

HYDROLYSIS: IMPLICATIONS FOR RADIOCARBON PRETREATMENT AND CHARACTERIZATION OF BLACK CARBON

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ABSTRACT. Charcoal is the result of natural and anthropogenic burning events, when biomass is exposed to elevated temperatures under conditions of restricted oxygen. This process produces a range of materials, collectively known as pyrogenic carbon, the most inert fraction of which is known as black carbon (BC). BC degrades extremely slowly and is resistant to diagenetic alteration involving the addition of exogenous carbon, making it a useful target substance for radiocarbon dating particularly of more ancient samples, where contamination issues are critical. We present results of tests using a new method for the quantification and isolation of BC, known as hydrolysis (hypy). Results show controlled reductive removal of non-BC organic components in charcoal samples, including lignocellulosic and humic material. The process is reproducible and rapid, making hypy a promising new approach not only for isolation of purified BC for ¹⁴C measurement but also in quantification of different labile and resistant sample C fractions.

INTRODUCTION

Charcoal is the product of biomass exposed to high temperatures (>300 °C) in reducing conditions (pyrolysis). Due to a global distribution and high apparent environmental recalcitrance, charcoal is one of the most common materials submitted for radiocarbon age measurement (e.g. Bird 2006). As a result of global biomass burning, charcoal is an important component of terrestrial and marine sediments. Estimates of dry biomass consumed in modern burning events are on the order of 8700 teragrams yr⁻¹ (Levine et al. 1992), while the long-term use of fire by human populations also means charcoal is prevalent in archaeological sites. The longevity of charcoal results from formation chemistry, as H/N/O/S content falls, residual plant carbon (C) is converted into highly aromatic condensed ring formations (Eckmeier et al. 2007). The most recalcitrant fraction of pyrolyzed biomass is known as black carbon (BC). BC is operationally defined as highly aromatic, with high resistance to oxidative degradation (Schmidt et al. 2001; Simpson and Hatcher 2004; Preston and Schmidt 2006). The BC aromatic structure confers high stability as aromatic ring bond strengths are ~812 kJ mol⁻¹, versus ~368 kJ mol⁻¹ in C-C bonds (McMurry 1996; Maitland et al. 2005). Estimates for the half-life of pyrolyzed carbon in soils are 5–7 kyr (Preston and Schmidt 2006), compared to mean residence times for bulk soil organic carbon of ~300 and ~2500 yr in the surface and subsoil regions, respectively (Fontaine et al. 2007).

It has become apparent that charcoal forms a continuum of materials and is not a chemically homogeneous or inert substance. Pyrolysis temperature dictates the degree of aromaticity and range of chemical forms in freshly produced charcoal (Antal and Grønli 2003; Ascough et al. 2008a). In addition, it is clear that at least some charcoal has the potential to undergo postdepositional degradation (e.g. Cohen-Ofri et al. 2006), resulting in loss of charcoal from sediments and soils over decadal and longer timescales (Bird et al. 1999a, 2002). It has also been demonstrated that depositional environment controls rates of charcoal degradation; for example, several studies suggest that degradation rates may be increased in tropical regions due to generally hot and humid conditions (e.g. Bird et al. 2002; Higham et al. 2009a). Charcoal from environmental deposits may therefore comprise a range

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of substances, including material derived from the original (unpyrolyzed) biomass, degraded components of altered charcoal, and exogenous material from the depositional environment.

A key requirement for charcoal ^{14}C pretreatment is the removal of all (contaminating) C not corresponding to the pyrolysis event, as this is the fraction most likely to have a different ^{14}C activity to that of the original biomass. Contamination sources include soil carbonates, humic acids, and microbial and lignocellulosic material. These may physically adhere to the sample structure, but may also be sorbed or bonded chemically to the sample; for example, porous charcoal structures are predisposed to sorptions of mobile organic carbon (e.g. DeLuca et al. 2006). Therefore, removal of all contamination types is necessary. Routine pretreatment involves washing sequentially with acid for sediment carbonate removal, followed by alkali to extract organic contaminants (including humic acids), and a final acid wash to remove any atmospheric CO_2 absorbed in the base step (acid-base-acid [ABA] pretreatment). This technique is relatively rapid and appears to provide robust results for a large number of samples. However, retention and analysis of material removed into solution during ABA treatment may be impractical, and in some instances the alkali step results in the virtually complete dissolution of charred plant samples (Hedges et al. 1989; Rebollo et al. 2008). Also, the ABA technique does not always remove all contaminating carbon (e.g. Goh 1979; Gillespie et al. 1992; Harkness et al. 1994; Chappell et al. 1996; Higham et al. 1998).

This lack of control over the outcome of ABA pretreatment becomes critical with increasing sample ^{14}C age, when even small quantities of exogenous carbon can have a significant impact upon the measured sample ^{14}C activity. In some ancient charcoal samples, ABA pretreatment results in erroneously young ages (e.g. Higham et al. 2009b). Unfortunately, the age range for which sample contamination issues are particularly acute (beyond ~ 30 ka BP), also relates to a timeframe of key prehistoric chronologies, e.g. the spread of modern humans in Australia and western Eurasia. Bird et al. (1999b) described an alternative pretreatment technique, replacing the final ABA acid wash with oxidation in acidified potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$ in H_2SO_4) to isolate the most resistant, highly aromatic fraction of the sample (Wolbach and Anders 1989; Bird and Gröcke 1997). This is followed by stepped combustion (typically at 300°C , 600°C and 900°C), to remove any final traces of labile C. The acid-base-oxidation-stepped combustion (ABOX-SC) method appears effective in removing contamination from older samples, resulting in charcoal ^{14}C ages that are more consistent with complementary dating evidence than the ABA method (Turney et al. 2001; Bird et al. 2003; Higham et al. 2009a,b). However, the rigorous ABOX-SC pretreatment is very harsh and results in large losses of sample material in many cases. This means that where available sample quantities are low or the charcoal is poorly preserved, insufficient material for dating may survive ABOX-SC (Brock and Higham 2008). This is due to the difficulty in reliably identifying a point during the ABOX-SC treatment when all contaminants are removed, but at which oxidation of the BC structure itself (and hence loss of the target material of interest for dating) has not yet commenced. Thus, there is a requirement of typically >100 mg of well-preserved charcoal for ABOX-SC pretreatment (Higham et al. 2009b).

