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Artificial formation of benzene polycarboxylic acids during sample processing of black carbon analysis: the role of organic carbon amount

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Abstract

Black carbon is also known as pyrogenic carbon formed by partial combustion of organic material under limited oxygen supply. It occurs along a continuum from original organic slightly charred material to highly aromatic combustion residues such as charcoal, graphite, and soot. Black carbon is extensively studied in various environments due to its ubiquity. It is also important for the biochar community because it can specifically trace the stable polycondensed part of biochar. Different methods have been adopted for black carbon determination; among them using benzene polycarboxylic acids (BPCA) as molecular markers for the polycondensed aromatic moieties of charred materials. However, different researchers have shown interferences from organic matter during BPCA analysis. Therefore, the aim of this work was to assess if artificial formation of BPCA occurs in soil samples when the organic carbon load exceeds 5–10 mg. For this purpose, we conducted black carbon analysis of different soil samples with varying TOC contents of up to 20 mg. In addition, organic matter-rich plant materials were used as a black carbon-free control (leaves of Ivy and Beech, leaves/needles of Spruce and needles of Thuja). To exclude the high-pressure digestion as source of artificial black carbon formation, a comparison between the conventional and a microwave-assisted extraction (MAE) oxidation process was included. Our results show that for soil samples, no artificial BPCA formation occurred at least up to 20 mg of total organic carbon. Higher sample weights are unrealistic for BPCA analysis of soils using current methodology. Therefore, our results clearly demonstrate that there is no artificial BPCA formation during properly performed black carbon analysis of soil samples. On the contrary, for some samples, BPCA contents tended to decrease with increasing sample weight, and thus increasing amount of TOC. In contrast, for plant samples, artificial BPCA formation of up to 3 g kg^{-1} occurred when more plant material equivalent to 10 mg total organic carbon was used. However, there was no amount dependence of artificial BPCA formation. The reason for artificial BPCA formation was not the high-pressure digestion, as microwave-assisted digestion showed comparable results. However, for real-world analysis, this artificial BPCA formation is not relevant because such high soil sample weights cannot be used. Nevertheless, when using organic-rich material such as peat and charred materials, the samples should contain less than 10 mg of total organic carbon.

Keywords Pyrogenic carbon, BPCA, Biochar, Highly aromatic combustion residues, Plant material, Conventional digestion, Microwave-assisted digestion

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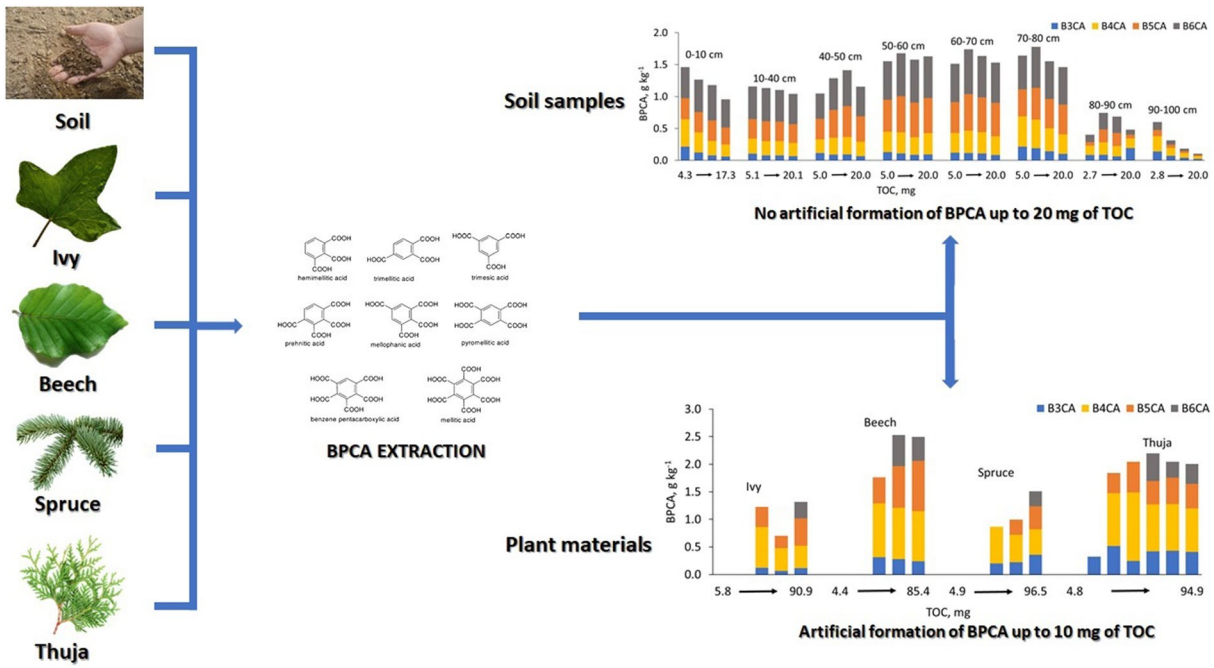
Graphical Abstract



Introduction

Black carbon, also known as pyrogenic carbon, is formed by incomplete combustion of organic matter under limited oxygen supply. Its chemical structure is characterized by increasing aromaticity from slightly charred material over char and charcoal to graphite and soot particle recondensed from the gas phase [1–5]. Black carbon can also reach soil as deposition of emissions from biomass combustion or traffic [6] and today also from biochar applications [5, 7, 8]. It is estimated that worldwide up to 383 Tg of black carbon is produced by pyrolysis of biomass [5]. The application of biochar to soils for soil improvement and C sequestration is continuously increasing [5, 9]. The same is true for environmental remediation, i.e., the removal of organic and inorganic pollutants [10, 11].

