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RAPID REPORT

Microwave-assisted combustion to produce benzene polycarboxylic acids as molecular markers for biochar identification and quantification

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Abstract
Biochar is a promising carbon dioxide removal (CDR) technology for climate change mitigation. Current procedures for its determination are lengthy, labor-intensive, and difficult to conduct. Benzene polycarboxylic acids (BPCA) are the most promising molecular markers for identification and quantification of biochar and its quality as they specifically represent the stable polyaromatic backbone of biochar. Therefore, using the BPCA method, its stability and, thus, its C sequestration potential could be used for CDR accounting. The current BPCA method relies on a specific high-pressure digestion apparatus, which is not available around the world. Therefore, the aims of the present work were (i) to compare the conventional high-pressure nitric acid oxidation with a microwave-assisted digestion technique and optimize the oxidation conditions in such a way that previous results are comparable with future ones, and (ii) to significantly reduce the digestion time of soil samples of 8 h and to develop a suitable routine method that produces comparable and reproducible results. For this purpose, soil and control sample series were prepared for different temperature–time-program. Obtained results were compared with the values of the conventional method both for individual samples and for the whole dataset separately. To ensure the representativeness of the results, in addition to various soil samples with different properties, we included two reference materials into our data set, one without biochar (wheat flour) and a biochar sample. Our results showed that conventional nitric acid oxidation in the BPCA determination at 170 °C and 8 h can be substituted by digestion in a microwave reaction system (CEM Mars6) at 190 °C and 1 h. Our results further showed that this condition needs to be strictly matched, because, otherwise, over- or underestimation of biochar quantity and/or quality will be the consequence. The goal of a less time-consuming BPCA extraction from soil samples was achieved by reducing the extraction time from 8 to 1 h using the microwave-assisted method. However, one disadvantage of the new method is that five times more sample material and chemicals are needed for further BPCA analysis, compared to the original method.

Keywords Biochar · Black carbon · Pyrogenic carbon · Analysis · Molecular marker · Microwave-assisted combustion

1 Introduction

Biochar has high potential for C sequestration and could be used as climate change mitigation option (Tisserant and Cherubini 2019) if the amount of stable carbon can be quantified correctly. The chemical structure of stable biochar consists of polycondensed aromatic moieties identical to black carbon, pyrogenic carbon, or charcoal (Glaser et al. 2002; Schimmelpfennig and Glaser 2012). Compared to other soil organic carbon compounds, it is chemically much more recalcitrant and thus offers the potential to bind carbon into the global carbon cycle in the long term and to reduce the concentration of greenhouse gases in the atmosphere (Brodowski et al. 2005; Glaser et al. 1998). Biochar can therefore function as a promising carbon dioxide removal (CDR) technology for climate change mitigation. In addition, there are other positive effects making biochar a game changer, such as the improvement of many other ecosystem services including soil fertility as impressively shown by the Terra Preta phenomenon (Glaser et al. 2002).

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However, the charred organic material spans a continuum from light charring over charcoal to graphite (Di Rauso Simeone et al. 2018), which makes biochar analysis quite complicated. In addition to optical, thermal, and spectroscopic techniques, the investigation of chemical degradation products of biochar has become established (Schmidt et al. 2001; Hammes et al. 2007). Benzene polycarboxylic acids (BPCA), which characterize the polyaromatic backbone of biochar, are analyzed as molecular markers for the quantification of black carbon, pyrogenic carbon, or biochar (Glaser et al. 1998). The so-called BPCA method is based on the principle of nitric acid oxidation of the polyaromatic building blocks of biochar with concentrated nitric acid under high pressure and high temperature. In detail, the highly aromatic core is decomposed with the formation of BPCA, which can be used as molecular markers for both quantification and detection or the degree of aromatization (Glaser et al. 1998). According to Glaser et al. (1998), it is urgently necessary to remove the polyvalent ions at least partially before BPCA production, since BPCA can precipitate and the black carbon content would later be underestimated. Since biochar has no fixed molecular structure, individual BPCA ratios can additionally be used to estimate the quality of biochar and thus, its stability, which can be used for carbon dioxide removal (CDR) accounting.

Depending on how much aromatic carbon is involved in the molecular structure, four BPCA groups are classified (Fig. 1). BPCA with three carboxyl groups are called benzenetricarboxylic acids (B3CAs, ∑ hemimellitic, trimellitic, and tremesic acids). The next degree of aromatization is represented by the benzene tetracarboxylic acids (B4CAs, ∑ pyromellitic, mellophanic, and prehnitic acids). Benzene pentacarboxylic acid (B5CA) is characterized by five and benzenehexacarboxylic acid (B6CA, mellitic acid) by six carboxyl groups. The differentiation of the BPCA structures enables statements to be made about the degree of condensation of biochar and the pyrolysis temperature. It is also necessary to eliminate the polyvalent ions using cation-exchange resins after the digestion, since BPCA belong to the complexing agents. Finally, the extracted BPCA are prepared for gas chromatographic analysis by derivatization. The polyaromatic portion of the sample can finally be analyzed on a molecular level.

It should be mentioned, however, that the BPCA procedure does not provide any information about their origin. The proportion of polycondensed aromatic carbon in soil can either be caused by the influence of temperature, i.e., pyrogenic, or can be of biological origin (Glaser and Knorr 2008). To confirm whether it really is of pyrogenic carbon, a further variable needs to be introduced such as the use of (stable) isotope labeling techniques.