Here, we report results of an alternative high-pressure hydrogen pyrolysis charcoal pretreatment technique, known as hydrolysis (hypy), which rapidly and reproducibly isolates the condensed aromatic structure of BC. Hypy uses catalytic addition of hydrogen to reductively separate labile and refractory components of samples. Previous work demonstrated the potential of hypy to separate and isolate BC within both pyrolyzed biomass and complex matrices such as soils (Ascough et al. 2008b). This identified operating conditions under which labile sample carbon was converted, but the resistant BC component was not degraded, facilitating removal of ^{14}C contamination while minimizing sample loss. Ascough et al. (2008b) noted that further research was required, including the need to

chemically characterize the solid residue and products of hypy with methodologies commonly used in BC-containing matrices (e.g. Simpson and Hatcher 2004). Here, we present the results of hypy applied to a suite of charcoal samples from environmental deposits up to ~50 ka in age. Sample hypy chemical changes are characterized, and the ^{14}C age of sample fractions isolated by hypy are compared with results following ABA and ABOX-SC pretreatment. Implications of the results for ^{14}C dating and characterization of charcoal samples from depositional environments are discussed.

METHODOLOGY

Sample Materials

Samples were selected to i) test the capacity of hypy to remove potential contaminants; ii) assess chemical changes during hypy; iii) compare the ^{14}C ages of different hypy fractions from selected samples; and iv) compare the ^{14}C ages of residue from ancient charcoal following hypy with ages for the same samples following ABA and ABOX-SC.

Samples of microcrystalline cellulose, a humic acid standard (Elliott soil; International Humic Substances Society [IHSS]) and melanoidin were used to test the capacity of hypy to remove potential contaminants. Cellulose and humic acids are regularly isolated during routine ^{14}C pretreatment techniques. Melanoidins are dark-colored heterogeneous polymers of high molecular weight produced from the Maillard reaction between sugars and amino acids, used as a proxy for organic materials likely to interfere with BC isolation (Hammes et al. 2007). The Elliott soil humic acid standard was extracted from soil under prairie vegetation where fire has been prevalent, and contains significant quantities of charcoal (6.6 g C kg⁻¹ soil) (Skjemstad et al. 2002). Solid-state ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization magic angle spinning (^{13}C -CP-SSNMR) shows the Elliott soil humic acid has a high concentration of aromatic C (~50%), relative to aliphatic C (~26%) (Thorn et al. 1989). A proportion of soil humic acids may be derived from BC via oxidation of pyrogenic carbon (e.g. charcoal) (Haumaier and Zech 1995). Therefore, the Elliott soil humic acid is likely to contain a proportion of native BC.

Chemical changes during hypy were assessed in 5 charcoal samples (MA, AZ, OH, TSB, and PF; Table 1). All of these samples were available in relatively large (>1 g) quantities, permitting multiple analyses. For MA, weight loss, %C loss, and ^{14}C measurements following hypy and ABA pretreatment are described in Ascough et al. (2008b); the results for the other samples are presented in Table 2. Charcoal samples were physically cleaned of all adhering materials, crushed to pass a 500- μm mesh and placed in 0.5M HCl made with Milli-QTM ultrapure water to remove soil carbonates, then washed 3 times in ultrapure water before drying to constant weight at 50 °C. Samples AZ and PF were also used to compare ^{14}C ages obtained after ABA and hypy pretreatment, and the age of labile material removed from the sample during hypy (termed “hypy product”) in samples likely to be of Holocene age.

Charcoal samples FUM and ESQ (Table 1) were used to compare ^{14}C ages in charcoal residue obtained after i) hypy, ii) ABA, and iii) ABOX-SC pretreatment in samples likely to be >25 ka BP. It was not possible to perform analysis of chemical changes during hypy on these samples due to the limited available sample quantities. FUM was obtained from within the one of the deepest Upper Paleolithic (Aurignacian) levels at Grotte de Fumane, north Italy (Bartolomei et al. 1992; Broglio et al. 2006; Peresani et al. 2008) and was subjected to ABA and ABOX-SC by Higham et al. (2009b). Previous charcoal ABA ^{14}C dates from this site are very inconsistent, with an indeterminate age-depth relationship and a range of ~5000 ^{14}C yr for samples collected from within a single hearth (Higham et al. 2009b). ESQ was obtained from a layer deep within the stratigraphic profile at Cueva

del Esquilleu, a Mousterian site in northern Spain (Baena et al. 2005). Previous ABA charcoal ages at ESQ range between $34,380 \pm 670$ and $49,700 \pm 1600$ ^{14}C yr BP for levels stratigraphically higher than the charcoal sample used here (Baena et al. 2005; Zilhão 2006). Charcoal was washed in 1M HCl at 80 °C for 20 min, followed by washing 3 times in ultrapure water to remove carbonates before hydrolysis.

Table 1 Details for samples analyzed in this study.