Consequently, due to its persistence in the environment, black carbon certainly contributes to a significant global carbon sink [3, 7]. Forbes et al. [12] and Preston and Schmidt [13] reported that more than 80% of black carbon that ends up in the soil could reside there for hundreds to thousands of years, due to its resistance to chemical and biological breakdown [7].

Several methods have been reported in literature for the analysis of black carbon in soil such as thermal oxidation, pre-extraction and thermal oxidation, chemical oxidation, oxidation with UV and CP MAS ^{13}C NMR spectroscopy [3, 14, 15]. Among these methods, the oxidation of the material and the subsequent use of benzene polycarboxylic acids (BPCA) as molecular marker for black carbon is widely used [2, 16, 17]. The BPCA method is based on the principle that the polycondensed aromatic structure of black carbon is converted to BPCA after acid hydrolysis to remove polyvalent cations which disturb further analysis, and HNO_3 oxidation of organic matter and the polyaromatic backbone of black carbon. The advantage of the BPCA method is the use of a molecular marker specific for polycondensed aromatic moieties [2]. Nevertheless, BPCA could also be formed artificially during the analytical procedure [16, 17]. One explanation could be the co-occurrence of black carbon and soil organic matter at sites with large amount of biomass production [17]. Another one could be a yet unknown methodological artefact that could lead to the production of BPCA especially B3CA and B4CA during BPCA analysis. Another systematic error (of all black carbon methods) is the biological formation of polycondensed aromatic moieties, e.g., through *Aspergillus niger* [16]. It was shown that biological black carbon production is significant in the environment and that it could contribute 3–9% of annual black carbon deposition in soils [18].

The history of the BPCA method to determine black carbon can be described as follows. Glaser et al. [2] proposed the evaluation of black carbon using BPCA as molecular markers for the polyaromatic backbone of black carbon. Brodowski et al. [16] showed an artificial black carbon formation using 32% HCl hydrolysis to remove polyvalent cations for plant material but not for soil samples. Therefore, the HCl treatment was substituted by 4 M triflouroacetic acid (TFA), showing no artificial black carbon production even for plant material, at least up to 5 mg of total organic carbon (TOC) [16]. Kappenberg et al. [17] modified the BPCA method only by considering penta- and hexacarboxylic acids (B5CA and B6CA, respectively) as black carbon-derived because BPCA with three and four carboxylic groups (B3CA and B4CA, respectively) may originate from non-black carbon materials. But they missed that B6CA could be produced biologically from *Aspergillus niger* [16, 18] and the pyrogenic carbon content based on B5CA and B6CA may contain also biologically produced black carbon, thus being not of pyrogenic origin.

In addition, Chang et al. [19] evaluated the black carbon content in soil through BPCA method and also in other materials including lignin and humic substances (humic acids and fulvic acids). They found that humic substances could contribute to the BPCA formation. In particular, the mature humic substances could contribute to the formation of B5CA and B6CA. This finding is no contradiction to the specificity of BPCA for polycondensed aromatic moieties, i.e., black carbon, as it is certainly incorporated into soil organic matter and thus, humic substances, which represent on average ~0.4% in terms of weight and the ~50% as percentage of TOC [20–22]. This is an important point that should be discussed also in future studies. Black carbon is the recalcitrant highly aromatic fraction of carbon in soil from char, charcoal, graphite, soot and biological black pigments, and also humic substances can of course contain condensed aromatic structures of these sources and thus it is no contradiction that humic substances also contain black carbon or BPCA.

On the basis of the works of Kappenberg et al. [17] and Chang et al. [19], the aim of our work was (i) to assess if organic matter load that exceeds 5 mg of organic carbon from soil samples could artificially produce BPCA although this aspect was already assessed by Brodowski et al. [16] but there is a contradiction of the works of Kappenberg et al. [17] and Brodowski et al. [16]; (ii) to verify if BPCA could be produced artificially from a non-pyrogenic organic matter at high quantity (Ivy, Beech, Spruce, Thuja) when the organic carbon amount exceeds 5 mg; (iii) to assess the artificial formation of BPCA and then of the black carbon content of soil and organic matter-rich material through microwave-assisted extraction (MAE) oxidation process [23] to identify the digestion technique as sources of artificial BPCA formation.

We hypothesize (i) that no artificial BPCA formation occurs up to 10 mg TOC, which is in the range of any practical BPCA analysis procedure with respect to reasonable sample aliquot as already shown by Brodowski et al. [16]. Further, we hypothesize (ii) that any artificial BPCA formation is dependent the type of organic matrix (e.g., green tissue such as leaves, or woody tissue such as needles). Organic matrix with abundant aromatic structures (lignin) could artificially produce a greater amount of BPCA during the oxidation process compared to the green tissue. Last but not least, we hypothesize (iii) that artificial BPCA formation is independent from hot nitric acid oxidation technique.