Due to the long digestion time of 8 h with conventional analysis technology (Glaser et al. 1998), alternative and less time-consuming procedures are required. The present work focused on accelerating, optimizing, and replacing the existing routine method for the determination of black carbon in soil samples using a microwave digestion system. The main difference between the two methods is that the black carbon digestion in the microwave and the BPCA extraction in a pressure digestion block kept at a certain

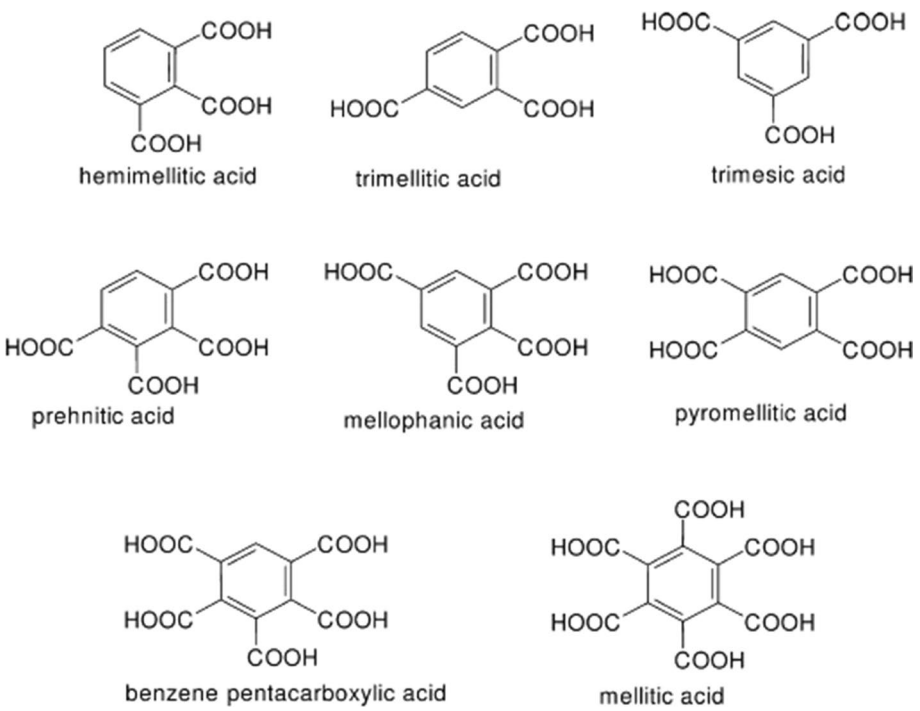

Fig. 1 Structural formulas of the various positional isomers of benzene polycarboxylic acids, which are used as markers for black carbon analysis (Glaser et al. 1998)

temperature are based on a different type of heating. In the conventional method, the principle of heat conduction and convection come into play, whereby a transfer of heat energy from the source to the object is characteristic. In this case, heating takes place from the outside in. The electromagnetic energy of the microwave, on the other hand, acts directly on the molecules inside by causing dipole rotations and ion conduction, which is then converted into heat energy. The exothermic reaction therefore takes place from the inside out. The electromagnetic waves are defined in a frequency range between 0.3 and 300 GHz (Lopez-Avila 2000). With this process technology, the enthalpy of reaction is basically adsorbed in the solvent and transferred to other molecules in the form of heat to change the rotational states in the molecule (Kou and Mitra 2000). Ion conduction describes the movement of electrophoretic ions in solution when an electromagnetic field is applied. The ionic conduction and the solution's resistance to ion flow create friction and ultimately thermal energy. Dipole rotation is a re-orientation of the dipoles under the influence of microwave radiation. This means that the molecules rotate permanently through their dipoles to align themselves with the electromagnetic field. This characteristic of molecules and ionic solutions, absorbing microwave energy, creates permanent dipole moments. From this, it can be concluded that non-polar solvents cannot be heated by microwaves, because there are no dipoles. In comparison, the microwave heats the solvent mixture of the sample much faster thanks to its special reaction mechanisms, thus reducing the extraction time. Furthermore, the sample throughput is much higher, since several samples can run through a single extraction process at the same time in the multi-vessel system suitable for the microwave. Another major positive side effect of microwave extraction is the reduction in the consumption of chemicals. This means that the associated contamination and the costs for sample preparation can be minimized. In addition, the recovery of the analytes and their reproducibility are better (Eskilsson and Bjorklund 2000). Furthermore, this analysis technology offers precise and controlled temperature regulation in the extraction process with significantly reduced workload.

2 Material and methods

2.1 Soil samples

All samples were air-dried (30–40 °C), sieved (≤ 2 mm) and ground before analysis. Four soils with different properties (Table 1) and two reference materials were selected for method comparison. Each sample was replicated three times. A laboratory standard was used as a test control, because its content of black carbon was known from several previous analyses. It is a Chernozem from Etzdorf (Germany), characterized by a silty, clayey loam texture and consisting mainly of silt (63%) and clay (31%). The Luvic Chernozem from Bornhoeck (Germany) had a sandy clay loam texture. In addition, the sample contained carbonate and had the highest pH value of 7.7 among the tested soils, as well as the lowest level of total organic carbon (TOC) and black carbon. The Ferralsol was from Santarem (Brazil) with a heavy clay texture consisting of 70% clay. Terra Preta is a famous Anthrosol from Brazil (Santarem) and also a heavy clay soil with a clay content of 96%. Furthermore, it contained the highest amounts of TOC (65.2 g kg^{-1}) and black carbon (16.3 g kg^{-1}) compared to the other soils examined (Di Rauso Simeone et al. 2018). Wheat flour was used as a negative control with a black carbon yield of 0% to check for artificial BPCA generation during analysis. The charcoal sample represented the positive control and should have ideally a black carbon content of 100%.

