Characterization, Quantification and Compound-specific Isotopic Analysis of Pyrogenic Carbon Using Benzene Polycarboxylic Acids (BPCA)

Daniel B. Wiedemeier1, Susan Q. Lang2, Merle Gierga3, Samuel Abiven1, Stefano M. Bernasconi3, Gretchen L. Früh-Green5, Irka Hajdas4, Ulrich M. Hanke1, Michael D. Hilf1, Cameron P. McIntyre4, Maximilian P. W. Scheider1, Rienk H. Smittenberg1, Lukas Wacker4, Guido L. B. Wiesenberg1, Michael W. I. Schmidt1

1Department of Geography, University of Zurich
2Department of Earth and Ocean Sciences, University of South Carolina
3Department of Earth Sciences, ETH Zurich
4Laboratory of Ion Beam Physics, ETH Zurich
5Department of Geological Sciences, Stockholm University

Correspondence to: Daniel B. Wiedemeier at daniel.wiedemeier@uzh.ch

URL: http://www.jove.com/video/53922
DOI: doi:10.3791/53922

Keywords: Chemistry, Issue 111, Black carbon, fire-derived organic matter, molecular marker, compound-specific isotopic analysis, 14C age, 13C signature, biochar, global carbon cycle, char, pyrogenic carbon, charcoal

Date Published: 5/16/2016

Citation: Wiedemeier, D.B., Lang, S.Q., Gierga, M., Abiven, S., Bernasconi, S.M., Früh-Green, G.L., Hajdas, I., Hanke, U.M., Hilf, M.D., McIntyre, C.P., Scheider, M.P.W., Smittenberg, R.H., Wacker, L., Wiesenberg, G.L.B., Schmidt, M.W.I. Characterization, Quantification and Compound-specific Isotopic Analysis of Pyrogenic Carbon Using Benzene Polycarboxylic Acids (BPCA). J. Vis. Exp. (111), e53922, doi:10.3791/53922 (2016).

Abstract

Fire-derived, pyrogenic carbon (PyC), sometimes called black carbon (BC), is the carbonaceous solid residue of biomass and fossil fuel combustion, such as char and soot. PyC is ubiquitous in the environment due to its long persistence, and its abundance might even increase with the projected increase in global wildfire activity and the continued burning of fossil fuel. PyC is also increasingly produced from the industrial pyrolysis of organic wastes, which yields charred soil amendments (biochar). Moreover, the emergence of nanotechnology may also result in the release of PyC-like compounds to the environment. It is thus a high priority to reliably detect, characterize and quantify these charred materials in order to investigate their environmental properties and to understand their role in the carbon cycle.

Here, we present the benzene polycarboxylic acid (BPCA) method, which allows the simultaneous assessment of PyC's characteristics, quantity and isotopic composition (13C and 14C) on a molecular level. The method is applicable to a very wide range of environmental sample materials and detects PyC over a broad range of the combustion continuum, i.e., it is sensitive to slightly charred biomass as well as high temperature chars and soot. The BPCA protocol presented here is simple to employ, highly reproducible, as well as easily extendable and modifiable to specific requirements. It thus provides a versatile tool for the investigation of PyC in various disciplines, ranging from archeology and environmental forensics to biochar and carbon cycling research.

Introduction

In a complete combustion process, biomass or fossil fuel is converted into CO2, H2O and inorganic residues (ash). However, under local or temporal oxygen limitations, combustion becomes incomplete and pyrolysis takes place, producing a solid organic residue known as char1. These charred residues are also referred to as pyrogenic organic matter (PyOM) and mainly consist of pyrogenic carbon (PyC) or, synonymously, black carbon (BC)2-4. Charring processes are omnipresent and can be part of both natural and anthropogenic combustion1-5,6. Wildfire is an important natural process, intrinsic to most ecosystems, which produces a significant quantity of PyC each year7,10. Similarly, the burning of fossil fuel for energy production in industry and transport presents an important anthropogenic source of PyC11-13. Both sources contribute to the ubiquity of PyC in the environment; PyC is present in the air, in the form of aerosols13-14, in water as particulate or dissolved organic matter2,5-11, as well as in ice cores15-19, soils20-21, and sediments22-24 in sizes varying from m to nm (e.g., large charred tree trunk after a forest fire or nano-scale soot particles that escape a diesel engine exhaust). The ubiquity of PyC in the environment is not only due to large production rates but also to its long persistence and relative stability against degradation25-26. Although exact turnover times have not yet been established and may depend upon specific environmental conditions27-28, it seems clear that PyC is less readily decomposed into CO2 than most other forms of organic carbon29-30. This observation has an important implication for the global C cycle: as charred materials store PyC for a relatively long time, they sequester C in organic forms that would otherwise be rapidly respired as CO2, thus reducing atmospheric greenhouse gas concentrations over time29-32.
Besides the climate mitigating aspect, chars have further environmentally relevant properties. Their high porosity, large surface area and negative surface charge can immobilize hazardous compounds\(^{37}\) and improve soil fertility\(^{44-35}\). The recognition of chars as a potentially beneficial soil amendment led to the emerging field of so-called biochar technology\(^{46}\). Biochar will likely be produced on large scales in the coming years and thus significantly increase PyC abundance in soils\(^{37}\). Moreover, the occurrence of wildfires and the burning of fossil fuels are also projected to remain high over the course of the 21\(^{st}\) century, continuously contributing large quantities of PyC to the environment\(^{1,38-39}\). Another increasingly important source of PyC is likely to be nanotechnology that also uses PyC-like compounds\(^{40-44}\). It is thus crucial to detect, characterize and quantify these pyrogenic materials accurately in order to investigate their properties and understand their role in the environment.