Sample	Location	Description	Reference
AZ	Faial Island, Azores	Charcoal concentration in the matrix of a lahar deposit	F Tempera, personal communication
PF	Pedra Furada, Brazil	Charcoal extracted from 1.04–1.07 m depth of sand and clay deposits in a sandstone rockshelter	Guidon and Delibrias 1985, 1986; Delibrias et al. 1988; Parenti 2001; Santos et al. 2003
OH	Oursi-hubeero, Burkina Faso	Charcoal within sand and clay deposits in an archaeological structure dating to ~AD 1050	Hallier and Petit 2000, 2001
TSB	Toca do Serrote da Bastiana, Brazil	Charcoal from deposits overlying burials dated to 200–150 yr BP, in a limestone rockshelter	N Guidon, personal communication
MA	Maninjau caldera, Sumatra	Charcoal from <i>in situ</i> charring of trees dated to ~50 ka BP	Alloway et al. 2004
ESQ	Esquilleu, northern Spain	Charcoal from a Mousterian layer (Level XIX), within an archaeological stratigraphic profile	Baena et al. 2005
FUM	Grotte de Fumane, northern Italy	Charcoal from a Proto-Aurignacian level (layer A2, structure 17) from a cave deposit	Bartolomei et al. 1992; Broglio et al. 2006; Peresani et al. 2008; Higham et al. 2009b
Cellulose	—	Pure cellulose (Sigma Aldrich)	—
Humic acid	Illinois, USA	Prairie soil, IHSS soil standard	http://ihss.gatech.edu/ihss2 ; Thorn et al. 1989
Melanoidin	—	BC ring trial sample; negative BC control	Hammes et al. 2007

Table 2 Results of elemental analysis (on an ash-free basis) on charcoal samples prior to and following hypy treatment, showing atomic O/C ratios.

Sample	Ash content	Untreated charcoal			Hypy residue		
		%C	%O	O/C	%C	%O	O/C
AZ	3.4	62.0	29.2	0.37	95.3	5.0	0.04
PF	16.5	69.8	27.4	0.35	79.3	5.9	0.07
OH	1.4	65.8	21.9	0.25	89.6	4.2	0.04
TSB	0.1	60.7	25.9	0.32	85.3	5.4	0.05
MA	0.3	78.0	9.1	0.09	86.5	3.1	0.03

Hydrolysis (hypy)

Hypy was performed using the apparatus and procedure described in Ascough et al. (2008a) (see Figure 1). Aliquots for analysis were combined with a 0.2M solution of ammonium dioxodithiomolybdate $[(\text{NH}_4)_2\text{MoO}_2\text{S}_2]$ and placed on pre-cleaned quartz wool within shortened borosilicate pipette tips in the hypy reactor. Samples were heated resistively at $300\text{ }^\circ\text{C min}^{-1}$ from 50 to 250 °C, then at $8\text{ }^\circ\text{C min}^{-1}$ to the final temperature, which was held for 120 s. Hypy was performed under a H_2 pressure of 15 MPa and sweep gas flow of 5 L min^{-1} (ATP). Hypy products were collected on silica in a dry-ice cold trap (Meredith et al. 2004). Hypy weight loss curves were produced for cellulose, humic acid, and melanoidin every 50 °C between final temperatures of 300 to 600 °C. Hypy

treatment of charcoal samples was at 600 °C, on the basis of previous work suggesting this regime enabled effective removal of trace contaminants (Ascough et al. 2008a).

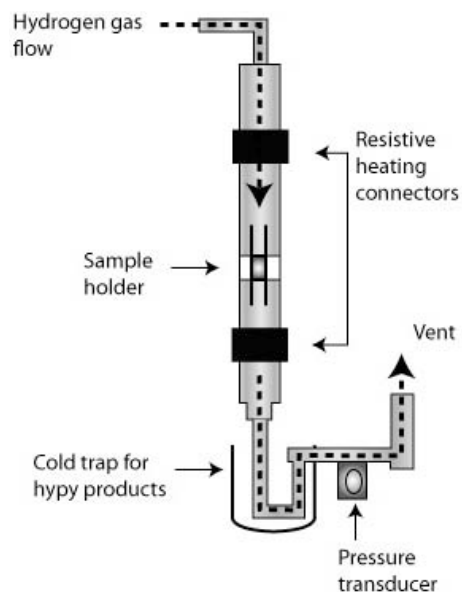


Figure 1 Schematic diagram of hydropyrolysis apparatus used for treatment of samples within this study (after Ascough et al. 2008b).

Elemental Analysis

The mineral ash content of the samples was determined by loss on ignition at 1020 °C under air, with a multiple replicate measurement precision of $\pm 0.5\%$. Charcoal elemental carbon (C) and oxygen (O) content was analyzed before and after hpy with a Costech elemental analyzer for %C and a ThermoFinnigan high-temperature conversion elemental analyzer for %O. Samples were measured in duplicate with laboratory standards and blanks; elemental abundances were calculated by comparing the gas pulse peak area to acetanilide (IAEA/Sigma Aldrich, %C: 71.09%, %O: 11.84%), with an external reproducibility of better than 0.5% for C and 0.7% for O.