2.2 Extraction of benzene polycarboxylic acids

For the BPCA analysis, the conventional and microwave-assisted digestion methods were compared in this work. For this purpose, all samples, both process technologies, were subjected to hydrolysis with 4 M TFA before extraction. After adding 10 mL of 4 M TFA each, the samples were heated for 4 h at 105 °C in a drying cabinet (Brodowski et al. 2005). Subsequently, both the sediments and the hydrolysates were pipetted onto a binder-free glass fiber filter with a broken-off Pasteur pipette and filtered. The filter was placed in a Buchner funnel on a suction bottle with a

Table 1 Relevant properties of the soils used in the course of the method comparison (Di Rauso Simeone et al. 2018)

Soil type	Country	Location	Texture	Sand (%)	Silt (%)	Clay (%)	pH (KCI)	TOC[a] (g kg^{-1})	BC[b] (g kg^{-1})
Chernozem	Germany	Etzdorf	Silty clay loam	6.0	63.0	31.0	5.7	22.0	3.25
Luvric Chernozem	Germany	Bornhöck	Sandy clay loam	48.1	25.3	25.9	7.7 ($Cacl_2$)	1.0	0.38
Ferrasol	Brasil	Santarem	Heavy clay	15.0	15.2	69.7	3.8	14.0	1.05
Anthrosol	Brasil	Santarem	Heavy clay	2.9	1.6	95.6	6.0	65.2	16.3

[a]Content of the total organic carbon

[b]Content of black carbon

pump. To counteract quantitative loss of the soil samples, the hydrolysis glasses (25 mL) to be pipetted were rinsed at least three times with deionized water, as were the glass filters to eliminate multivalent cations. The resulting filtrates were discarded and only the filter residues were placed on the large watch glasses corresponding to the filter. The cleaned samples were then placed in the drying cabinet at 30–40 °C for at least 2 h. All samples were subject to the same conditions prior to the BPCA extraction.

2.3 Conventional nitric acid oxidation

For the conventional nitric acid oxidation process, about 500 mg of soil samples and about 10 mg of wheat flour or charcoal were used. With this method, the dried sample residues from the previous filtration were transferred with a spatula onto creased weighing paper or the part covered with the sample was cut out. The samples were then transferred to suitable digestion glasses made of quartz glass with a lid for the pressure digestion apparatus. Furthermore, 2 mL of 65% nitric acid (HNO_3) was added to the filter residues, the samples being completely wetted with the concentrated acid. The solution was then kept at 170 °C in a pressure digestion block for 8 h and digested. The pyrogenic carbon in the samples was oxidized to the corresponding aromatic acids (Fig. 1). After removal from the digestion cabinet, the samples had cooled down and were ready to be rinsed and filtered off with the Dowex columns for the next cleaning step.

2.4 Microwave-assisted nitric acid oxidation

In the case of microwave-assisted extraction, about 2.5 g of soil samples or 50 mg of wheat flour or charcoal was used. After TFA hydrolyzed as described above, the samples were placed in 55 mL Teflon reaction vessels (Xpress vessels). The CEM Mars6 microwave reaction system enables up to 40 samples to be digested in one run. The containers used, however, have a volume of 55 mL and stipulate a fill quantity of at least 5 mL, since the sample must be completely wetted with the solvent for the volume-dependent microwave extraction. For this reason, the weights for the special digestion vessels were scaled up by a factor of 2.5, which corresponds to the concentration ratio of the conventional method and thus enables a proper method comparison. As a result, 5 mL of 65% nitric acid was added to these filter residues, transferred to the special digestion vessels, and tightly sealed. Several series of measurements, which were subjected to different temperature and time programs, were examined to optimize the method. The sample sets were oxidized for 0.5, 1, 2, or 4 h both at 170 °C and 190 °C in the CEM microwave digestion instrument. The respective sets of samples for each test series were all simultaneously heated to the desired temperatures (170 °C and 190 °C) within 30 min and held there for the selected periods of time (0.5, 1, 2, or 4 h). Further parameter settings were tested, such as higher oxidation temperatures (210 °C) or longer digestion times, such as the 8 h of the conventional method (data not shown). However, these proved to be unsuitable, because the samples decomposed under these conditions and no analytes were therefore available for evaluation.