Material and methods

Soil and plant samples

Soil samples from a Calcaric Regosol at different soil depths (Amsdorf, Saxony-Anhalt, Germany) and a Chernozem topsoil (Etzdorf, Saxony-Anhalt, Germany) were selected to cover a wide range of total organic carbon contents (Table 1). We used also black carbon-free plant material from four different plant species: leaves of Ivy (*Hedera helix* L.), Beech (*Fagus sylvatica* L.), leaves/needles of Thuja (*Thuja occidentalis* L.) and needles of Spruce (*Picea abies* L.) collected from Bindlach, Bavaria, Germany (Table 2). The fresh material was dried at 40 °C and milled.

Black carbon analysis

Black carbon was analyzed using BPCA as molecular marker [2, 16]. Different amount of ground soil (0.2–3.7 g) and plant samples (10–201 mg) corresponding to a different range of organic carbon amounts (3–22 mg and 4–97 mg in soil and plant, respectively) were hydrolyzed with 10 mL of 4 M trifluoroacetic acid (TFA) at 105 °C for 4 h to remove polyvalent cations. The dried residue was oxidized with 2 mL of 65% nitric acid at 170 °C for 8 h. The digested solution was diluted to 10 mL with deionized water. An aliquot of 2 mL was cleaned up by a cation exchange procedure using a Dowex 50 W X 8, 200–400 mesh. After freeze-drying, the BPCA were analyzed as trimethylsilyl (TMS) derivatives [2] using capillary gas chromatography using a Shimadzu GC-2010 instrument (Shimadzu Ltd., Tokyo, Japan) equipped with a DB5 capillary column (30 m · 0.32 mm · 0.25 µm film thickness) and a Flame ionization detector. Phthalic acid (1 mg mL^{-1} in deionized water) was used as first internal standard and added prior to the sample clean-up; 2.2′-biphenyldicarboxylic acid (1 mg mL^{-1} in methanol, second internal standard) was added prior to

Table 1 Soil physical and chemical properties of Calcaric Regosol (Amsdorf) and Chernozem (Etzdorf)

Depth (cm)	Horizon	Sand (%)	Silt (%)	Clay (%)	pH (Salt)	CEC pH 7 (mmolc kg^{-1})	Base saturation (%)	TOC (g kg^{-1})	Carbonate-C (g kg^{-1})	*N* (total) (g kg^{-1})	TOC/N (–)
Regosol											
0–40	Ap	35.0 ± 1.8	39.0 ± 2.0	27.0 ± 1.4	7.6 ± 0.4	119 ± 6	100 ± 5	24.5 ± 1.2	52.6 ± 5.3	1.9 ± 0.1	12.9 ± 0.6
40–50	reFh1	4.0 ± 0.2	72.0 ± 3.6	23.0 ± 1.2	7.6 ± 0.4	172 ± 9	100 ± 5	24.1 ± 1.2	9.5 ± 1.0	1.6 ± 0.1	15.1 ± 0.8
50–60	reFh2	8.0 ± 0.4	72.0 ± 3.6	20.0 ± 1.0	6.7 ± 0.3	166 ± 8	100 ± 5	19.3 ± 1.0	1.9 ± 0.2	1.7 ± 0.1	11.3 ± 0.6
60–70	2Ab1	10.0 ± 0.5	68.0 ± 3.4	22.0 ± 1.1	5.2 ± 0.3	166 ± 8	100 ± 5	16.4 ± 0.8	0.0 ± 0.1	1.3 ± 0.1	12.6 ± 0.6
80–80	2Ab2	8.0 ± 0.4	70.0 ± 3.5	21.0 ± 1.1	4.9 ± 0.2	154 ± 8	100 ± 5	13.9 ± 0.7	0.0 ± 0.1	1.0 ± 0.1	13.9 ± 0.7
80–90	2Ab3	6.0 ± 0.3	67.0 ± 3.4	27.0 ± 1.4	4.9 ± 0.2	194 ± 10	100 ± 5	5.4 ± 0.3	0.0 ± 0.1	1.0 ± 0.1	5.4 ± 0.3
90–100	IIelCv(Go)	8.0 ± 0.4	67.0 ± 3.4	25.0 ± 1.3	7.2 ± 0.4	168 ± 8	100 ± 5	5.6 ± 0.3	9.0 ± 0.9	0.6 ± 0.1	9.4 ± 0.5
Chernozem											
0–30	rAxp	6.0 ± 0.3	63.0 ± 3.0	31.0 ± 2.0	7.1 ± 0.4	220 ± 11	100 ± 5	21.4 ± 1.1	0.0 ± 0.1	1.7 ± 0.1	12.6 ± 0.6

Table 2 Characteristics of plant samples. Cellulose and lignin content were obtained from Buitrago et al. [24], Welker et al. [25], Xiao et al. [26], Brinkmann et al. [27]

Plant sample	Scientific name	Location	Country	TOC (g kg^{-1})	Cellulose (%)	Lignin (%)
Ivy	*Hedera helix* L.	Bindlach	Germany	453 ± 44	24.0 ± 2.4	13.5 ± 3.5
Beech	*Fagus sylvatica* L.	Bindlach	Germany	429 ± 38	27.0 ± 2.8	20.0 ± 1.4
Spruce	*Picea abies* L.	Bindlach	Germany	482 ± 49	15.0 ± 1.5	23.0 ± 5.7
Thuja	*Thuja occidentalis* L.	Bindlach	Germany	474 ± 43	19.0 ± 1.4	30.0 ± 2.9

derivatization. The sum of BPCA after nitric acid oxidation is a relative measure of black carbon and when multiplied with 2.27 to correct for the loss of C during oxidation, a charcoal equivalent can be calculated [2].