Here, we present the use of a state-of-the-art compound-specific approach to analyze PyC in various samples: the most recent generation of the benzene polycarboxylic acid (BPCA) method\(^{42}\). This method is broadly applicable within PyC research as it directly targets the "backbone" of PyC: its polycyclic condensed structures that form during the thermal treatment\(^{43-45}\) and that are therefore inherent to all the various forms of PyC\(^{5-46}\). However, these structures are not directly assessable by chromatographic means, due to their size and heterogeneity. In order to chromatographically analyze such pyrogenic compounds, PyC is first digested with nitric acid under high temperature and pressure, which breaks the large polycyclic structures down into its building blocks, the individual BPCAs\(^{47}\). The BPCAs are then, after a few purification steps, amenable to chromatographic analysis\(^{48,42}\). PyC is thus isolated and analyzed on a molecular level and can be used to quantify PyC abundance in environmental compartments\(^{39-42}\). The BPCA method additionally characterizes the investigated PyC when relative yields of B3-, B4-, B5- and B6CA are compared\(^{49}\). Compound-specific isotopic analysis of PyC is of great interest\(^{50}\), as it can be used, e.g., to distinguish between the precursor biomass of chars in tropical regions\(^{51-52}\), to derive the age of charred materials\(^{53-54}\) or to trace PyC in C cycling studies with an isotopic label\(^{55,56}\). Further information about PyC as well as the BPCA method’s history, development and applications in particular can be found in Wiedemeier, 2014\(^{57}\), from where part of the above paragraphs and part of the discussion were compiled.

### Protocol

1. **General Precautions and Preparations**

   1. Use only clean, decalcified (10% HCl bath) and combusted glassware (500 °C for 5 hr), thoroughly cleaned tools and ultrapure, high pressure liquid chromatography (HPLC) grade water and solvents for the entire procedure.
   2. Freeze dry and homogenize samples with a carbon-free ball mill\(^{59,60}\) and determine their total organic carbon (TOC) content by elemental analysis\(^{59,60}\).

   Note: Purity requirements for chemicals and laboratory equipment are especially high for compound-specific \(^{14}\)C analysis of BPCAs. Include blank assessments\(^{49}\) and swipe tests\(^{61}\) to monitor potential sources of sample contamination.

2. **HNO\(_3\) Digestion**

   1. Weigh freeze-dried and homogenized samples (cf. 1.2.) into quartz digestion tubes and cover against dust with aluminum foil.
      1. For PyC quantification and characterization purposes, use samples containing > 1 mg TOC\(^{52}\). Thus, in the case of soils and sediments, use ca. 200 - 400 mg and in the case of organic-rich samples, such as pure charcoals, use ca. 10 - 20 mg per digestion tube.
      2. For subsequent compound-specific isotopic analysis of PyC (\(^{14}\)C and \(^{13}\)C), make sure the sample contains enough BPCA-C to meet the detection limits of the particular isotope-ratio mass spectrometer that will be used after step 6. If there is no \textit{a priori} information about a sample's PyC quantity available (e.g., from previous measurements), first quantify its PyC content (steps 1 - 5) and prepare more sample later if the BPCA-C yields are too low for isotopic analysis.
         Note: Include blank and reference samples with known PyC and \(^{13}\)C and \(^{14}\)C content (e.g., from the "black carbon reference materials", cf. results section). This will allow to check the reproducibility of the PyC quantification and enable blank correction calculations of the compound-specific isotopic measurements after analysis.
   2. Add 2 ml of 65% HNO\(_3\) into the digestion tubes, use a vortex mixer to assist thorough wetting of the sample and then insert the digestion tubes into the pressure chamber. Close the pressure chambers according to the manual\(^{62}\) and put them into a pre-heated oven at 170 °C for 8 hr.
      CAUTION: After digestion, let the chambers cool down inside the oven and only open them under the fume hood after they reached room temperature because harmful gases may escape.
   3. Filter the samples with water into volumetric flasks using disposable glass fiber filters (< 0.7 microns), for instance in glass syringes, and adjust volume to 25 ml. The dilution is needed to stop further digestion.
      Note: The 25 ml solutions containing the BPCAs can be stored in the refrigerator for up to 2 months before further processing. Digestion can in principle also be performed using other instrumentation, for example with a pressurized microwave system\(^{16}\). In that case, tests should be run with reference materials to check BPCA recoveries and method reproducibility (cf. representative results section).