2.5 Cleaning in glass columns with ion-exchange resin

The elimination of polyvalent cations (such as Fe^{3+}, Al^{3+}, Ca^{2+} or Mg^{2+}) is particularly important in BPCA analysis and is achieved using cation-exchange chromatography (Dowex 50 W × 8, 200–400 mesh, Sigma Aldrich, St. Louis, MO, USA). After the BPCA had been successfully extracted with both methods, the solutions were filtered off with the help of a cellulose filter in volumetric flasks and rinsed at least three times with deionized water. The volumetric flask of the conventional method was made up to 10 mL and to 25 mL for the microwave-assisted extraction (MAE), respectively. The solutions were then transferred to a tightly sealed storage vessel. The Dowex columns were then prepared by placing glass wool in the columns to prevent the cation-exchange resin from permeating. This is intensively rinsed before and after being introduced into the column and then conditioned. Then, 2 mL aliquots of the digestion solutions were pipetted into pointed flasks (25 mL) and diluted with 4 mL distilled water and 100 µL internal standard 1 (IS1, phthalic acid) was added. Furthermore, pointed flasks (100 mL) were placed under the prepared columns to collect the purified eluates. The sample solutions were then poured onto the cation-exchange resin in the Dowex columns using a Pasteur pipette. After the first 6 mL had run through completely, the pointed flasks (25 mL) from which the sample solutions were pipetted were each rinsed three times with 1 mL of deionized water and also transferred to the columns. This was followed by five further rinsing steps of 10 mL deionized water for cleaning polyvalent cations via the columns. It must be ensured that the columns have run through completely before each elution, but that they have not run dry either. Finally, the eluates were collected in the 100 mL pointed flask, deep-frozen, and freeze-dried for approximately 15–20 h.

2.6 Derivatization

A derivatization with a strong ensiling reagent before the measurement on a gas chromatograph with a flame ionization detector (GC/FID) was necessary. During derivatization, strongly polar groups are converted into less strongly polar groups. The derivatives formed have increased volatility and increased thermal stability. For this purpose, the

purified sample solutions were transferred with 1 mL of methanol into reaction vials (5 mL) with a septum and lid and the pointed flasks were rinsed three times with methanol using a Pasteur pipette. They were then exposed to an ultrasonic bath to dissolve them further. In addition, 100 µL of IS2 solution (internal standard 2, biphenyl-2,2-dicarboxylic acid) was added to each sample and evaporated under nitrogen until it was completely dry. For the subsequent derivatization of the samples and standards, 98 µL BSTFA (*N*,*O*-bis(trimethylsily)-trifluoroacetamide), 2 µL TSIM (*N*-trimethylsilylimidazole), and 50 µL Toluene were added. Then, the vials were closed tightly and shaken with a vortex shaker. Next, the sample solutions were heated for 2 h in an aluminum block preheated to 90 °C. Finally, after cooling, the derivatives were transferred to GC autosampler vials with inlets and tightly sealed with tongs, so that they were ready for the subsequent GC/FID analysis. To determine possible losses of BPCA during the previous analysis steps, a defined amount of IS1 was added prior to the glass column sample cleanup, of which a certain proportion was found during the measurement. The difference between the added and measured IS1 is defined as recovery. All results were corrected to a recovery of 100%. The IS2 contains information about the quality of the measurement preparation process. The external standards included all aromatic acids, as well as IS1 and IS2. The IS1 was previously added in the concentration of the external standard (10 µL, 25 µL, 50 µL, 100 µL, 250 µL, and 500 µL). This means that the concentration of IS1 is steadily increasing, while the concentration of IS2 remained constant, as 100 µL of it was always added to samples and standards.

2.7 Gas chromatography separation and flame ionization detection

After the trimethylsilyl (TMS) derivatives had cooled, the GC analysis was carried out by capillary gas chromatography using the Shimadzu GC 2010 instrument (Shimadzu Ltd., Tokyo, Japan). The device was equipped with a Sulpeco SPB5 capillary column (length: 30 m, inner diameter: 0.32 mm, film thickness: 0.25 µm) and a flame ionization detector. Helium was used as carrier gas with a constant flow rate of 0.8 mL min^{-1}. The detector temperature was kept at 310 °C and the injector temperature at 300 °C. 2 µL aliquots were injected at a split ratio of 1:30 into the fully deactivated inlet system, where the samples were vaporized on a silylated liner and transported with the carrier gas. The temperature program began by holding the column temperature at 100 °C for 2 min. The temperature was then raised to 240 °C at 20 °C per minute and held for 7 min. Finally, the temperature was increased further to 300 °C at 30 °C per minute and was held for 10 min. This resulted in a total running time of 28 min for GC analysis with FID. Black carbon content was obtained by multiplying sum of BPCA content by 2.27 (Glaser et al. 1998). Recovery was between 81 and 113%. Linearity ranged from 5 to 250 µg BPCA per vial. Reproducibility is given in Fig. 2 (standard error, $N=3$).

2.8 Visualization and statistical evaluation

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 reliability of the results, the regression coefficients intercept and slope, the correlation coefficient r; the determination coefficient R^2, and the significance value p were determined and presented in a table for the total black carbon content and for the yield of each BCPA. In addition, since some samples for the digestions at 190 °C and 4 h had been completely decomposed during the extraction process, there were no measured data for evaluation and were therefore marked as "n.d." (no data/not determinated/detected) in the tables.