^{13}C -CP-SSNMR

Solid-state ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization magic angle spinning (^{13}C -CP-SSNMR) was used to compare the C molecular environments of AZ, TSB, and PF in: i) the untreated sample; ii) following oxidation in 0.1M $\text{K}_2\text{Cr}_2\text{O}_7/2\text{M H}_2\text{SO}_4$ at 60 °C for 4 hr (as per the oxidation step of the ABOX-SC pretreatment); and iii) following hpy at 600 °C. Spectra were also obtained of AZ and PF following digestion with 1M NaOH at 60 °C for 1 hr (as per the base step of the ABA pretreatment). After oxidation or NaOH treatment, samples were washed 3 times to reach neutral pH with ultrapure water, with centrifugation between each step. The supernatant was decanted, and the residue freeze-dried prior to analysis. ^{13}C -CP-SSNMR spectra were recorded using a 4-mm MAS probe at a ^{13}C frequency of 100.56 MHz on a Varian VNMRS instrument. Samples were spun at 10 or 12 kHz with a CP contact time of 1 or 2 ms and a 1-s recycle time. Spectra were referenced to neat tetramethylsilane. A rotor-synchronized spin-echo was used to suppress a broad probe background signal.

Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) was used to analyze the combined aliphatic and aromatic compounds retained as hypy products on silica in charcoal samples AZ, TSB, OH, and MA. These analyses were undertaken to characterize and compare the non-organized, labile carbon fraction in these samples. The products analyzed from these samples were those following the hypy program used to treat charcoal samples for ^{14}C measurement in this paper (i.e. a final hold temperature of 600 °C). The aliphatic and aromatic fractions of the hypy products were separated by silica gel/alumina adsorption chromatography with successive elutions of *n*-hexane, *n*-hexane/DCM (3:2 v/v), and DCM/methanol (1:1 v/v). GC-MS analyses in full-scan mode were performed on a Varian CP-3800 gas chromatograph, interfaced to a 1200 mass spectrometer (EI mode, 70 eV). Separation was achieved on a VF-5MS fused silica capillary column (50 m \times 0.25 mm ID, 0.25 μm thickness), with helium as the carrier gas, and an oven program of 50 °C (hold for 2 min) to 300 °C (hold for 20.5 min) at 4 °C min^{-1} .

^{14}C Pretreatment and Measurement

ABA and ABOX-SC pretreatment techniques of FUM are described in Higham et al. (2009b). One aliquot each of AZ, PF, and ESQ was pretreated by ABA, comprising sequential washes with HCl and NaOH; following each wash, samples were rinsed with ultrapure water. One aliquot of ESQ was treated by ABOX-SC. ESQ and FUM were subjected to both pretreatments, while AZ and PF were only treated with the former. Specific treatment methodologies were consistent between different samples to which they were applied. To compare the ^{14}C ages of different hypy fractions from selected samples for which chemical characterizations of these fractions were available, 1 aliquot each of AZ and PF was treated with hypy at 600 °C as described above, with both the hypy residue and the hypy product collected on silica being retained for ^{14}C measurement. A ^{14}C measurement was also obtained of TSB following ABA pretreatment, in order to obtain an absolute age for this sample. To compare the ^{14}C ages of ancient charcoal residue following hypy with those following ABA and ABOX-SC, 1 aliquot each of ESQ and FUM was treated with hypy at 600 °C as described above, and the hypy residue retained for ^{14}C measurement.

ABA and ABOx-SC pretreatment techniques are described in Higham et al. (2009b) and Brock et al. (2010). FUM and ESQ were treated with both protocols, while AZ and PF were treated only with the ABA protocol.

RESULTS

Conversion of Non-Charcoal Material During Hypy

The weight loss profiles for cellulose, humic acid, and melanoidin are presented in Figure 2. Catalyst weight loss was consistent at 35% for individual runs, and sample hypy weight losses were corrected to account for remaining catalyst. Cellulose weight loss was 100% by 600 °C, with the majority of degradation occurring below 450 °C. Replicate analyses confirmed that full conversion of cellulose was achieved via hypy. Humic acid conversion was ~96% by 600 °C, where a proportion of residue weight (~1%) was due to ash content. In the melanoidin sample, 100% weight loss was achieved by 525 °C.

Elemental Analysis

Ash contents were generally <3%, with a higher content in PF. All elemental abundance values were calculated on an ash-free basis. The initial %C of the samples was 78.0% to 60.7% (Table 2). The

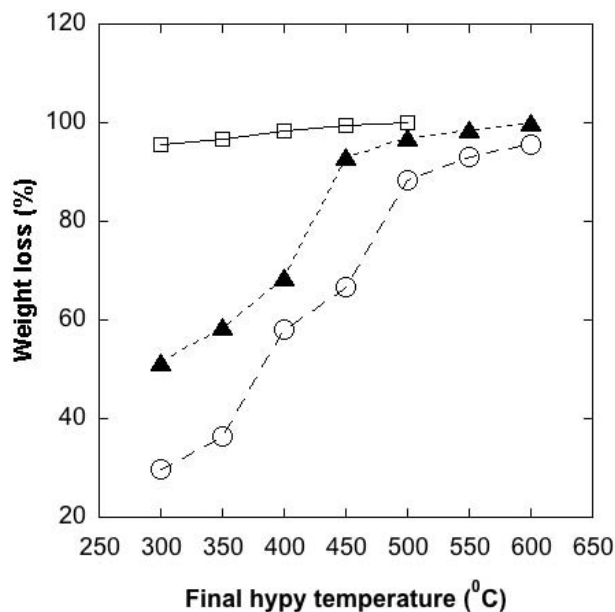


Figure 2 Weight loss profiles during hypy treatment at increasing final hold temperatures for cellulose (black triangles), humic acid (white circles) and melanoidin (white squares).

initial %O of MA (9.1%) was much lower than the other 4 charcoals (21.9% to 29.2%). Atomic O/C ratios of untreated charcoal samples ranged from 0.37 to 0.09. Following hypy, sample %C increased dramatically and the O/C ratios of all samples fell to between 0.03 and 0.07.