3. **Removal of Cations**

   1. For each sample, prepare two glass columns (400 mm height, 15 mm diameter) with 11 g of cation exchange resin per column. Condition the resin inside the columns by consecutively rinsing it with: 2 column volumes of water, 1 column volume of 2 M NaOH, 2 column volumes of water for neutralizing pH, 1 column volume of 2 M HCl, and eventually 2 column volumes of water.
2. Check the conductivity of the water, which is rinsed through the resin after its conditioning. The resin is considered as properly conditioned when the conductivity is below 2 µS cm⁻¹.
3. Put one half of the sample (i.e., 12.5 ml, cf. step 2.3) on each column, rinse sequentially 5 times with 10 ml water and freeze dry the aqueous solution afterwards. The sample is stable after the freeze-drying and can be stored up to a week before further processing if it is kept dry in a dark and cool place.
   Note: Use liquid nitrogen to freeze the samples (‘snap freezing’) as it avoids the freezing out of HNO₃, which can result in a puddle of strong non-freezing acid solution. Make sure the freeze drier is acid proof to a good degree and test for potential contamination by vacuum pump fumes if compound-specific¹⁴C analysis of BPCAs is intended.

4. Removal of Apolar Compounds

1. Condition the C18 solid phase extraction cartridges according to the manufacturer’s instruction manual, i.e., consecutively rinse them with 2.5 ml of methanol, 2.5 ml of water and eventually with 2.5 ml of methanol/water (1:1 v/v).
2. Redissolve the freeze-dried residue in 3 ml methanol/water (1:1 v/v). Elute each half of it (1.5 ml) over a separate C18 solid phase extraction cartridge into 2.5 ml test tubes. Rinse the cartridges with another 1 ml of methanol/water (1:1 v/v).
3. Dry the test tubes with the sample solution, for instance using a vacuum concentrator, heated to 45 °C and with a vacuum of ca. 50 mbar.
4. Express findings of PyC quantity in BPCA-C/dry weight of the sample [g/kg] or BPCA-C/TOC [%]. Moreover, the qualitative characteristics of the PyC in the samples can be described using proportions of individual BPCAs, e.g., the proportion of B6CA (B6CA/BPCA [%]) indicates the degree of aromatic condensation of the PyC⁴⁴.

5. Chromatography

1. Prepare solvent A by mixing 20 ml of 85% orthophosphoric acid with 980 ml of water and filter the solution through a disposable glass fiber filter using vacuum. Do not expose solvent A to sunlight and use it within 24 hr in order to avoid algal growth. Use pure HPLC grade acetonitrile as solvent B.
2. Prepare standard solutions of commercially available BPCAs (hemimellitic, trimellitic, pyromellitic, pentacarboxylic and mellitic acid) to produce an external standard concentration series (e.g., 6 vials containing 5, 20, 60, 100, 150 and 250 µg of each BPCA mixed together in 1 ml water, respectively).
3. Conduct the chromatography using the settings in Table 1 and Table 2 and quantify the BPCA contents by comparing the respective BPCA peak areas to the measurements of the external standard series⁶³.
4. Express findings of PyC quantity in BPCA-C/dry weight of the sample [g/kg] or BPCA-C/TOC [%]. Moreover, the qualitative characteristics of the PyC in the samples can be described using proportions of individual BPCAs, e.g., the proportion of B6CA (B6CA/BPCA [%]) indicates the degree of aromatic condensation of the PyC⁴⁴.