BPCA determination through microwave-assisted oxidation process

Black carbon was additionally analysed in all samples through microwave-assisted oxidation according to Glaser et al. [23]. Different amounts of ground soil (25–506 mg) and plant samples (25–504 mg) corresponding to a different range of organic carbon (0.5–11 mg and 11–243 mg in soil and plant, respectively) were digested with 10 mL of 4 M trifluoroacetic acid (TFA) at 105 °C for 4 h. The dried residue was oxidized with 5 mL of 65% nitric acid at 190 °C for 1 h. The digested solution was diluted to 25 mL with deionized water. Purification and analysis were performed as described in "Black carbon analysis" section.

Data analysis

Data analysis was carried out using XLSTAT (2016). Before ANOVA analysis, data were tested for normal distribution using the Shapiro–Wilk test and Levene's test was performed to assess the homogeneity of variance. If normal distribution and homogeneity of variance were given, significant differences between means at $p < 0.05$ were assessed according to Fisher (LSD) test. Non-parametric Kruskal–Wallis and Dunn rank tests were used when normal distribution and homogeneity of variance were violated. Furthermore, the differences between microwave and conventional oxidation processes among individual samples were assessed by paired t-test. Pearson or Spearman correlation matrix for normally or non-normally distributed data were evaluated within the same sample for BPCA amounts as function of TOC amount. For the evaluation and comparison of the methods with regard to the extraction performance of BPCA, regression analysis of the data was conducted. To check the variability of the results, the coefficient of variation (CV) calculated by the ratio between the standard deviation and the mean of the sample in percent was evaluated ($N = 3$).

Results

Individual benzenepolycarboxylic acids content in soil samples as function of total organic carbon

The content of the sum of BPCA with 3 carboxylic groups (B3CA) ranged between 0.02 and 0.22 g kg^{-1} (Fig. 1 and Additional file 1: Table S1). The B4CA values ranged between 0.04 and 0.54 g kg^{-1} (Fig. 1 and Additional file 1: Table S2). For B5CA and B6CA, the values ranged

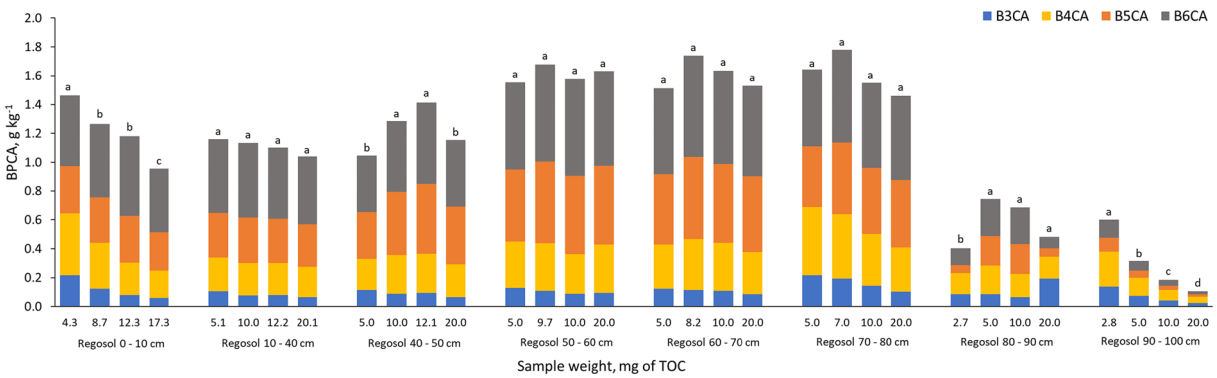


Fig. 1 Individual and sum of BPCA with three (B3CA), four (B4CA), five (B5CA) and six (B6CA) carboxylic groups of Regosol samples as function of amount of total organic carbon (TOC) and thus, sample weight. Different lowercase letters indicate significant differences among BPCA in the same soil profile $p < 0.05$

from 0.02 and 0.57 and 0.02 and 0.70 g kg^{-1}, respectively (Fig. 1, Additional file 1: Tables S3 and S4). The coefficient of variation (CV) of individual BPCA contents decreased by increasing degree of aromaticity of BPCA (B3CA > B4CA > B5CA > B6CA; Additional file 1: Tables S1–S4). Furthermore, CV increased with increasing soil depth of the Regosol profile. By comparing the B3CA and B4CA contents in different soil samples, a greater value of 0.16 ± 0.05 and 0.40 ± 0.08 g kg^{-1}, respectively, were observed in Regosol at 70–80 cm sample (Additional file 1: Tables S1 and S2). The highest B5CA contents were registered in Regosol 50–60 and 60–70 cm (Additional file 1: Table S3). The highest B6CA contents were observed in Regosol 50–60, 60–70 and 70–80 cm (Additional file 1: Table S4).