^{13}C -CP-SSNMR

The ^{13}C -CP-SSNMR spectra of untreated PF, AZ, and TSB (Figure 3) all contain a signal in the aromatic region (110–160 ppm), centered on ~130 ppm, indicative of aromatic ring structures in pyrogenic carbon (Simpson and Hatcher 2004). The weak signal at 110 ppm in the spectrum from PF is due to interference from the Teflon[®] rotor cap. PF gives a single aromatic signal centered on ~125 ppm, with high- and low-frequency spinning sidebands (* in Figure 3), characteristic of charcoal produced at >500 °C (Ascough et al. 2008a). NaOH and hypy treatment do not affect the spectra of this sample. After $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation, low-intensity signals appear at 170 and 190 ppm, indicating formation of carboxylic and ketone groups, respectively.

Along with aromatic C, AZ contains a large amount of alkyl-C at 10–50 ppm, and a peak at 55 ppm typical of the $-\text{OCH}_3$ group in lignin (Hatcher et al. 1989). The signal at 147 ppm (aromatic C-O) may indicate guaiacyl lignin (Kringstad and Mörck 1983), and the signal at 170 and 200 ppm demonstrates the presence of carboxyl carbon and aldehyde or ketone groups, respectively. $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation removed the 55-ppm $-\text{OCH}_3$ peak; otherwise, the spectrum was similar to the unoxidized sample, with a broad, indistinct aromatic signal suggesting carbon in a large range of aromatic structures. NaOH treatment resulted in a large mass loss (~50%) and very dark supernatant, indicating a high quantity of base-soluble material in AZ. After NaOH treatment, a high quantity of alkyl-C and the 55-ppm $-\text{OCH}_3$ peak remained. After hypy treatment, the only remaining signal was for (pyrogenic) condensed aromatic carbons at ~130 ppm. The range of condensed aromatic structures present in this sample appears lower than that after the dichromate or alkali treatment.

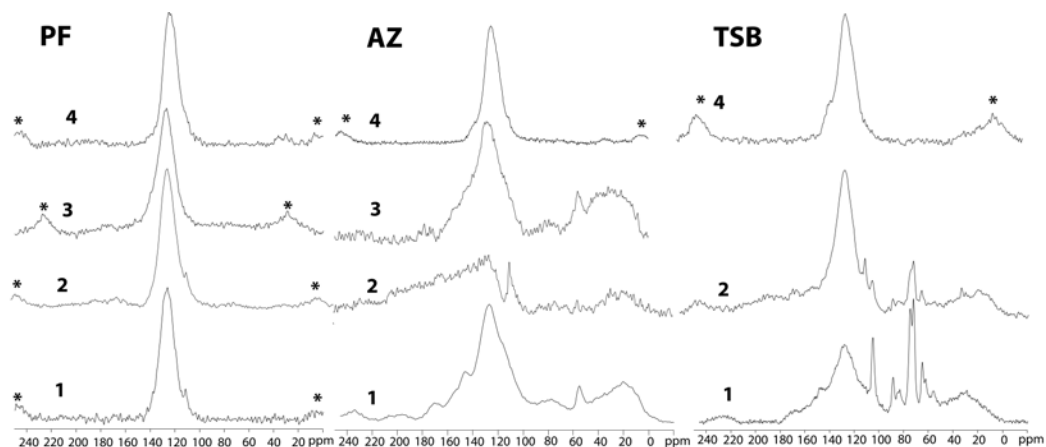


Figure 3 ^{13}C -CP-SSNMR spectra of charcoal samples (left to right: PF, AZ, TSB), 1: charcoal, 2: charcoal following 4 hr of $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation, 3: charcoal following NaOH treatment, 4: charcoal hypy treatment. Asterisks indicate spinning sidebands in the spectra of highly aromatic samples.

Along with the aromatic signal at ~ 130 ppm, TSB also contains peaks in the alkyl and O-alkyl C region (0–110 ppm). A broad signal at 10–40 ppm indicates the presence of aliphatic carbon ($-\text{CH}_3$, $>\text{CH}_2$, $>\text{CH}-$, and $>\text{C}<$), and resonances at 60–105 ppm are diagnostic for cellulose and hemicellulose. These include carbon in the glucopyranose ring (72–75 ppm), in the CH_2OH group (62 and 65 ppm), and in glycosidic bonds (~ 105 , ~ 89 , and 84 ppm) (Earl and Vanderhart 1981; Atalla and Vanderhart 1999). After $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation, overall spectral aromaticity increased, but a significant amount of alkyl-C remained in a broad, unresolved band at 10–50 ppm. Although the relative height of the cellulosic signal decreased (indicating some degradation), peaks at 60–80 and 105 ppm suggest intact pyranose rings and glycosidic linkages. In contrast, the signal range after hypy treatment is dramatically reduced, consisting of those from condensed aromatic structures centered on 127 ppm.

Gas Chromatography-Mass Spectrometry

GC-MS revealed that the hypy products consisted predominantly of a complex distribution of aromatic compounds (Table 3 and Figure 4). These were mainly polycyclic in nature and ranged from compounds comprising 1 aromatic ring (trimethylbenzene) to 7 peri-fused rings (coronene), with varying degrees of alkylation. The degree of alkyl substitution decreases with increasing size of polycyclic aromatic moieties. Comparison of products from the charcoal samples shows that the degree of alkyl substitution is higher in AZ and OH than it is in TSB and MA. For example, in AZ and OH the content of pyrene is broadly equivalent to that of methylpyrene, whereas in TSB and MA the ratio of this parent polycyclic aromatic hydrocarbon (PAH) to the alkylated PAH is much higher. It is therefore apparent from the results that GC-MS analysis of the aromatic fraction released as labile product during hypy forms a useful proxy for the non-graphitic C component in charcoal samples.