6. Wet Oxidation of Purified BPCAs for Subsequent¹³C and¹⁴C Analysis

1. Following step 5.3., collect the individual BPCAs in sufficient quantity (e.g., > 30 µg BPCA-C for current accelerator mass spectrometers⁴⁹,⁶⁴) using a fraction collector connected to the HPLC⁴⁹ and then remove the solvents by blowing down the fractions with a gentle N₂ stream while heating them to 70 °C. Only tiny amounts of liquid phosphoric acid, including the BPCAs, will remain in the vial.
2. Prepare the oxidizing reagent by dissolving 2 g of Na₅S₂O₄ in 50 ml of water, freshly prepared within 24 hr of use.
   Note: Recrystallize the sodium persulfate twice to improve its purity by fully dissolving several grams in hot water and then collecting the solid after the water has cooled⁵⁵-⁶⁶.
3. Redissolve the blown down residue (step 6.1) with 4 ml water and transfer sample to 12 ml gas-tight borosilicate vial. Add 1 ml of oxidizing reagent and close with standard cap containing a butyl rubber septum.
4. Purge the gas-tight vial including the aqueous solution with He for 8 min to remove CO₂ from the vial and the solution⁶⁶.
5. Oxidize samples in the gas-tight vials by heating them at 100 °C for 60 min.
6. Directly analyze the CO₂ from the oxidation on isotope-ratio mass spectrometers for¹³C content⁶⁵-⁶⁶ and on accelerated mass spectrometers for¹⁴C content⁶⁷,⁶⁸.
   Note: Oxidized samples can be stored for at least one week⁶⁶ before¹³C and/or¹⁴C analysis.

Representative Results

We recommend to test the method set-up by measuring a suite of well-described PyC materials (“black carbon reference materials”) that have extensively been used for various method developments and comparisons in the literature⁴⁴,⁴⁸,⁶⁹-⁷⁷. Information on the reference materials is available from the University of Zurich (http://www.geo.uzh.ch/en/units/physische-geographie-boden-biogeographie/services/black-carbon-reference-materials).

The described procedure allows baseline separation of all BPCA target compounds by HPLC. The chromatograms of the reference materials ‘chernozem’ (silty soil with a significant PyC content) and grass char (made from Oryza Sativa) are shown in Figure 2. By adjusting the chromatography parameters in Tables 1 and 2 (e.g., chromatography temperature, pH of solvent A or flow rate, etc.), the separation can be further modified for specific needs⁴²,⁶³.

Quantitative analysis of the reference materials’ chromatograms with external standards (step 5.3.) should yield the PyC values depicted in Figure 3. Please note that slight changes in the procedure (e.g., the omission of step 3 or 4 in specific cases), can lead to higher PyC values. Generally, recoveries should be checked with pure BPCA standards/spiked reference materials can help to detect disproportionate losses in steps 3 and 4 and yield information about the chromatography performance in step 5.⁴²,⁶³.
Table 3 shows the $^{13}$C and $^{14}$C values that are obtained when purified BPCAs of reference materials are analyzed for their carbon isotopic content after step 6. For reliable results, it is imperative to collect sufficient amounts of BPCA-C (e.g., > 30 µg BPCA-C for current accelerator mass spectrometers, cf. Figure 4) and to take all possible measures to minimize contamination of the sample by extraneous C.

Besides checking the method set-up with reference materials as described above, it is highly advisable to prepare and measure samples in replicates, both for PyC quantification (step 5) and subsequent compound-specific $^{13}$C and $^{14}$C analyses of BPCAs (step 6).

Figure 1: The BPCA Analysis Procedure. In the protocol step 2, the PyC polycyclic aromatic condensed structures are digested, producing the different BPCAs, which are then further cleaned (steps 3 and 4) and chromatographically analyzed and separated (step 5). After wet oxidation (step 6), the purified BPCAs are amenable to compound-specific isotopic analysis ($^{13}$C and $^{14}$C) on isotope-ratio mass spectrometers. Please click here to view a larger version of this figure.

Figure 2: Chromatograms for BPCA Separation. Shown are the black carbon reference materials "chernozem" (a) and "grass char" (b). Baseline separation is achieved for all the BPCA target compounds (B6CA; B5CA; 1,2,4,5-, 1,2,3,5-, 1,2,3,4-B4CA; 1,2,4-, 1,2,3-B3CA). Information on the black carbon reference materials is available from the University of Zurich (http://www.geo.uzh.ch/en/units/physische-geographie-boden-biogeographie/services/black-carbon-reference-materials). This figure was modified from Wiedemeier et al. 2013 and is reprinted with permission from Elsevier. Please click here to view a larger version of this figure.
Figure 3: Replicated PyC Measurements of Different Black Carbon Reference Materials. Error bars for laboratory replicates are smaller than symbol size and the coefficient of variation averaged 5% (min: 1%, max: 10%). This figure was modified from Wiedemeier et al. 2013 \(^2\) and is reprinted with permission from Elsevier. Please click here to view a larger version of this figure.

