Video Article

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

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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 (^{13}C and ^{14}C) 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.

Video Link

The video component of this article can be found at https://www.jove.com/video/53922/

Introduction

In a complete combustion process, biomass or fossil fuel is converted into CO_2, H_2O 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 char[1]. 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 combustion[5-6]. Wildfire is an important natural process, intrinsic to most ecosystems, which produces a significant quantity of PyC each year[4,7-10]. Similarly, the burning of fossil fuel for energy production in industry and transport presents an important anthropogenic source of PyC[11-13]. Both sources contribute to the ubiquity of PyC in the environment: PyC is present in the air, in the form of aerosols[13-14], in water as particulate or dissolved organic matter[15-17], as well as in ice cores[18-19], soils[20-21], and sediments[22-24] 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 degradation[25-26]. Although exact turnover times have not yet been established and may depend upon specific environmental conditions[27-28], it seems clear that PyC is less readily decomposed into CO_2 than most other forms of organic carbon[29-30]. 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 CO_2, thus reducing atmospheric greenhouse gas concentrations over time[31-32].

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[33] and improve soil fertility[34-35]. The recognition of chars as a potentially beneficial soil amendment led to the emerging field of so-called biochar technology[36]. 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 21th century, continuously contributing large quantities of PyC to the environment[11,38-39]. Another increasingly important source of PyC is likely to be nanotechnology that also uses PyC-like compounds[40-41]. 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 (*cf*. **Figure 1**). The BPCAs are then, after a few purification steps, amenable to chromatographic analysis[20,42]. PyC is thus isolated and analyzed on a molecular level and can be used to quantify PyC abundance in environmental compartments[20,42]. The BPCA method additionally characterizes the investigated PyC when relative yields of B3-, B4-, B5- and B6CA are compared (*cf*. **Figure 1**): The respective proportion of differently carboxylated BPCAs is linked to the size of the original polycyclic structures and is therefore indicative of PyC's quality and pyrolysis temperature[44,47-48]. Moreover, the presented method allows for the determination of the C isotopic composition (^{13}C and ^{14}C) of PyC because the individual BPCAs, deriving directly from pure PyC structures, can be isotopically analyzed after isolation (*cf*. **Figure 1**, steps 5 and 6)[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[26,55-56]. 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[58] 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. HNO₃ 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[42]. 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 (^{13}C and ^{14}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 *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% HNO₃ 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 HNO_3, 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-specific ^{14}C 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. Other means of evaporation can also be used, for instance a blow-down system with N_2 gas as in step 6.
4. Redissolve the residue in the test tube with 1 ml water. Support dissolution with vortex mixer and transfer to 1.5 ml auto sampler vials.
Note: Samples can be stored in the refrigerator for up to 3 months at this stage[42].

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 series[63].
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 PyC[44].

6. Wet Oxidation of Purified BPCAs for Subsequent ^{13}C and ^{14}C Analysis

1. Following step 5.3., collect the individual BPCAs in sufficient quantity (*e.g.,* > 30 µg BPCA-C for current accelerator mass spectrometers[49,64]) using a fraction collector connected to the HPLC[49] and then remove the solvents by blowing down the fractions with a gentle N_2 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 $Na_2S_2O_8$ 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 cooled[65-66].
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 CO_2 from the vial and the solution[66].
5. Oxidize samples in the gas-tight vials by heating them at 100 °C for 60 min.
6. Directly analyze the CO_2 from the oxidation on isotope-ratio mass spectrometers for ^{13}C content[65-66] and on accelerated mass spectrometers for ^{14}C content[67-68].
Note: Oxidized samples can be stored for at least one week[66] before ^{13}C and/or ^{14}C 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 literature[44,48,69-77]. 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 needs[42,63].

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 [49].

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).
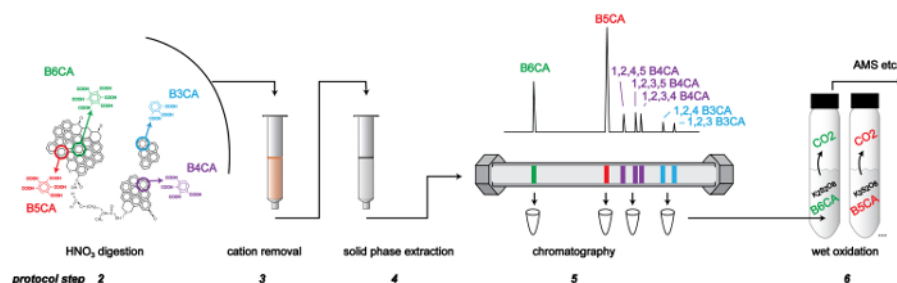

Figure 1: The BPCA Analysis Procedure. In the protocol **step 2**, the PyC polycyclic aromatic condensed structures are digested, producing the different BPCAs, which are then further cleaned (**steps 3** and **4**) and chromatographically analyzed and separated (**step 5**). After wet oxidation (**step 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.
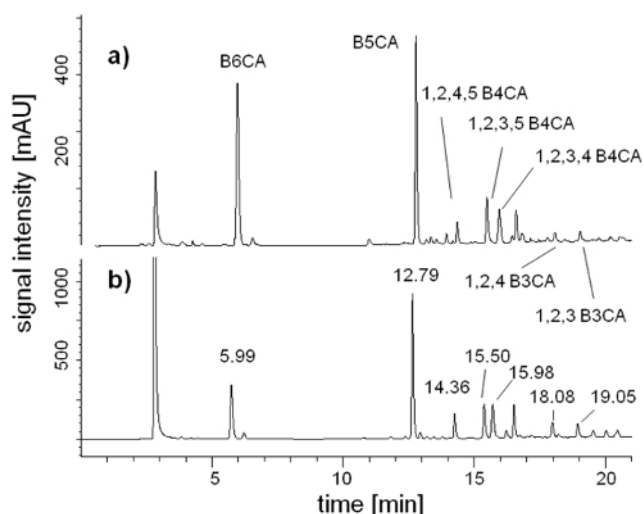