3 Results

3.1 Sum of all BPCA and conversion to black carbon (biochar)

In Fig. 2, the black carbon content of all samples and procedures under study is shown comparatively. At first glance, it could be seen that the various black carbon digestions provided relatively comparable results within individual samples except for the Luvic Chernozem. The values of Chernozem, Ferrasol, Terra Preta, wheat flour, and charcoal samples all approach those of the conventional method. It is obvious that the results of the MAE at 190 °C and 1 h were closest to those of the conventional procedure (dashed line in Fig. 2), especially for the Chernozem, Ferrasol, Terra Preta, and charcoal. However, here too, there are irregularities regarding the Luvic Chernozem sample, which were neglected in the method evaluation. The missing values of the wheat flour samples proved that none of the methods artificially produced black carbon. We also tested 210 °C and 150 °C. However, both were not suitable because of either drying out or no sufficient digestion of biochar samples (data not shown). Results of the regression analysis are summarized in Table 2. All regressions were highly significant ($p \leq 0.001$). The coefficient of determination R^2 in terms of the total content of black carbon was between 0.9935 (190 °C and 2 h) and 1 (190 °C and 1 h) and numerically confirmed the functionality of the MAE under all presented conditions (except 190 °C and 4 h), as they approached the value 1. As a result, it is also possible to carry out black carbon determinations under the remaining conditions. In particular, the MAE with a coefficient of determination R^2

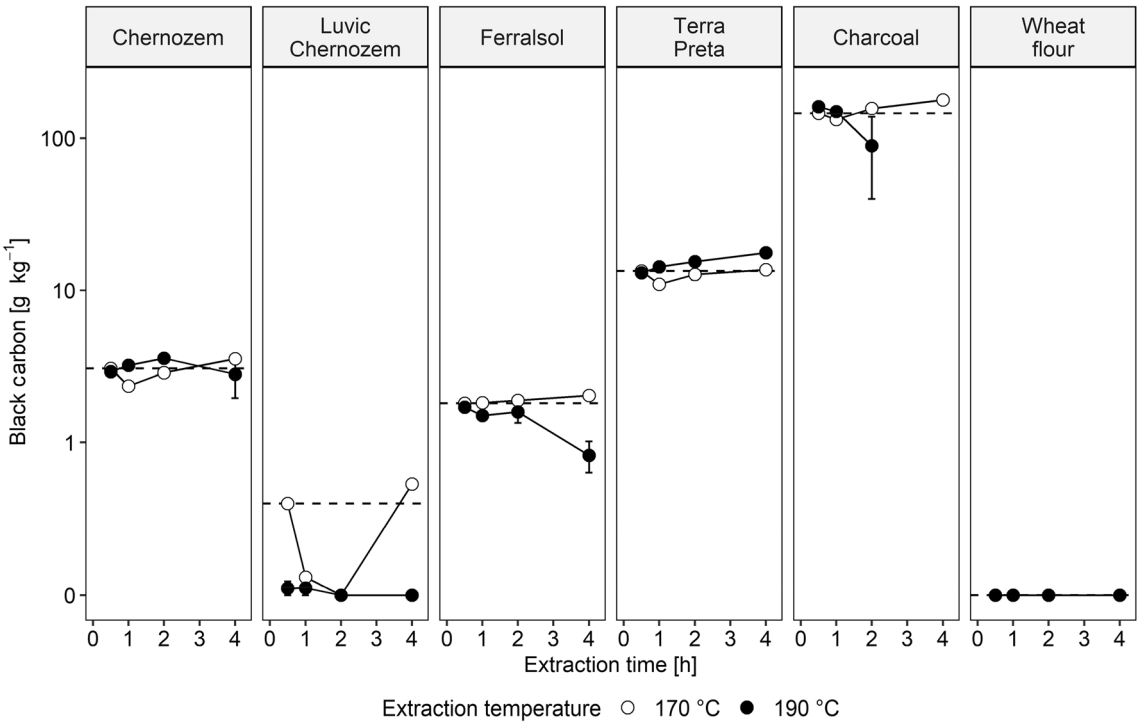

Fig. 2 Comparison of black carbon content in the samples between the conventional nitric acid oxidation (dashed lines) and the microwaved-assisted digestions differed according to the various extraction times at 170 °C (white) and 190 °C (black). Error bars represent standard errors of replicated digestions ($n = 3$)

of 1 at an oxidation temperature of 190 °C and an extraction time of 1 h stand out in Table 2 and provide the most comparable results. The intercept ranged between -0.59 (170 °C and 4 h) and 1.77 (190 °C and 2 h) and the extraction at 190 °C and 1 h in the microwave achieved again the best result in the data evaluation with an intercept of − 0.05. The slope of the total black carbon results ranged from 0.60 (190 °C and 2 h) to 1.23 (170 °C and 4 h). And here again, the most ideal values were found for the MAE at 1 h and 190 °C with a slope of 1.03, which almost described a 1:1 correlation. Furthermore, the correlation coefficient revealed that it approached the value 1 in all cases and that it must therefore always be a positive linear relationship. Using the values in Table 2, it can also be deduced that the weaker the oxidation condition, the longer the required digestion time for BPCAs in the microwave.

3.2 Individual benzene polycarboxylic acids

The graphic representation of the results of the B3CA yields (Fig. 3) revealed excellent comparability for the soil samples but not for the charcoal sample. The series of measurements of the charcoal samples showed that the data from the conventional method could not be

Table 2 Results of the correlation analysis of the black carbon content in the respective sample series differentiated according to the extraction conditions using various temperature–time programs in the microwave (axis intercept, slope coefficients, coefficient of determination R^2, correlation coefficient r, and p value)

	170 °C				190 °C			
	0.5 h	1 h	2 h	4 h	0.5 h	1 h	2 h	4 h
Slope	0.85	0.91	1.08	1.23	1.11	1.03	0.60	n.d.*
Intercept	− 0.36	− 0.35	− 0.52	− 0.59	− 0.58	− 0.05	1.77	n.d
R^2	0.9998	0.9999	0.9999	0.9998	0.9999	1.0000	0.9935	n.d
r	0.9999	0.9999	0.9999	0.9999	0.9999	1.0000	0.9967	n.d
p	0.001	0.001	0.001	0.001	0.001	0.001	0.001	n.d