Figure 4: Radiocarbon \(^{14}C\) Values for B5CA and B6CA Isolated from a Modern and a Fossil Char. The given error is composed of corrections for instrumental accelerator mass spectrometer background and of the blank for wet oxidation. The solid gray line represents an idealized line for the mixture of the real \(^{14}C\) value of the respective sample and the determined mean external contamination. This figure was modified from Gierga et al. 2014 \(^4\) and is reprinted with permission from Elsevier. Please click here to view a larger version of this figure.

| mobile phase A | 20 ml ortho phosphoric acid (85%) in 980 ml ultrapure water |
| mobile phase B | acetonitrile |
| column         | C18 reversed phase (cf. material list for details) |
| column temperature | 15 °C |
| flow rate      | 0.4 ml min \(^{-1}\) |
| identification | retention time, UV absorption at 216 nm |
| quantification | external standards of BPCAs |
| pressure       | ca. 120 bar |

Table 1: Chromatography Settings.
Table 2: Mixing Gradient of Mobile Phases.

|                | bulk char | BPCA |
|----------------|-----------|------|
| $^{13}$C [‰ vs. VPDB] |           |      |
| chestnut char  | -27.4 a   | -27.7 ±0.8 |
| maize char     | -12.9 ±0.4 | -13.0 ±0.4 |
| F $^{14}$C [%]  |           |      |
| modern char    | 1.142 b   | 1.13 ±0.003 ±0.013 |
| fossil char    | 0.003 b   | 0.014 ±0.001 ±0.001 |

Table 3: Carbon Isotopic Values ($^{13}$C and F $^{14}$C) of Reference Char Materials and Compound-Specific Isotopic Analysis of the Corresponding BPCAs. The BPCA values represent B6CA and B5CA that were collected simultaneously in step 5. However, isotopic analysis of individual BPCAs can be achieved analogously when BPCAs are collected separately. Bulk char data is from Yarnes et al. (2011) 20 for the chestnut char (a) and from Gierga et al. (2014) 49 for the fossil and modern char (b). Errors for the $^{13}$C measurements are standard errors from triplicates while errors for the F $^{14}$C measurements (bulk char: ETH-50456, ETH-50458; BPCA: ETH-62324, ETH-62335) are derived from error propagation 84.

Discussion

The BPCA method has several important advantages when compared to other available PyC methods 78-79: i) it detects PyC over a broad range of the combustion continuum, i.e., it is sensitive to slightly charred biomass as well as high temperature chars and soot 42,70, ii) it can simultaneously characterize and quantify PyC and isotopically analyze PyC 26.42,50,56,71,83-85, iii) it is applicable to a very wide range of environmental sample materials 42,70 and iv) its methodology has been intensely reviewed and could be put in a consistent framework with the assessments of other PyC methods 44,47,70,83-85. For these reasons, the BPCA approach is arguably the most versatile PyC method available to date, whose underlying assumptions are well constrained and have been continuously tested against other methods.

The above protocol consolidates the strengths of previous BPCA methods into a single procedure, is highly reproducible, simple to employ and can easily be extended and modified to specific requirements. For example, when chromatography is conducted with a pH gradient instead of an organic solvent, on-line isotope-ratio monitoring of BPCAs is possible 42, obviating the need for the wet oxidation step. Similarly, the removal of cations and/or apolar compounds (steps 3 and 4) may be skipped when it is known that particular samples do not contain any such compounds (e.g., in some cases of laboratory-produced chars).

Like every PyC method, the BPCA procedure has some limitations, too. In this regard, it is important to note that the BPCA approach inherently underestimates total PyC quantity in the samples: the method destroys large parts of the PyC polymeric structures in order to extract their BPCA building blocks, thus not quantitatively recovering all PyC in the form of BPCAs 30,86. Conversion factors had been proposed in the past to translate BPCA yields into total PyC contents. However, finding one correct conversion factor is practically impossible because of the heterogeneous degree of aromatic condensation in most chars 16,44,50,56. In many cases, PyC quantities of samples are compared relative to each other 25,67-68. We then suggest not to use any conversion factors and to simply report BPCA data "as measured" 44. In particular cases, when BPCA yields are taken to estimate absolute PyC quantities 24,89-90, the originally published conversion factor 25 of 2.27 seems appropriate as it converts the BPCA yields into conservative estimates of PyC contents 30.

Another difficulty with PyC methods is that they are potentially sensitive to interfering, non-PyC materials and/or that PyC is produced during the analysis itself, leading to an overestimation of the actual PyC content in samples 70. The BPCA approach is very robust against such interfering materials 70, does not produce any PyC by itself 16,70,86 and is conservative in nature (cf. above paragraph). Even graphite, a chemically very similar material to PyC but of petrogenic origin, does not interfere with BPCA measurements 84. So far, the only known non-PyC interferences for the BPCA method are some condensed, aromatic pigments of fungi 91, which should be quantitatively negligible for the vast majority of studies 86. The BPCA method with its simultaneous qualitative, quantitative and 13C and 14C isotopic information is thus an excellent tool for the investigation of PyC in various disciplines.
Disclosures

The authors have nothing to disclose.

Acknowledgements

The authors thankfully acknowledge support by the following funding sources: the University of Zurich Research Priority Program "global change and biodiversity", the Swiss National Science Foundation projects 134452, 131922, 143891, 119950 and 134847, and the Deep Carbon Observatory - Deep Energy award 60040915.

