Radiocarbon* 43, 177–182.
- Higham, T., 2011. European Middle and Upper Palaeolithic radiocarbon dates are often older than they look: problems with previous dates and some remedies. *Antiquity* 85, 235–249.
- Higham, T.F., Barton, H., Turney, C.S., Barker, G., Ramsey, C.B., Brock, F., 2009. Radiocarbon dating of charcoal from tropical sequences: results from the Niah Great Cave, Sarawak, and their broader implications. *J. Quat. Sci.* 24, 189–197.
- Huang, Y., Eglinton, G., Van der Hage, E., Boon, J., Bol, R., Ineson, P., 1998. Dissolved organic matter and its parent organic matter in grass upland soil horizons studied by analytical pyrolysis techniques. *Eur. J. Soil Sci.* 49, 1–15.
- IUSS Working Group, 2014 WRB, 2014. World reference base for soil resource 2014. In: International soil classification system for naming soils and creating legends for soil maps.
- Jansen, B., Nierop, K.G., Hageman, J.A., Cleef, A.M., Verstraten, J.M., 2006. The straight-chain lipid biomarker composition of plant species responsible for the dominant biomass production along two altitudinal transects in the Ecuadorian Andes. *Org. Geochem* 37, 1514–1536.
- Kaal, J., Nierop, K.G., Kraal, P., Preston, C.M., 2012. A first step towards identification of tannin-derived black carbon: conventional pyrolysis (Py-GC-MS) and thermally assisted hydrolysis and methylation (THM-GC-MS) of charred condensed tannins. *Org. Geochem* 47, 99–108.
- Kaal, J., Rumpel, C., 2009. Can pyrolysis-GC/MS be used to estimate the degree of thermal alteration of black carbon? *Org. Geochem* 40, 1179–1187.
- Kaal, J., Brodowski, S., Baldock, J.A., Nierop, K.G., Cortizas, A.M., 2008a. Characterisation of aged black carbon using pyrolysis-GC/MS, thermally assisted hydrolysis and methylation (THM), direct and cross-polarisation ^{13}C nuclear magnetic resonance (DP/CP NMR) and the benzenepolycarboxylic acid (BPCA) method. *Org. Geochem* 39, 1415–1426.
- Kaal, J., Martínez-Cortizas, A., Nierop, K.G., Buurman, P., 2008b. A detailed pyrolysis-GC/MS analysis of a black carbon-rich acidic colluvial soil (Atlantic ranker) from NW Spain. *Appl. Geochem.* 23, 2395–2405.
- Kaiser, K., Hilgers, A., Schlaak, N., Jankowski, M., Kühn, P., Bussemer, S., Przegietka, 2009. Palaeopedological marker horizons in northern central Europe: characteristics of Lateglacial Usselo and Finow soils. *Boreas* 38, 591–609.
- Kalblitz, K., Solinger, S., Park, J., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils: a review. *Soil Sci.* 165, 277–304.
- Kisand, V., Gebhardt, S., Rullkötter, J., Simon, M., 2013. Significant bacterial transformation of riverine humic matter detected by pyrolysis GC-MS in serial chemostat experiments. *Mar. Chem.* 149, 23–31.
- Knicker, H., Müller, P., Hilscher, A., 2007. How useful is chemical oxidation with dichromate for the determination of "Black Carbon" in fire-affected soils? *Geoderma* 142, 178–196.
- Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol. Biochem.* 34, 139–162.
- Ludwig, M., Achtenhagen, J., Miltner, A., Eckhardt, K., Leinweber, P., Emmerling, C., Thiele-Bruhn, S., 2015. Microbial contribution to SOM quantity and quality in density fractions of temperate arable soils. *Soil Biol. Biochem.* 81, 311–322.
- Meredith, W., Ascough, P., Bird, M., Large, D., Snape, C., Song, J., Sun, Y., Tilston, E., 2013. Direct evidence from hydropyrolysis for the retention of long alkyl moieties in black carbon fractions isolated by acidified dichromate oxidation. *J. Anal. Appl. Pyrolysis* 103, 232–239.
- Mook, W.G., Streuman, H.J., 1983. Physical and chemical aspects of radiocarbon dating. In: *Proceedings of the First International Symposium ^{14}C and Archaeology Vol. 8* Council of Europe, Groningen.
- Nguyen, T.H., Brown, R.A., Ball, W.P., 2004. An evaluation of thermal resistance as a measure of black carbon content in diesel soot, wood char, and sediment. *Org. Geochem.* 35, 217–234.
- Nierop, K., Buurman, P., 1999. Water-soluble organic matter in incipient podzols: accumulation in B horizons or in fibres? *Eur. J. Soil Sci.* 50, 701–711.
- Nierop, K., Buurman, P., 1998. Composition of soil organic matter and its water-soluble fraction under young vegetation on drift sand, central Netherlands. *Eur. J. Soil Sci.* 49, 605–615.
- Olsson, I.U., 2009. Radiocarbon dating history: early days, questions, and problems met. *Radiocarbon* 51, 1–43.
- Rebollo, N.R., Cohen-Ofri, I., Popovitz-Biro, R., Bar-Yosef, O., Meignen, L., Goldberg, P., Weiner, S., Boaretto, E., 2008. Structural characterization of charcoal exposed to high and low pH: implications for ^{14}C sample preparation and charcoal preservation. *Radiocarbon* 50, 289–307.