Results of regression analysis were summarized for the BPCA content in the Additional file 1: Tables S1–S4. B3CA, B4CA, B5CA and B6CA data were normally distributed with a no significant p-value of the Shapiro–Wilk test, so it was possible to run Pearson correlation with the exception of Regosol 80–90 cm for B5CA evaluation (Additional file 1: Table S3) in which Shapiro–Wilk test was significant and Spearman correlation test was evaluated. Significant ($p < 0.05$) negative correlation between sample weight as mg of TOC and B3CA content was observed in the Regosol 40–50 cm ($r = -0.969$) and 60–70 cm ($r = -0.999$) samples (Additional file 1: Table S1). For B4CA, a significant negative Pearson correlation with sample weight as mg of TOC in Regosol 0–10 cm and 10–40 cm and Chernozem 0–30 cm was registered (Fig. 2, Additional file 1: Table S5). Apart from the Regosol sample at 90–100 cm, no significant correlation among sample weight as mg of TOC and B5CA and B6CA contents were observed, so the amount of TOC did not affect the BPCA content in soil samples up to 20 mg of TOC, which is already much higher than appropriate for a good laboratory practice (Additional file 1: Tables S3 and S4).

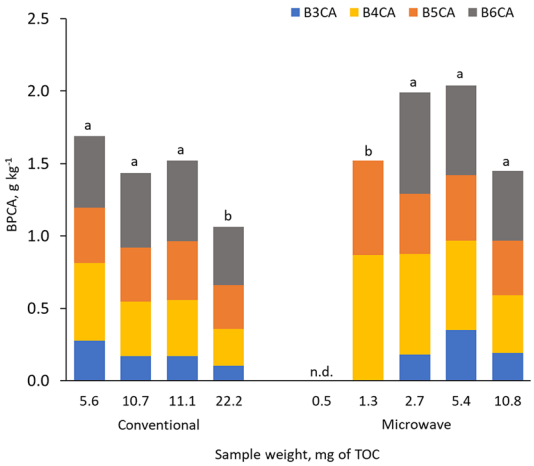


Fig. 2 Individual and sum of BPCA with three (B3CA), four (B4CA), five (B5CA) and six (B6CA) carboxylic groups of Chernozem sample, as function of amount of total organic carbon (TOC) and thus, sample weight after high pressure digestion (conventional) and microwave digestion (Microwave). Different lowercase letters indicate significant differences among BPCA in the same soil profile $p < 0.05$

Total black carbon content in soil samples as function of total organic carbon

The variation of black carbon content (0.2–4.0 g kg^{-1}) as function of sample weight as mg of TOC (3–20 mg) is given in Fig. 1 and Additional file 1: Table S5. The highest amount of black carbon was observed in 50–60, 60–70 and 70–80 cm of the Regosol (Fig. 1) and 0–30 cm of the Chernozem (Additional file 1: Table S5). The coefficient of variation (CV) indicating the relative variability of the results was greater at 0–50 cm (14 and 18%) except for 10–40 cm and at 80–100 cm (31 and 71%) in Regosol and Chernozem samples, conversely, little variability was observed at 50–80 cm (Additional file 1: Table S5).

A significant negative Pearson correlation between sample weight as mg of TOC and black carbon content was observed in Regosol 0–10 and 90–100 cm ($r = -0.995$ and $r = -0.992$) samples and in the Chernozem 0–30 cm sample ($r = -0.987$; Additional file 1: Table S5).

Chernozem soil sample at 0–30 cm was also subjected to microwave-assisted oxidation process [23], in which there were no artificial formation of the BPCA and no amount dependency based on the results of the regression analysis (Additional file 1: Tables S1–S5). In this work, Chernozem soil sample was tested and no significant differences were observed between microwave-assisted oxidation process and conventional one (Fig. 2). In fact, by weighting up to 11 mg TOC (more TOC was analytically not feasible), there were no significant differences between the conventional oxidation process and the MAE one, respectively (Fig. 2).

Individual benzenepolycarboxylic acids and black carbon content in plant samples as function of total organic carbon with conventional oxidation process

To further understand if artificial formation of BPCA occurred during oxidation process, different black carbon-free plant materials with different chemical composition were analysed. Leaves of Ivy and Beech (angiosperms), leaves/needles of Thuja and needles of Spruce were chosen to cover, as reported in Table 2, different chemical structures i.e., contents of cellulose and lignin [24–27]. Furthermore, before running correlation analysis, normality test of the data showed that B3CA and B5CA data were normally distributed,

instead B4CA and B6CA registered in some cases non-normal distributed data. The BPCA were evaluated on different content of sample weight as mg of TOC ranged between 4 and 97 mg (Fig. 3). Until 11, 11, 13 and 5 mg of TOC in Ivy, Beech, Spruce and Thuja, respectively, no artificial formation of BPCA occurred (Fig. 3). By increasing the TOC load (> 10 mg of TOC) different behaviour in the BPCA content was observed (Fig. 3). For B3CA, by increasing the TOC load, no further increase of these biomarkers was registered in all plant samples excluding the Spruce sample that registered a significant positive correlation of between B3CA and sample weight as mg of TOC ($r = 0.921$, Additional file 1: Table S6; Fig. 3a). The B4CA content did not show the same behaviour and was not related to the cellulose, lignin contents or sample weight as mg of TOC of the plant material (Additional file 1: Table S7).

In Additional file 1: Table S10, the black carbon content of the plant material was reported. No significant Pearson or Spearman correlations between black carbon content and sample weight as mg of TOC in Ivy, Beech and Thuja samples was observed (Additional file 1: Table S10). A significantly positive Pearson correlation between black carbon content and sample weight as mg of TOC was only observed in the Spruce sample ($r = 0.913$; Fig. 3a; Additional file 1: Table S10). The CV in all of the biomarkers were ranged between 33 and 52%.