References

1. Shafizadeh, F. Introduction to pyrolysis of biomass. *Journal of Analytical and Applied Pyrolysis*. 3 (4), 283-305 (1982).
2. Simoneit, B. R. T. Organic matter of the troposphere - III. Characterization and sources of petroleum and pyrogenic residues in aerosols over the western United States. *Atmos. Environ.* 18 (1), 51-67 (1984).
3. Goldberg, E. D. Black carbon in the environment. *Wiley* (1985).
4. Preston, C. M., & Schmidt, M. W. I. Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. *Biogeoosciences*. 3 (4), 397-420 (2006).
5. Schmidt, M. W. I., & Noack, A. G. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. *Global Global Biogeochem. Cycles*. 14 (3), 777-793 (2000).
6. Scott, A. C., Bowman, D. M. J. S., Bond, W. J., Pyne, S. J., & Alexander, M. E. *Fire on Earth: An Introduction*. *Wiley* (2014).
7. Tinner, W., Hubischmid, P., Wehrli, M., Ammann, B., & Conedera, M. Long-term forest fire ecology and dynamics in southern Switzerland. *J. Ecol*. 87 (2), 273-28 (1999).
8. Forbes, M. S., Raison, R. J., & Skjemstad, J. O. Formation, transformation and transport of black carbon (charcoal) in terrestrial and aquatic ecosystems. *Sci. Total Environ*. 370 (1), 190-206 (2006).
9. Bowman, D. M. J. S. et al. Fire in the Earth System. *Science*. 324 (5926), 481-484 (2009).
10. Krawchuk, M. A., Moritz, M. A., Parisien, M. A., Van Dorn, J., & Hayhoe, K. Global pyrogeography: The current and future distribution of wildfire. *PloS ONE*. 4 (4), (2009).
11. Bond, T. C. et al. A technology-based global inventory of black and organic carbon emissions from combustion. *J. Geophys. Res.: Atmos.* 109 (14), (2004).
12. Cao, G., Zhang, X., & Zheng, F. Inventory of black carbon and organic carbon emissions from China. *Atmos. Environ.* 40 (34), 6516-6527 (2006).
13. Bond, T. C. et al. Bounding the role of black carbon in the climate system: A scientific assessment. *J. Geophys. Res.: Atmos.* 111 (11), 5380-5552 (2013).
14. Ramanathan, V., & Carmichael, G. Global and regional climate changes due to black carbon. *Nat. Geosci*. 1 (4), 221-227 (2008).
15. Dittmar, T., & Koch, B. P. Thermogenic organic matter dissolved in the abyssal ocean. *Mar. Chem.* 102 (3-4), 208-217 (2006).
16. Dittmar, T. The molecular level determination of black carbon in marine dissolved organic matter. *Org. Geochem.* 39 (4), 396-407 (2008).
17. Ziolkowski, L., & Druffel, E. Aged black carbon identified in marine dissolved organic carbon. *Geophys. Res. Lett.* 37 (16), L16601 (2010).
18. McConnell, J. R. et al. 20th-Century Industrial Black Carbon Emissions Altered Arctic Climate Forcing. *Science*. 317 (5843), 1381-1384 (2007).
19. Ming, J. et al. Black carbon record based on a shallow Himalayan ice core and its climatic implications. *Atmos. Chem. Phys.* 8 (5), 1343-1352 (2008).
20. Glaser, B., Haumaier, L., Guggenberger, G., & Zech, W. Black carbon in soils: the use of benzenecarboxylic acids as specific markers. *Org. Geochem.* 29 (4), 811-819 (1998).
21. Knicker, H. Pyrogenic organic matter in soil: Its origin and occurrence, its chemistry and survival in soil environments. *Quat. Int.* 243 (2), 251-263 (2011).
22. Masiello, C. A., & Druffel, E. R. M. Black carbon in deep-sea sediments. *Science*. 280 (5371), 1991 (1999).
23. Gustafsson, Ö. et al. Evaluation of a protocol for the quantification of black carbon in sediments. *Global Biogeochem. Cycles*. 15 (4), 881-890 (2001).
24. Sánchez-Garcia, L., de Andréas, J. R., Gélinas, Y., Schmidt, M. W. I., & Louchoeur, P. Different pools of black carbon in sediments from the Gulf of Cádiz (SW Spain): Method comparison and spatial distribution. *Mar. Chem.* 151, 13-22 (2013).
25. Marschner, B. et al. How relevant is recalcitrance for the stabilization of organic matter in soils? *J. Plant Nutr. Soil Sci.* 171 (1), 91-110 (2008).
26. Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., & Xu, X. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling. *Soil Biol. Biochem.* 41 (2), 210-219 (2009).