*No data (sample decomposed)

represented satisfactorily by the strongly fluctuating B3CA yields of the microwave digestions. For the Chernozem sample, the microwave-assisted digestion at 190 °C and 1 h, for the Ferrasol at 170 °C and 2 h and for the Terra Preta at 190 °C and 0.5 h achieved the most comparable results. Regarding to the B3CA yields (Table 3), the coefficient of determination R^2 was between 0.9584 (190 °C and 2 h) and 0.9989 (170 °C and 0.5 h) and the slope between 0.51 (190 °C and 2 h) and 0.94 (170 °C and 4 h). The intercept showed a minimum value of 0.02 (190 °C and 1 h) to a maximum value of 0.07 (190 °C and 2 h) for the B3CA content. The MAE at 190 °C and 1 h presents the best intercept of 0.02. All of the microwave digestions are suitable for hemimellitic acid, trimellitic acid, and tremesic acid, but none of them stand out extraordinary well.

The results of B4CA in the soil samples confirmed graphically that the nitric acid oxidation at 190 °C and 1 h digestion time fitted best to the results of the conventional method and again, discrepancies among different digestion conditions were highest for the charcoal sample (Fig. 4). The B4CA results in Table 4 show values of R^2 between 0.9927 (190 °C and 0.5 h) and 0.9998 (170 °C and 1 h and 4 h), with all R^2; values determined here. The data analysis provided an acceptable result and did not make any clear differences between the methods. The slope ranged from 0.72 (170 °C and 2 h) to 1.08 (190 °C and 2 h) and the intercept

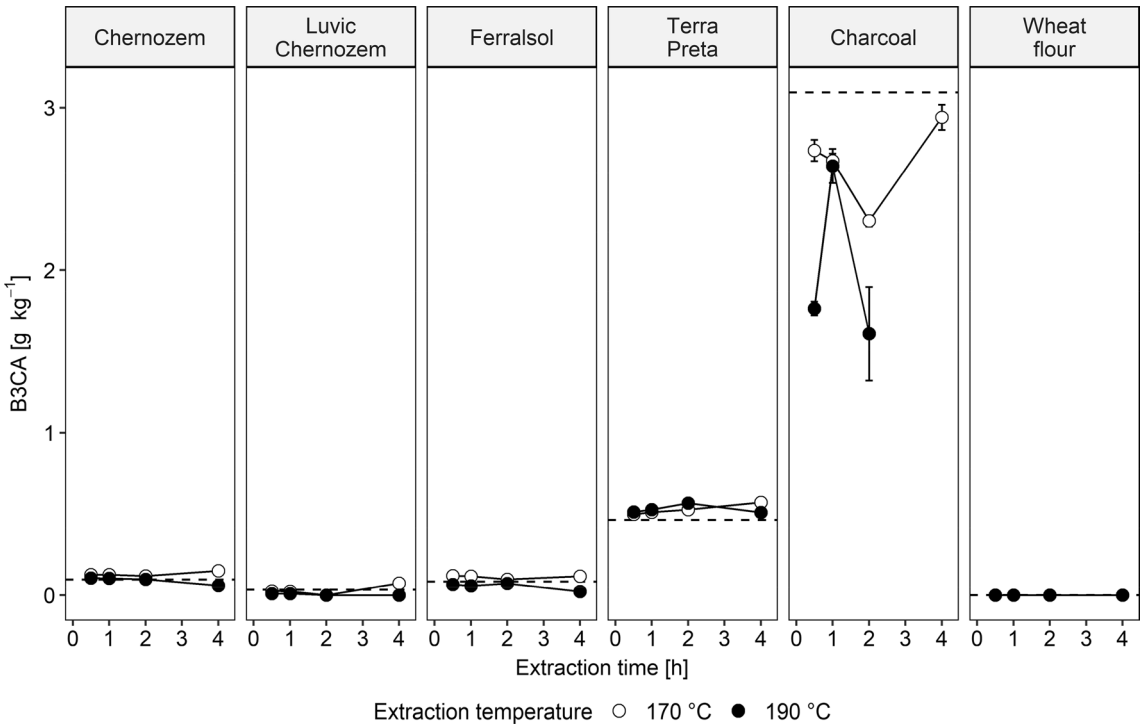

Fig. 3 Amount of B3CA (∑ hemimellitic, trimellitic, trimesic acids) in the samples extracted by conventional nitric acid oxidation (dashed lines) compared to the B3CA content in the microwave digestions at 170 °C (white) and 190 °C (black) for different extraction times. Error bars represent standard errors of replicated digestions ($n = 3$)

Table 3 Correlation analysis for the proportions of B3CAs (benzene tricarboxylic acids, ∑ hemimellitic, trimellitic, and trimesic acids) in the respective sample series differentiated according to the extraction conditions using various temperature–time programs in the microwave

	170 °C				190 °C			
	0.5 h	1 h	2 h	4 h	0.5 h	1 h	2 h	4 h
Slope	0.88	0.86	0.74	0.94	0.56	0.85	0.51	n.d.*
Intercept	0.03	0.04	0.05	0.05	0.06	0.02	0.07	n.d
R^2	0.9989	0.9983	0.9933	0.9984	0.9798	0.9971	0.9584	n.d
r	0.9994	0.9991	0.9966	0.9992	0.9898	0.9985	0.9790	n.d
p	0.001	0.001	0.001	0.001	0.001	0.001	0.001	n.d