Figure 2: Chromatograms for BPCA Separation. Shown are the black carbon reference materials "chernozem" **(a)** and "grass char" **(b)**. Baseline separation is achieved for all the BPCA target compounds (B6CA; B5CA; 1,2,4,5-. 1,2,3,5-, 1,2,3,4-B4CA; 1,2,4-, 1,2,3-B3CA)[42]. Information on the black 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[42] and is reprinted with permission from Elsevier. Please click here to view a larger version of this figure.
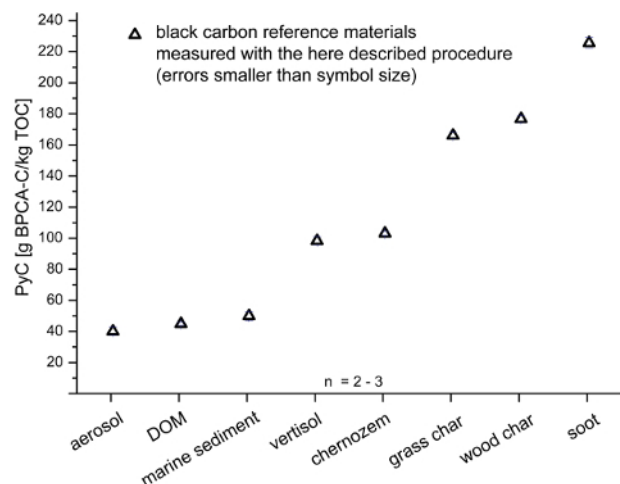
Figure 3: Replicated PyC Measurements of Different Black Carbon Reference Materials. Error bars for laboratory replicates are smaller than symbol size and the coefficient of variation averaged 5% (min: 1%, max: 10%).This figure was modified from Wiedemeier *et al.* 2013[42] and is reprinted with permission from Elsevier. Please click here to view a larger version of this figure.
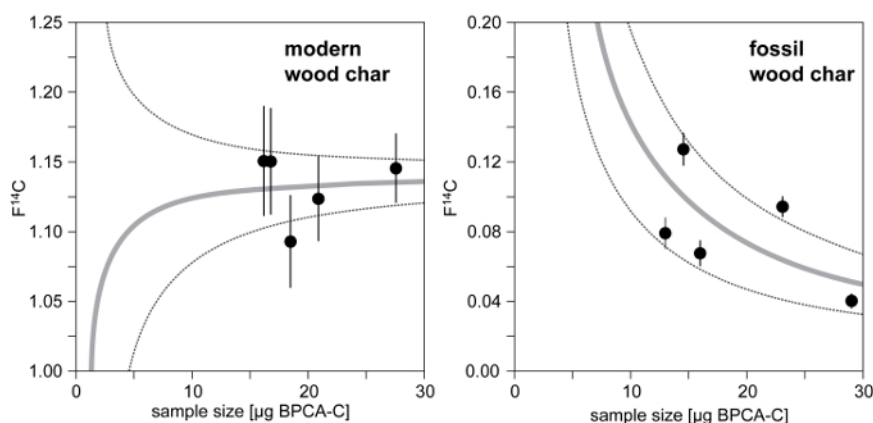

Figure 4: Radiocarbon (^{14}C) Values for B5CA and B6CA Isolated from a Modern and a Fossil Char. The given error is composed of corrections for instrumental accelerator mass spectrometer background and of the blank for wet oxidation. The solid gray line represents an idealized line for the mixture of the real F^{14}C value of the respective sample and the determined mean external contamination. This figure was modified from Gierga *et al.* 2014[49] 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.

time	mobile phase B
[min]	[vol %]
0	0.5
5	0.5
25.9	30
26	95
28	95
28.1	0.5
30	0.5

Table 2: Mixing Gradient of Mobile Phases.

	bulk char		BPCA	
	$\delta^{13}C$ [‰ vs. VPDB]			
chestnut char	-27.4[a]	±0.4[a]	-27.7	±0.8
maize char	-12.9	±0.4	-13.0	±0.4
	$F^{14}C$ [%]			
modern char	1.142[b]	±0.004[b]	1.13	±0.013
fossil char	0.003[b]	±0.001[b]	0.014	±0.001

Table 3: Carbon Isotopic Values ($\delta^{13}C$ 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) [73] for the chestnut char **(a)** and from Gierga *et al.* (2014) [49] for the fossil and modern char **(b)**. Errors for the $\delta^{13}C$ 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[64].

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[16,44,80-81], quantify[20,42] and isotopically analyze PyC[49-50,66,73,82-83], 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[44,47,70,84-85]. For all 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 polycyclic structures in order to extract their BPCA building blocks, thus not quantitatively recovering all PyC in the form of BPCAs[20,86]. 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[41,48,80,86]. In many cases, PyC quantities of samples are compared relative to each other[42,81,87-88]. We then suggest not to use any conversion factors and to simply report BPCA data "as measured"[48]. In particular cases, when BPCA yields are taken to estimate absolute PyC quantities[24,89-90], the originally published conversion factor[20] of 2.27 seems appropriate as it converts the BPCA yields into conservative estimates of PyC contents[86].

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[(Schneider, M. P. W. *unpublished results.* Zurich, (2013))]. 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 ^{13}C and ^{14}C isotopic information is thus an excellent tool for the investigation of PyC in various disciplines.

Disclosures

The authors have nothing to disclose.

Acknowledgements

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