27. Schmidt, M. W. I. et al. Persistence of soil organic matter as an ecosystem property. *Nature*. 478 (7367), 49-56 (2011).
28. Singh, N., Abiven, S., & Torn, M. S. Fire-derived organic carbon in soil turns over on a centennial scale. *Biogeoosciences*. 9 (8), 2847-2857 (2012).
29. Santos, F., Torn, M. S., & Bird, J. A. Biological degradation of pyrogenic organic matter in temperate forest soils. *Soil Biol. Biochem.* 51, 115-124 (2012).
30. Kuzyakov, Y., Bogomolova, I., & Glaser, B. Biochar stability in soil: Decomposition during eight years and transformation as assessed by compound-specific 14C analysis. *Soil Biol. Biochem.* 70, 229-236 (2014).
31. Kuhbusch, T. A. Black Carbon and the Carbon Cycle. *Science*. 280 (5371), 1903-1904 (1998).
32. Liang, B. et al. Stability of biomass-derived black carbon in soils. *Geochim. Cosmochem. Acta.* 72 (24), 6069-6078 (2008).
33. Beesley, L. et al. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environ. Pollut.* 159 (12), 3269-3282 (2011).
34. Biederman, L. A., & Harpole, W. S. Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. *GCB Bioenergy*. 5 (2), 202-214 (2013).
35. Glaser, B., & Birk, J. J. State of the scientific knowledge on properties and genesis of Anthropogenic Dark Earths in Central Amazonia (terra preta de indio). *Geochim. Cosmochim. Acta*. 82, 39-51 (2012).
36. Lehmann, J., & Joseph, S. in *Biochar for Environmental Management: Science and Technology*. Earthscan (2009).
37. Marris, E. Putting the carbon back: Black is the new green. *Nature*. 442 (7034), 626-626 (2006).
38. Flannigan, M. et al. Global wildland fire season severity in the 21st century. *For. Ecol. Manage.* 294, 54-61 (2013).
39. Kelly, R. et al. Recent burning of boreal forests exceeds fire regime limits of the past 10,000 years. *Proc. Natl. Acad. Sci. U. S. A.* 110 (32), 13055-13060 (2013).
40. Hoet, P. H. M., Brûlske-Holfeld, I., & Salata, O. V. Nanoparticles - Known and unknown health risks. *J. Nanobiotechnol.* 2, (2004).
41. Ziolkowski, L. A., & Duffel, E. R. M. The feasibility of isolation and detection of fulerenes and carbon nanotubes using the benzene polycarboxylic acid method. *Mar. Pollut. Bull.* 59 (4-7), 213-218 (2009).
42. Wiedemeier, D. B., Bloesch, U., & Hagedorn, F. Stable forest-savanna mosaic in north-western Tanzania: local-scale evidence from δ13C revealed by the 13C/12C isotopic ratio in a Cerrado’s oxisol. *J. Biogeogr.* 39 (10), 1194-1202 (2012).
43. Maestrini, B., Herrmann, A. M., Nannipieri, P., Schmidt, M. W. I., & Abiven, S. Ryegrass-derived pyrogenic organic matter changes organic carbon and nitrogen mineralization in a temperate forest soil. *Eur. J. Soil Sci.* 65 (1), 1-9 (2014).
44. Ziolkowski, L. A., Druffel, E. R. M. The feasibility of isolation and detection of fullerenes and carbon nanotubes using the benzene polycarboxylic acid method. *Radiocarbon*. 38 (2), 191-201 (1996).
45. Bird, M. I. New insights into pyrogenic carbon by an improved benzene polycarboxylic acid molecular marker method. University of Zurich (2014).
46. Perttila, M., & Pedersen, B. in *Quality Assurance in Environmental Monitoring*. Wiley (2007).
47. Baldo, J., & Smernik, R. Chemical composition and bioavailability of thermally altered Pinus resinosa (Red pine) wood. *Org. Geochem.* 33 (9), 1003-1108 (2002).
48. Nelson, D. W., & Sommers, L. E. in *Soil Analysis Part 3. Chemical methods*. SSSA Book Series (1996).
49. Buchholz, B. A., Freeman, S. P. H. T., Haack, K. W., & Vogel, J. S. Tips and traps in the 14C bio-AMS preparation laboratory. *Nucl. Instrum. Methods Phys. Res., Sect. B*. 172 (1-4), 404-408 (2000).
50. Ney, D., Wacker, L. et al. A versatile gas interface for routine radiocarbon analysis with a gas ion source. *Nucl. Instrum. Methods Phys. Res., Sect. B*. 294, 315-319 (2013).
51. Hammes, K., Smernik, R., Skjemstad, J., Herzog, A., & Vogt, U. Synthesis and characterisation of laboratory-charred grass straw (Oryza saliva) and chestnut wood (Castanea sativa) as reference materials for black carbon quantification. *Org. Geochem.* 37 (11), 1629-1633 (2006).