*No data (sample decomposed, limit conditions for analytes have been exceeded)

of at least − 0.04 (170 °C and 0.5 h) to a maximum of 0.09 (170 °C and 2 h). The MAE at 190 °C and 1 h showed again the best results to replace the conventional method (intercept 0.00; slope 1.02). These values most closely suggest a 1:1 relationship between the data for the conventional and microwave-assisted methods. The results of the distribution of the B5CA content in the various soil samples also showed that the nitric acid oxidation taking place in the microwave at 190 °C and 1 h extraction time represented the results of the conventional digestion most satisfactorily (Fig. 5). The samples Chernozem, Terra Preta, and charcoal in particular visualize an almost perfect match, while the discrepancies for all other conditions are obvious. The coefficient of determination R^2 for the data from B5CA (Table 5) is between 0.9291 (190 °C and 2 h) and 0.9999 (170 °C and 2 h). It follows that the coefficient of determination R^2 of the values of the B5CA analysis confirms the comparability of the results of the microwave-supported method with those of the conventional method. The slope in B5CA yields ranged from 0.72 (190 °C and 2 h) to 1.11 (190 °C and 0.5 h) and the intercept from − 0.12 (190 °C and 0.5 h) to 0.12 (190 °C and 2 h). With an intercept of 0.00 and a slope of 1.01, the MAE at 190 °C and 1 h also provided the best results for comparability with the conventional treatment.

The data of B6CA among the different methods and conditions also showed digestion at 190 °C and 1 h in the

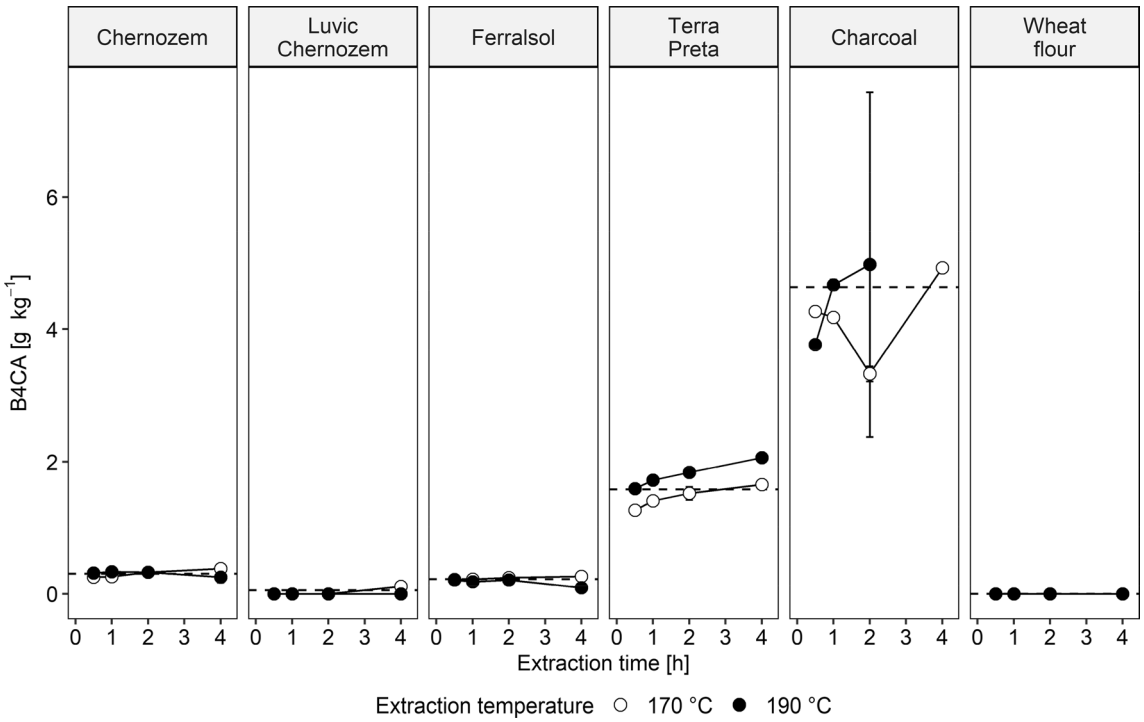

Fig. 4 Yield of B4CA (∑ pyromellitic, mellophanic, prehnitic acids) in the sample series extracted by conventional nitric acid oxidation (dashed lines) compared to the B4CA content in the microwave digestions at 170 °C (white) and 190 °C (black) for different extraction times. Error bars represent standard errors of replicated digestions ($n = 3$)

Table 4 Correlation analysis for the proportions of B4CAs (benzene tetracarboxylic acids, ∑ pyromellitic, mellophanic, and prehnitic acids) in the respective sample series differentiated according to the extraction conditions using various temperature–time programs in the microwave

	170 °C				190 °C			
	0.5 h	1 h	2 h	4 h	0.5 h	1 h	2 h	4 h
Slope	0.92	0.90	0.72	1.05	0.82	1.02	1.08	n.d*
Intercept	− 0.04	− 0.01	0.09	0.03	0.05	0.00	0.00	n.d
R^2	0.9980	0.9998	0.9859	0.9998	0.9927	0.9987	0.9988	n.d
r	0.9990	0.9999	0.9929	0.9999	0.9963	0.9993	0.9994	n.d
p	0.001	0.001	0.001	0.001	0.001	0.001	0.001	n.d