Individual benzenepolycarboxylic acids and black carbon content in plant samples as function of total organic carbon with microwave-assisted oxidation process

The plant materials were also digested through microwave-assisted oxidation process to check the artificial formation of BPCA during high pressure digestion (Fig. 3b). The TOC content of the analyzed plants ranged between

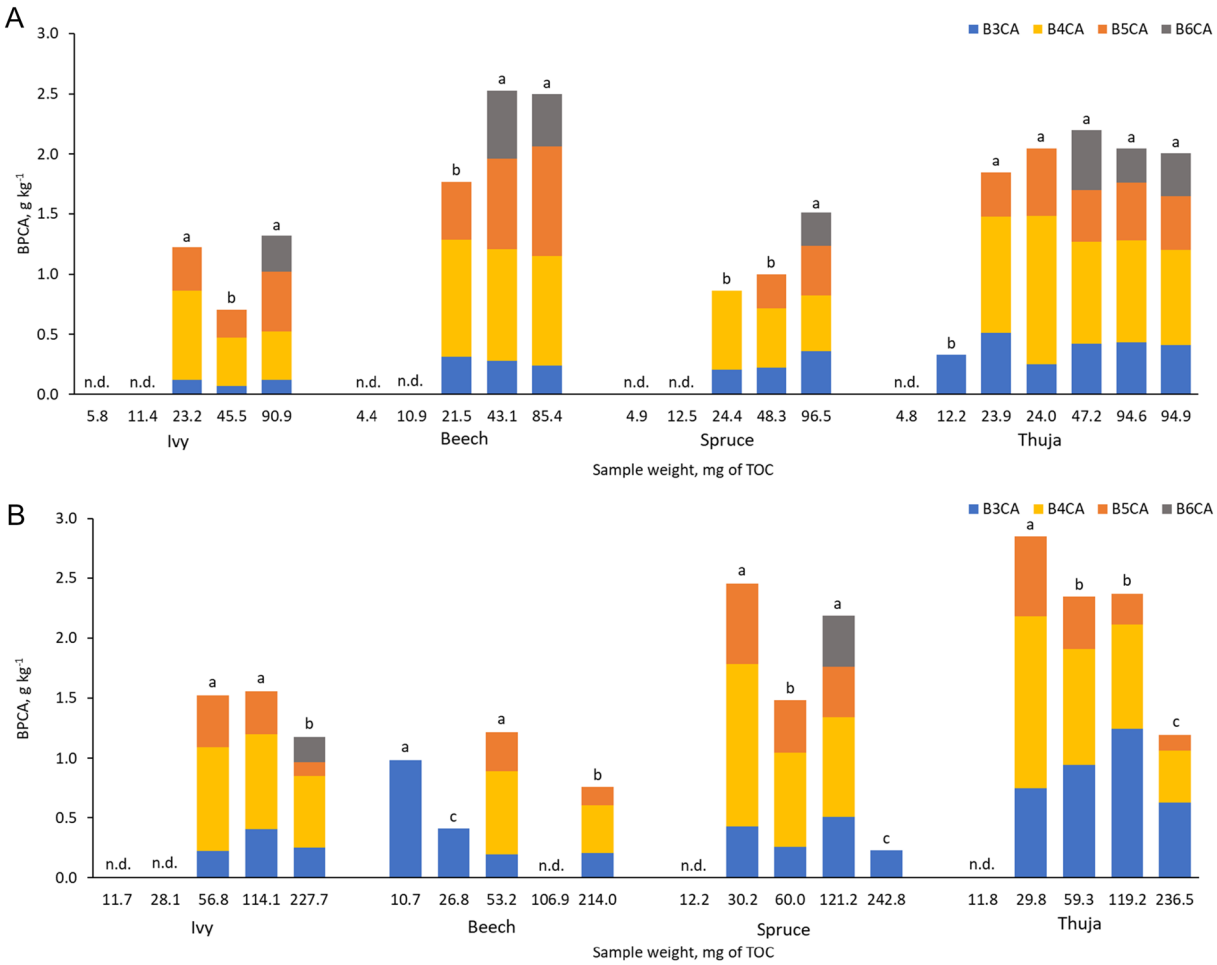


Fig. 3 Individual and sum of BPCA with three (B3CA), four (B4CA), five (B5CA) and six (B6CA) carboxylic groups of plant samples, as function of amount of total organic carbon (TOC) and thus, sample weight, after conventional high-pressure digestion (**a**) and microwave-assisted digestion (**b**). Different lowercase letters indicate significant differences among BPCA in the same soil profile $p < 0.05$