^{14}C Pretreatment ABA and ABOX Comparison Treatments

The ^{14}C measurement of TSB gave 129.7 ± 0.4 pMC, indicating this sample postdated AD 1950 (Levin and Kromer 2004) (Table 4). The ^{14}C age of AZ and PF (Table 4) following hypy treatment was within error of the same sample following standard ABA treatment (1049 ± 24 and 6500 ± 40 ^{14}C yr BP, respectively). In AZ, the ^{14}C age of the products extracted onto silica during hypy treat-

Table 3 Assignments for compounds (shown in Figure 4) identified via GC-MS of hypy products from AZ, OH, TSB, and MA.

Assignment	Compound
1	Trimethylbenzene
2	Indane
3	Naphthalene
4	Methylnaphthalene
5	Dimethylnaphthalene
6	Trimethylnaphthalene
7	Fluorene
8	Methylfluorene
9	Phenanthrene
10	Methylphenanthrene
11	Fluoranthene
12	Pyrene
13	Methylpyrene
14	Dimethylpyrene
15	Chrysene
16	Methylchrysene
17	Benzofluoranthene
18	Benzopyrene
19	Indeno(1,2,3-cd)pyrene
20	Benzo(ghi)perylene
21	Coronene

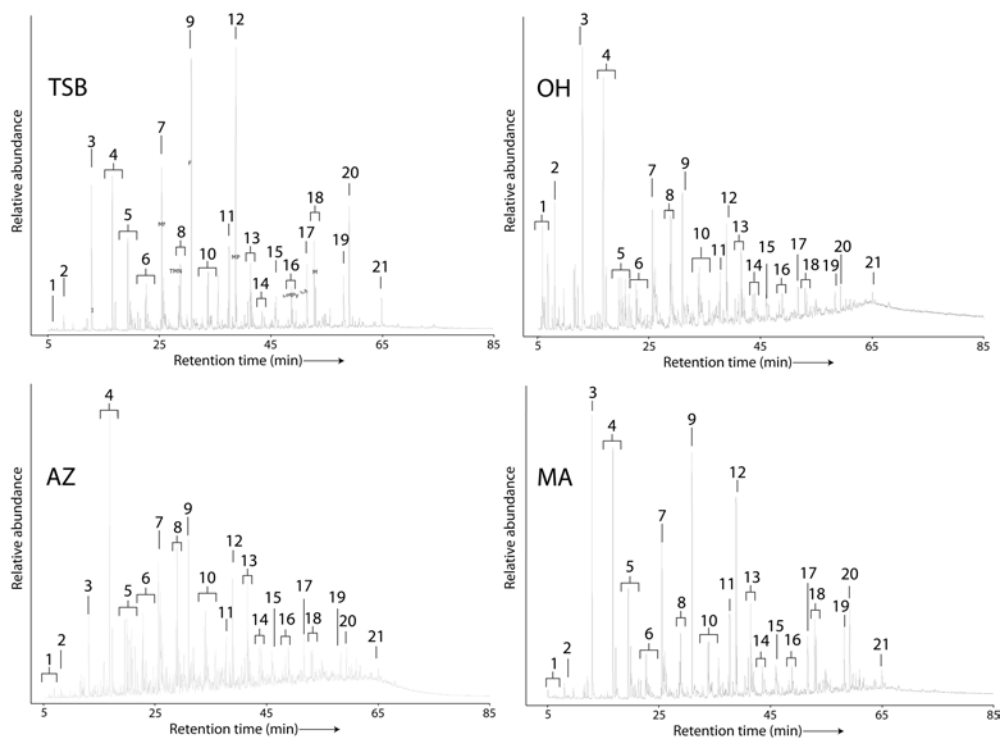


Figure 4 GC-MS chromatograms showing aromatic hydrocarbon distributions of hypy products from AZ, OH, TSB, and MA. Assignments for individual compounds numbered 1–21 are given in Table 3.

ment (1048 ± 23 ^{14}C yr BP) was within error of the hypy residue ^{14}C age. The ^{14}C age of hypy products from PF was 142 ± 48 ^{14}C yr older than the hypy residue and 151 ± 54 ^{14}C yr older than the ABA-treated charcoal.

Table 4 Results of ^{14}C measurement on charcoal samples following ABA and hypy treatment showing previous data obtained on pre-Holocene samples following ABA and ABOX-SC treatment (*Higham et al. 2009b).

Sample	Reporting nr	Treatment	^{14}C age $\pm 1 \sigma$	F $^{14}\text{C}\%$ $\pm 1 \sigma$	$\delta^{13}\text{C}$ (‰)
TSB	OxA-16026	ABA	—	129.72 ± 0.41	-26.7
PF	OxA-16028	ABA	6500 ± 40	44.52 ± 0.23	-26.6
PF	OxA-V-2268-53	Hypy (residue)	6509 ± 31	44.518 ± 0.172	-25.7
PF	OxA-V-2260-22	Hypy (product)	6651 ± 37	43.779 ± 0.209	-24.2
AZ	OxA-18945	ABA	1049 ± 24	87.767 ± 0.26	-22.6
AZ	OxA-V-2268-52	Hypy (residue)	1013 ± 23	88.154 ± 0.250	-21.4
AZ	OxA-V-2260-21	Hypy (product)	1048 ± 23	87.809 ± 0.251	-22.3
FUM	OxA-19411*	ABA	$32,530 \pm 240$	1.754 ± 0.071	-25.6
FUM	OxA-19412*	ABOX	$34,940 \pm 280$	1.3 ± 0.065	-24.2
FUM	OxA-V-2309-33	Hypy (residue)	$30,570 \pm 210$	2.377 ± 0.134	-21.7
ESQ	OxA-19085	ABA	$39,280 \pm 340$	0.763 ± 0.06	-23.5
ESQ	OxA-19086	ABOX	$>54,600$	0.077 ± 0.049	-23.0
ESQ	OxA-V-2309-41	Hypy (residue)	$45,200 \pm 1000$	0.517 ± 0.131	-22.1