52. Hammes, K. et al. Comparison of quantification methods to measure fire-derived (black/elemental) carbon in soils and sediments using reference materials from soil, water, sediment and the atmosphere. *Global Biogeochem. Cycles*. 21 (3), GB3016, (2007).
53. Meredith, W. R. Assessment of hydropyrolysis as a method for the quantification of black carbon using standard reference materials. *Geochim. Cosmochim. Acta*. 67, 131-147 (2012).
54. Kael, J., Schneider, M. P. W., & Schmidt, M. W. I. Rapid molecular screening of black carbon (biochar) thermosquences obtained from chestnut wood and rice straw: A pyrolysis-GC/MS study. *BioMass Bioenergy*. 45, 115-129 (2012).
55. Yarnes, C. et al. Stable isotopic analysis of pyrogenic organic matter in soils by liquid chromatography-isotope-ratio mass spectrometry of benzene polycarboxylic acids. *Rapid Commun. Mass Spectrom.* 25 (24), 3723-3731 (2011).
56. Han, Y. M. et al. Evaluation of the thermal/optical reflectance method for discrimination between char- and soot-EC. *Chemosphere*. 69, 569-574 (2007).
75. Leifeld, J. Thermal stability of black carbon characterised by oxidative differential scanning calorimetry. Org. Geochem. 38 (1), 112-127 (2007).
76. Roth, P. J. et al. Differentiation of charcoal, soot and diagenetic carbon in soil: Method comparison and perspectives. Org. Geochem. 46, 66-75 (2012).
77. Schmidt, M. W. I., Masiello, C. A., & Skjemstad, J. O. Final recommendations for reference materials in black carbon analysis. Eos. 84 (52), 582 (2003).
78. Bird, M. in Biochar for Environmental Management: Science and Technology. Earthscan (2009).
79. Hammes, K., & Abiven, S. in Fire Phenomena and the Earth System. Wiley (2013).
80. Schneider, M. P. W., Hilf, M., Vogt, U. F., & Schmidt, M. W. I. The benzene polycarboxylic acid (BPCA) pattern of wood pyrolyzed between 200 °C and 1000 °C. Org. Geochem. 41 (10), 1082-1088 (2010).
81. Schneider, M. P. W. et al. Toward a “molecular thermometer” to estimate the charring temperature of wildland charcoals derived from different biomass sources. Environ. Sci. Technol. 47 (20), 11490-11495 (2013).
82. Ziolkowski, L. A., & Druffel, E. R. M. Aged black carbon identified in marine dissolved organic carbon. Geophys. Res. Lett. 37 (16), (2010).
83. Coppola, A. I., Ziolkowski, L. A., Masiello, C. A., & Druffel, E. R. M. Aged black carbon in marine sediments and sinking particles. Geophys. Res. Lett. 41 (7), 2427-2433 (2014).
84. Wurster, C. M., Lloyd, J., Goodrick, I., Saiz, G., & Bird, M. I. Quantifying the abundance and stable isotope composition of pyrogenic carbon using hydrogen pyrolysis. Rapid Commun. Mass Spectrom. 26 (23), 2690-2696 (2012).
85. Wiedemeier, D. B., Brodowski, S., & Wiesenberg, G. L. B. Pyrogenic molecular markers: Linking PAH with BPCA analysis. Chemosphere. 119, 432-437 (2015).
86. Brodowski, S., Rodionov, A., Haumaier, L., Glaser, B., & Amelung, W. Revised black carbon assessment using benzene polycarboxylic acids. Org. Geochem. 36 (9), 1299-1310 (2005).
87. Singh, N. et al. Transformation and stabilization of pyrogenic organic matter in a temperate forest field experiment. GCB 20 (5), 1629-1642 (2014).
88. Abiven, S., Hengartner, P., Schneider, M. P. W., Singh, N., & Schmidt, M. W. I. Pyrogenic carbon soluble fraction is larger and more aromatic in aged charcoal than in fresh charcoal. Soil Biol. Biochem. 43 (7), 1615-1617 (2011).
89. Lehndorff, E. et al. Industrial carbon input to arable soil since 1958. Org. Geochem. 80, 46-52 (2015).
90. Lehndorff, E., Roth, P. J., Cao, Z. H., & Amelung, W. Black carbon accrual during 2000 years of paddy-rice and non-paddy cropping in the Yangtze River Delta, China. GCB 20 (6), 1968-1978 (2014).
91. Glaser, B., & Knorr, K.-H. Isotopic evidence for condensed aromatics from non-pyrographic sources in soils - implications for current methods for quantifying soil black carbon. Rapid Commun. Mass Spectrom. 22 (7), 935-942 (2008).