11 and 243 mg (Fig. 3b). No artificial formation of BPCA occurred when the TOC load was lower than 28 mg in Ivy leaves, 12 mg in Spruce leaves/needles, 12 mg in Thuja needles. In Beech leaves, only B3CA was produced when TOC load exceeded 10 mg (Fig. 3b). Unexpectedly, no artificial formation of BPCA was observed in Beech leaves by weighting 107 mg of TOC (Fig. 3b). Our results showed that by increasing the sample weight in all of the plant material no significant correlation between sample weight, as mg of TOC, and BPCA content, was observed (Fig. 3b). Furthermore, there was a greater variability of the results with this method compared to the conventional high-pressure digestion as shown by the CV (Additional file 1: Tables S11–S14). B3CA showed a positive correlation with lignin content ($r=0.911$; data not shown) and in fact a significant increase to 0.71 ± 0.46 g kg^{-1} in Thuja needles was observed compared with the other plant material (Additional file 1: Table S11). In this case, there was no clear relation of the B3CA content with the sample weight, as mg of TOC (Additional file 1: Table S11). Also for B4CA, B5CA and B6CA, no significant correlation with the amount of TOC was registered (Fig. 3b; Additional file 1: Tables S12–S14). Furthermore, the mean content of B4CA increased from 0.2 to 0.7 g kg^{-1} in Beech and Thuja samples, respectively (Additional file 1: Table S12), and for B5CA, the content increased from 0.1 and 0.3 g kg^{-1} in Beech and Thuja samples (Additional file 1: Table S13).

In addition, B6CA was produced only in Ivy leaves and in Spruce leaves/needles by weighting 228 (0.21 g kg^{-1}) and 121 (0.43 g kg^{-1}) mg of TOC (Fig. 3b). In Beech and in Thuja samples, no artificial formation of B6CA was observed when digestion was conducted with microwave-assisted oxidation (Fig. 3b; Additional file 1: Table S14).

Black carbon content ranged from 1.5 to 4.0 g kg^{-1} of Beech and Thuja samples, respectively (Fig. 3b; Additional file 1: Table S15). No significant correlation between black carbon content and sample weight, as mg of TOC, was observed (Fig. 3b; Additional file 1: Table S15).

Comparison between conventional and microwave-assisted oxidation process as function of organic carbon content

The results of the comparison assessed by a paired t-test between conventional and microwave-assisted oxidation processes are reported in Additional file 1: Table S16. It was not possible to run a t-test for B6CA, because in most cases of the microwave-assisted oxidation process, there was no artificial B6CA production (Fig. 3b and Additional file 1: Table S16).

A significantly higher B3CA content in the Ivy and Thuja samples (0.29 and 0.88 g kg^{-1}) treated with microwave-assisted oxidation process compared to the conventional method, was observed (Additional file 1: Table S16). In Beech and Spruce samples, no significant differences between the two methods were observed (Additional file 1: Table S16). Instead, B4CA and B5CA showed significantly higher contents in Beech leaves (0.95, and 0.74 g kg^{-1}, respectively), in the microwave-assisted oxidation process compared to the high-pressure digestion. Conversely, Ivy and Spruce leaves/needles showed a higher B4CA content in the high-pressure oxidation process (Additional file 1: Table S16). However, the black carbon content was not significantly different among the two oxidation methods in Ivy, Spruce and Thuja samples, with the exception of Beech leaves that showed a significantly higher black carbon content of 4.69 g kg^{-1} in the high-pressure oxidation process compared to the microwave-assisted process (Additional file 1: Table S16).

Summarizing, both oxidation methods generated BPCA when using plant material with more than about 10 mg of organic carbon. Although individual BPCA patterns are different for different oxidation methods and samples, there is no systematic difference between the two oxidation methods.

Discussion

The aim of this study was to asses if organic carbon load above 5 mg in soil and plant material could artificially produce BPCA during black carbon analysis. To check whether a potential artifact is caused by the high-pressure digestion, we analyzed samples also with microwave-assisted digestion in comparison.

The BPCA method was widely evaluated and discussed in literature [3, 14, 15, 17, 19]. Based on these discussions, there were several modifications of the original method published by Glaser et al. [2] regarding to sample weight used for analysis. Our analysis revealed no artificial BPCA formation when soil samples were analyzed up to the maximal sample weight possible for a good laboratory practice BPCA analysis corresponding to about 20 mg of TOC (Fig. 1). The same was true when high-pressure digestion was replaced by microwave-assisted digestion (Fig. 2). However, for plant samples, we observed nitric acid digestion-induced BPCA formation when TOC of digested plant samples exceeded about 20 mg TOC both with high-pressure digestion and microwave-assisted digestion (Fig. 3). Interestingly, this artifact did not increase when TOC amounts were further increased (Fig. 3).

Kappenberg et al. [17] found an amount-dependent BPCA formation of plant material and an amount effect on BPCA ratios. As possible explanation for digestion-induced BPCA formation they argued hydrothermal

carbonization, which is an exothermic process converting biomass at temperature from 180–250 °C and self-generated pressure for hydrochar [28]. However, the polycondensed aromatic moieties of chars are produced under oxygen-limiting conditions rather than in a strongly oxidizing environment such as nitric acid oxidation. Therefore, this explanation is not very likely. In addition, it has been shown that hydrochars are very low in BPCA content [29].

With respect to B6CA, we have observed an artificial production of mellitic acid only with TOC amount > 40 mg in Beech and Thuja and > 90 mg in Ivy and Spruce (Fig. 3), and there was no significant correlation among B6CA, lignin and cellulose contents (data not shown). During microwave-assisted oxidation process, B6CA was detected only in Ivy leaves when more than 200 mg of TOC was oxidized and in Spruce leaves/needles when more than 100 mg of TOC were oxidized (Fig. 3). Therefore, the BPCA patterns of plant material were different when high-pressure and microwave-assisted digestions were used (Fig. 3). One possible explanation could be that at high TOC amounts, the oxidation capacity of the acid used was not high enough to oxidize all organic materials thus producing BPCA through hydrothermal processes mentioned above.