ABA pretreatment of ESQ resulted in an age of $39,280 \pm 340$ ^{14}C yr BP (Table 4). The hypy-treated residue from this sample was significantly older, at $45,200 \pm 1000$ ^{14}C yr BP. After ABOX-SC treatment, ESQ yielded an infinite age ($>54,600$ ^{14}C yr BP). The ^{14}C age of FUM after hypy treatment was $30,570 \pm 210$ ^{14}C yr BP (Table 4). This is younger than the ABOX-SC ($34,940 \pm 280$ ^{14}C yr BP) and ABA ($32,530 \pm 240$ ^{14}C yr BP) age for this sample obtained by Higham et al. (2009b).

DISCUSSION

In Holocene-age samples, the ^{14}C age of the hypy residue is equivalent to the ABA-treated charcoal. This is consistent with previous results from the hypy residue of a Holocene charcoal from a Portuguese archaeological site (Ascough et al. 2008a). Contaminating C in these samples is therefore either present in insufficient quantities to significantly affect overall ^{14}C age, and/or of a chemical form that is labile in all pretreatments. In sample PF, small quantities of older contamination are removed by hypy. In this sample, the ^{14}C age of the hypy product from PF shows removal of older exogenous C. However, PF is dominantly composed of condensed aromatic C, and there are few ^{13}C -CP-SSNMR spectral changes during hypy, suggesting the older C in the hypy product comprised a minimal proportion of overall sample C, relative to the BC fraction.

Contamination that has little effect on the ^{14}C age could be a general feature of relatively young charcoal samples. For example, comparing the standard ABA and more aggressive ABOX-SC pretreatments, Higham et al. (2009a) found no significant difference in ^{14}C age in samples <25 ka BP. It is also important to consider that these results could also arise if a significant proportion of the labile C removed during charcoal pretreatment is contemporaneous with the sample BC component (i.e. relates to the same pyrolysis event). This could occur if the labile C represents degradation products of either incompletely charred initial sample lignocellulosic material (e.g. Kaal et al. 2008), or postdepositional charcoal alteration without addition of C of different ^{14}C activity. This appears to be the case in one of the samples (i.e. AZ) for which we obtained a ^{14}C age of the different hypy fractions. ^{13}C -CP-SSNMR spectra of AZ show that labile C is largely composed of humic

material (e.g. Laird et al. 2008). The ~50% mass loss during NaOH treatment shows this comprises a large proportion of the sample. GC-MS of the hypy products of AZ are dominantly aromatic, which suggests a pyrogenic origin for the humic material removed during pretreatment rather than exogenous substances of equivalent ¹⁴C age to the pyrolysis event.

These results highlight the importance of understanding the origin and variety of different chemical fractions that may exist within charcoal samples. An advantage of hypy in this regard is the ability to separate and retain different sample fractions for analysis, both in terms of ¹⁴C measurement and chemical characterization. The latter includes the ability to reproducibly quantify labile versus resistant (i.e. BC) sample C (e.g. Ascough et al. 2008b).

The results demonstrate that hypy treatment results in complete removal of cellulose and melanoidin by ~550 °C. Likewise, the vast majority of the Elliott soil humic acid is removed under these conditions, despite the highly aromatic nature of this sample. This is encouraging as it demonstrates that hypy removes aromatic chemical forms that do not consist of the condensed aromatic configurations of native BC. Furthermore, measured O/C atomic ratios and ¹³C-CP-SSNMR spectra prove that hypy facilitates removal of oxygen-containing functional groups and all non-aromatic C in charcoal samples (Table 2 and Figure 3). This is achieved in samples containing cellulosic carbon (e.g. TSB) and humic material (e.g. AZ). The lack of aliphatic-derived compounds identified via GC-MS of the hypy products from TSB indicates that cellulosic material is readily degraded during hypy and removed in gaseous form. In contrast, a significant proportion of non-aromatic carbon evidently survives the K₂Cr₂O₇ (ABOX) oxidation for at least 4 hr. This highlights the requirement for extended oxidation periods (e.g. ~20 hr, cf. Higham et al. 2009a) during application of the ABOX-SC technique. Cellulosic structures are chemically susceptible to such harsh oxidation; therefore, the persistence of this material may indicate protection from the K₂Cr₂O₇ solution. This may occur physically (e.g. in pore spaces), as non-BC biomass fractions can survive several hours of K₂Cr₂O₇ oxidation due to hydrophobicity (Knicker et al. 2007). Alternatively, this protection may arise chemically, by complexing with more resistant fractions such as lignin (Jin et al. 2006). Such mechanisms may also be responsible for the persistence of a substantial proportion of aliphatic alkyl-C within sample AZ following NaOH treatment, despite the increased proportion of condensed aromatic carbon. Hypy therefore seems to effectively degrade materials commonly targeted for isolation in ¹⁴C pretreatment at the tested operating conditions. Despite large differences in the chemistry of initial charcoal samples, hypy appears to isolate a highly consistent, condensed aromatic fraction in a range of samples.