- Rebollo, N.R., Weiner, S., Brock, F., Meignen, L., Goldberg, P., Belfer-Cohen, A., Bar-Yosef, O., Boaretto, E., 2011. New radiocarbon dating of the transition from the middle to the upper paleolithic in kebara cave, Israel. *J. Archaeol. Sci.* 38, 2424–2433.
- Reiß, S., Kelm, R., Bork, H.R., 2006. Economics and environmental change during the late mesolithic and neolithic periods—investigations in the valley of the gieselau near albersdorf, Schleswig-Holstein, Germany. *Environ. Archaeol.* 11, 7–17.
- Reimer, P.J., Bard, E., Bayliss, A., Beck, J.W., Blackwell, P.G., Bronk Ramsey, C., Buck, C.E., Cheng, H., Edwards, R.L., Friedrich, M., 2013. IntCal13 and Marine13 radiocarbon age calibration curves 0–50,000 years cal BP. *Radiocarbon* 55, 1869–1887.
- Richter, B.E., Jones, B.A., Ezzell, J.L., Porter, N.L., Avdalovic, N., Pohl, C., 1996. Accelerated solvent extraction: a technique for sample preparation. *Anal. Chem.* 68, 1033–1039.
- Roth, P.J., Lehdorff, E., Brodowski, S., Bornemann, L., Sanchez-García, L., Gustafsson, Ö., Amelung, W., 2012. Differentiation of charcoal, soot and diagenetic carbon in soil: method comparison and perspectives. *Org. Geochem* 46, 66–75.

- Rumpel, C., González-Pérez, J.A., Bardoux, G., Largeau, C., Gonzalez-Vila, F.J., Valentin, C., 2007. Composition and reactivity of morphologically distinct charred materials left after slash-and-burn practices in agricultural tropical soils. *Org. Geochem.* 38, 911–920.
- Sáiz-Jiménez, C., 1994. Production of alkylbenzenes and alkylnaphthalenes upon pyrolysis of unsaturated fatty acids. *Naturwissenschaften* 81, 451–453.
- Sander, M., Pignatello, J.J., 2005. Characterization of charcoal adsorption sites for aromatic compounds: insights drawn from single-solute and bi-solute competitive experiments. *Environ. Sci. Technol.* 39, 1606–1615.
- Schneider, M.P., Hilf, M., Vogt, U.F., Schmidt, M.W., 2010. The benzene polycarboxylic acid (BPCA) pattern of wood pyrolyzed between 200 C and 1000 C. *Org. Geochem.* 41, 1082–1088.
- Sevink, J., Koster, E.A., van Geel, B., Wallinga, J., 2013. Drift sands, lakes, and soils: the multiphase Holocene history of the Laarder Wasmeren area near Hilversum, The Netherlands. *Neth. J. Geosciences* 92, 243–266.
- Song, J., Peng, P., 2010. Characterisation of black carbon materials by pyrolysis–gas chromatography–mass spectrometry. *J. Anal. Appl. Pyrolysis* 87, 129–137.
- Suárez-Abelenda, M., Kaal, J., Camps-Arbestain, M., Knicker, H., Macías, F., 2014. Molecular characteristics of permanganate-and dichromate-oxidation-resistant soil organic matter from a black-C-rich colluvial soil. *Soil Res.* 52, 164–179.
- Tomiya, S., Minami, M., Nakamura, T., Mimura, K., Kagi, H., 2016. Changes of chemical structure and composition of charcoal by radiocarbon pretreatments: decontamination by ABA and ABOx treatments. *Radiocarbon* 58, 565–581.
- von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. *Soil Biol. Biochem.* 39, 2183–2207.
- Wood, R.E., Douka, K., Boscato, P., Haesaerts, P., Sinitsyn, A., Higham, T.F.G., 2012. Testing the ABOx-SC method: dating known-age charcoals associated with the Campanian Ignimbrite. *Quat. Geochronol.* 9, 16–26.
- Xiaoli, C., Shimaoka, T., Qiang, G., Youcai, Z., 2008. Characterization of humic and fulvic acids extracted from landfill by elemental composition, ¹³C CP/MAS NMR and TMAH-Py-GC/MS. *Waste Manage* 28, 896–903.
- Yizhaq, M., Mintz, G., Cohen, I., Khalaily, H., Weiner, S., Boaretto, E., 2005. Quality controlled radiocarbon dating of bones and charcoal from the early Pre-Pottery Neolithic B (PPNB) of Motza (Israel). *Radiocarbon* 47, 193–206.
- Zang, X., Brown, J.C., van Heemst, J.D., Palumbo, A., Hatcher, P.G., 2001. Characterization of amino acids and proteinaceous materials using online tetramethylammonium hydroxide (TMAH) thermochemolysis and gas chromatography–mass spectrometry technique. *J. Anal. Appl. Pyrolysis* 61, 181–193.
- Zencak, Z., Elmquist, M., Gustafsson, Ö., 2007. Quantification and radiocarbon source apportionment of black carbon in atmospheric aerosols using the CTO-375 method. *Atmos. Environ.* 41, 7895–7906.
- Zhang, X., Huang, D., Deng, H., Snape, C., Meredith, W., Zhao, Y., Du, Y., Chen, X., Sun, Y., 2015. Radiocarbon dating of charcoal from the Bianjiashan site in Hangzhou: new evidence for the lower age limit of the Liangzhu Culture. *Quat. Geochronol.* 30, 9–17.