Another explanation could be that in the conventional method the organic matter is oxidized for 8 h at 170 °C compared to the microwave-assisted digestion, which is only 1 h at 190 °C. Probably the time was essential for the production of artifacts such as high recalcitrant moieties especially in organic-rich material. In addition, we can also observe that in the microwave-assisted oxidation process there was a higher production of B3CA compared to the high-pressure digestion indicating that the artificially produced polyaromatic moieties have a lower degree of condensation.

The statement of Chang et al. [19] that mature humic substances with aromatic moieties could contribute to the BPCA and then to the black carbon content has nothing to do with method-induced BPCA formation. Instead, black carbon is certainly included as an integral constituent of soil organic matter during metabolization [3, 30].

It still remains unclear why strongly oxidizing hot nitric acid digestion produces BPCA when more than 20 mg TOC are digested alone (this study) while this does not occur when co-digested with mineral soils as shown by Brodowski et al. [16], which can be the focus of further studies. Nevertheless, we clearly showed that this effect does not occur when pure mineral soil samples are digested at quantities which can be handled with good laboratory practice, i.e., if the method is applied correctly.

Conclusion

We accept hypothesis (i) that no artificial BPCA formation occurs up to 10 mg TOC, which is in the range of any good laboratory practice BPCA analysis procedure with respect to reasonable sample aliquot. We accept hypothesis (ii) that artificial BPCA formation is dependent from the type of organic matrix. We demonstrated that lignin-rich organic materials such as Thuja produced more B3CA compared to other leaves in our study. Nevertheless, this contradicts our assumption that lignin would produce higher condensed aromatic moieties. We further accept hypothesis (iii) that artificial BPCA formation is independent from hot nitric acid oxidation technique that means that artificial BPCA formation must have reasons different from heating technique and heating time. Our results further demonstrated that method-induced BPCA formation does not only occur during high-pressure digestion, but also during microwave-assisted oxidation of black carbon to BPCA.

Overall, our results clearly show that there is no risk for artificial BPCA formation under good laboratory practice conditions for mineral soil samples. However, when using organic samples such as plant, peat, or biochar, sample equivalents containing not more than 10 mg of organic carbon should be used for BPCA analysis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00525-1.

Additional file 1. Table S1. Results (reported as mean ± SD) of the correlation between B3CA (Σ hemimellitic, trimellitic, trimesic acids) content and the respective soil samples differentiated according to different sample weight in terms of mg of TOC. **Table S2.** Results (reported as mean ± SD) of the correlation between B4CA (Σ pyromellitic, mellophanic, prehnitic acids) content and the respective soil samples differentiated according to different sample weight in terms of mg of TOC. **Table S3.** Results (reported as mean ± SD) of the correlation between B5CA (benzenepentacarboxylic acid) content and the respective soil samples differentiated according to different sample weight in terms of mg of TOC. **Table S4.** Results (reported as mean ± SD) of the correlation between B6CA (mellitic acid) content and the respective soil samples differentiated according to different sample weight in terms of mg of TOC. **Table S5.** Results (reported as mean ± SD) of the correlation between black carbon content and the respective soil samples differentiated according to different sample weight in terms of mg of TOC. **Table S6.** Results (reported as mean ± SD) of the correlation between B3CA (Σ hemimellitic, trimellitic, trimesic acids) content and the respective plant samples differentiated according to different sample weight in terms of mg of TOC. **Table S7.** Results (reported as mean ± SD) of the correlation between B4CA (Σ pyromellitic, mellophanic, prehnitic acids) content and the respective plant samples differentiated according to different sample weight in terms of mg of TOC. **Table S8.** Results (reported as mean ± SD) of the correlation between B5CA (benzenepentacarboxylic acid) content and the respective plant samples differentiated according to different sample weight in terms of mg of TOC. **Table S9.** Results (reported as mean ± SD) of the correlation between B6CA (mellitic acid) content and the respective plant samples differentiated according to different sample weight in terms of mg of TOC. **Table S10.** Results (reported as mean ± SD) of the correlation between black carbon content and the respective soil samples differentiated according to different sample weight in terms of mg of TOC. **Table S11.**

Results (reported as mean ± SD) of the correlation between B3CA (Σ hemimellitic, trimellitic, trimesic acids) content and the respective soil samples differentiated according to different sample weight in terms of mg of TOC. **Table S12.** Results (reported as mean ± SD) of the correlation between B4CA (Σ pyromellitic, mellophanic, prehnitic acids) content and the respective plant samples differentiated according to different sample weight in terms of mg of TOC. **Table S13.** Results (reported as mean ± SD) of the correlation between B5CA (benzene pentacarboxylic acid) content and the respective plant samples differentiated according to different sample weight in terms of mg of TOC. **Table S14.** Results (reported as mean ± SD) of the correlation between B6CA (mellitic acid) content and the respective plant samples differentiated according to different sample weight in terms of mg of TOC. **Table S15.** Results (reported as mean ± SD) of the correlation between black carbon content and the respective soil samples differentiated according to different sample weight in terms of mg of TOC. **Table S16.** Results of the comparison of the two methods: conventional and microwave assisted digestion process.

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BG and DRSG conceived the study, performed the initial literature search, and revised the manuscript. DRSG prepared the first draft of the manuscript, HM and TB performed the laboratory analysis and calculation of results, DRSG prepared the graphs.

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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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