The results indicate the potential advantages in further adaptation of the hypy program in order to remove possible fine exogenous BC contamination from charcoal samples. A small proportion of the Elliott humic acid sample is likely to consist of an original BC component introduced by repeated burning of the prairie soil; in such soils, a proportion of extractable humic acids appears to be derived from pyrogenic carbon (Haumier and Zech 1995). Depolymerization and surface oxidation of charcoal during diagenesis produces oxidized, weakly condensed aromatic structures, which appear chemically similar to highly aromatic humic substances extracted from soils (Haumier and Zech 1995; Kramer et al. 2004; Knicker et al. 2006; Knicker 2007; Kaal et al. 2008). Therefore, the ~3% highly resistant component in the Elliott soil sample is likely to represent a native BC component present in the humic acid itself. Such components are likely to comprise a very small component of bulk organic carbon in soils and sediments; however, they have the potential to influence charcoal ¹⁴C ages, particularly in more ancient samples. This is important as such a component is likely to be chemically highly resistant and structurally similar to the BC target material of interest in the charcoal samples, making isolation in pretreatment difficult. The thermal stability of BC materials increases in proportion to the size of polyaromatic domains and molecular weight (Venka-

taraman and Friedlander 1994). This means that removal of forms of exogenous BC may be possible if pretreatment were targeted to result in preferential loss of more weakly aromatized chemical forms, and those consisting of a smaller number of aromatic rings.

The hypy regime selected in this work (600 °C with hold time of 2 min) was chosen in order to optimize BC recovery while removing maximum amounts of C contamination. This results in loss of a small quantity of sample BC (Ascough et al. 2008b); however, a more aggressive regime may be required in older samples where contamination by exogenous BC is a problem. For example, increasing the hypy hold time at final temperature would result in a more aggressive treatment with the aim of preferentially removing smaller condensed aromatic domains, as in the combustion step of the ABOX-SC treatment. This could be a desirable approach, for example, in samples containing BC components derived from humic substances in soils with high native charcoal contents.

In the 2 charcoal samples >25 ka BP tested here, the results suggest that this material contains separate C fractions with significantly different ^{14}C activity. The fraction isolated by ABOX-SC in both ESQ and FUM has the lowest ^{14}C activity in both instances. Previous work shows that the combustion steps at 330 °C and above removes C contamination not isolated via the $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation (Bird et al. 1999b; Turney et al. 2001). In light of the above discussion, it is possible that sample polycyclic aromatic BC compounds of differing thermal stability have significantly different ^{14}C ages, and a key factor in pretreatment of such samples is removal of this proportion of the sample BC component. Although a ^{14}C age is not available for the hypy products (i.e. the labile C fraction) from samples FUM and ESQ, in ESQ the ^{14}C activity of material isolated by hypy is significantly older than that isolated by ABA. Both results presented here (e.g. consistency of elemental composition and ^{13}C -CP-SSNMR spectra of hypy residues from different charcoal samples) and previous work has established that hypy is a highly reproducible method (Ascough et al. 2008b). Therefore, the material isolated by hypy in ESQ and FUM is likely to be chemically similar. However, in FUM the ^{14}C activity of this fraction is higher than that of the fraction isolated by ABA. This highlights the fact that similar chemical components can have very different ^{14}C activities relative to overall sample ^{14}C activity. Such features also extend to the ABOX-SC method in certain instances; for example, in the Niah Cave sequence, ABOX-SC of charcoal from context 2079 (~21 ka BP) resulted in a younger ^{14}C age than either the untreated or ABA-treated charcoal (Higham et al. 2009a). However, in the remainder of the sequence, ABOX-SC returned the oldest ^{14}C ages. This highlights a key issue in ^{14}C measurement of ancient charcoal samples, where small amounts of carbon with a varying ^{14}C activity can have a large impact on measured sample age.

Hypy offers specific practical methodological advantages in pretreatment of charcoal samples for ^{14}C measurement, notably rapidity of processing and minimization of sample losses in pretreatment. The results suggest that hypy is more rapid and effective in removing non-BC sample components than even aggressive oxidation regimes. Following treatment, the sample hypy residue is suitable for direct combustion to CO_2 , with no further isolation steps required, representing a considerable advantage when processing samples rapidly degraded during conventional pretreatment. The results also highlight the ability to readily characterize different sample components via analysis of different hypy fractions, providing valuable information on the composition of labile versus resistant carbon in charcoal samples. For example, GC-MS analysis of hypy products suggests larger polyaromatic domains exist within samples MA and TSB, relative to AZ and OH. This may be related either to pyrolysis conditions (i.e. temperature) or be indicative of postdepositional degradation of the charcoal/BC structure. Improved pretreatment of charcoal samples close to the ^{14}C dating limit requires a better understanding of the chemistry of different potential environmental sources of contaminating ^{14}C , and the mechanisms by which different pretreatment methods remove or alter these carbon

forms. Such understanding will enable improvements in specificity of pretreatment techniques, and hence significant improvements in accuracy of charcoal ¹⁴C age measurement in key areas.

CONCLUSIONS

The results of this study support the interpretation that hypy is able to reproducibly and rapidly isolate the most resistant BC fraction from carbonaceous samples. A consistent highly aromatic component is isolated in charcoal with different starting compositions, including samples containing significant quantities of cellulosic and humic material. It is also apparent that hypy is able to effectively degrade compound classes routinely targeted for isolation in ¹⁴C pretreatment. In samples of Holocene age, the ¹⁴C age of solid charcoal residue isolated by hypy is equivalent to that of standard pretreatment procedures. In samples >30 ka BP, comparison of charcoal ¹⁴C ages following ABOX-SC, ABA, and hypy treatment highlights the importance of recognizing a continuum in the chemistry of different charcoal samples, and potential sources of ¹⁴C contamination that relates both to production conditions and depositional environment.

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