*No data (sample decomposed, limit conditions for analytes have been exceeded)

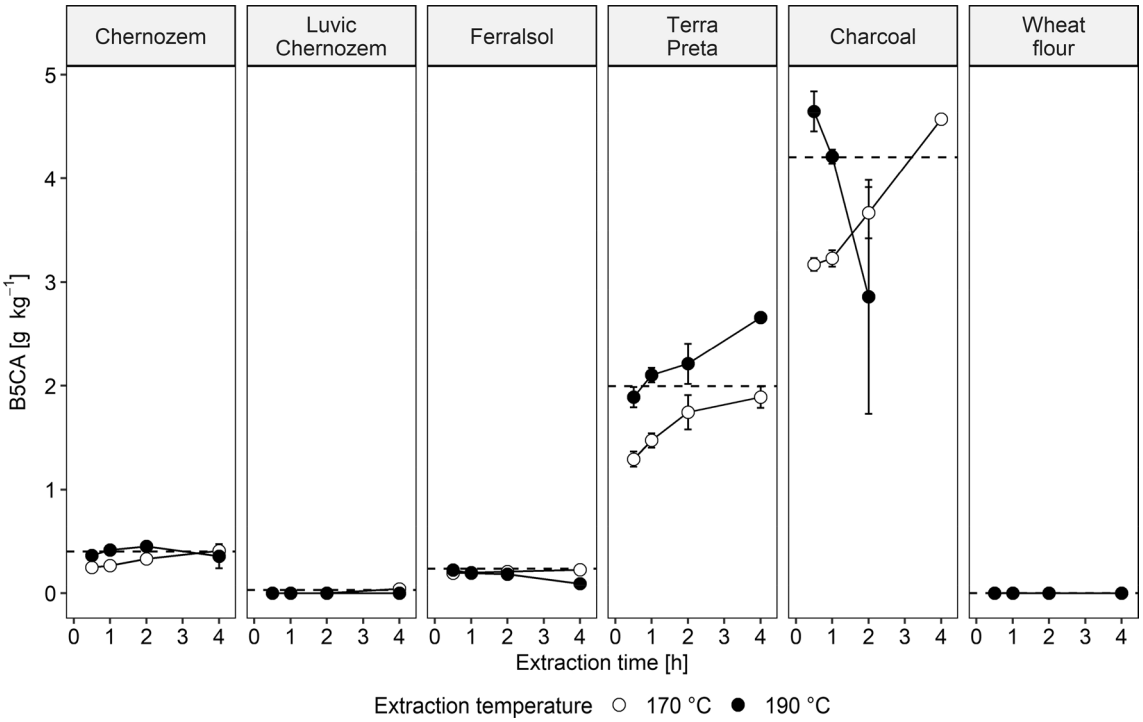

Fig. 5 B5CA content (benzene pentacarboxylic acid) in the sample series extracted by conventional nitric acid oxidation (dashed lines) compared to the B5CA yields of the microwave digestions at 170 °C (white) and 190 °C (black) for different extraction times. Error bars represent standard errors of replicated digestions ($n = 3$)

Table 5 Correlation analysis for the proportions of B5CA (benzene pentacarboxylic acid) in the respective sample series differentiated according to the extraction conditions using various temperature–time programs in the microwave

	170 °C				190 °C			
	0.5 h	1 h	2 h	4 h	0.5 h	1 h	2 h	4 h
Slope	0.74	0.77	0.88	1.07	1.11	1.01	0.72	n.d.*
Intercept	−0.03	−0.02	−0.01	−0.04	−0.12	0.00	0.12	n.d
R^2	0.9955	0.9995	0.9999	0.9964	0.9929	0.9992	0.9291	n.d
r	0.9977	0.9997	0.9999	0.9982	0.9964	0.9996	0.9639	n.d
p	0.001	0.001	0.001	0.001	0.001	0.001	0.001	n.d

*No data (sample decomposed, limit conditions for analytes have been exceeded)

microwave as a clear favorite (Fig. 6). Table 6 shows the statistical results for the B6CA regression analysis, with a coefficient of determination R^2 between 0.9987 (190 °C and 2 h) and 1 (all other except for 190 °C and 4 h). The slope of the B6CA yields was defined in a range between 0.57 (190 °C and 2 h) and 1.27 (170 °C and 4 h) and for the intercept between −0.13 (190 °C and 0.5 h) and 0.31 (190 °C and 2 h). Here, too, the MAE at 190 °C and 1 h showed the best results for the comparison of methods with an intercept of −0.03 and a slope of 1.04. It was noticeable in the data analysis that the intercept for the 190 °C digestion at 1 h always represented the most ideal results, which confirms the results of the data for black carbon in Table 2. Also when considering the slope, the values for the MAE at 190 °C and 1 h were almost always the most suitable, except for the B3CA yields.

4 Discussion

The aim of this study was to improve the existing BPCA method for black carbon determination in soil samples with respect to being less time-consuming and easier to use than conventional nitric acid oxidation, but at the same time producing reproducible and comparable results. For this purpose, the conventional digestion process was compared to a microwave-assisted analysis technique. In particular, the choice of digestion parameters was fundamental and a prerequisite for ideal conditions for the BPCA

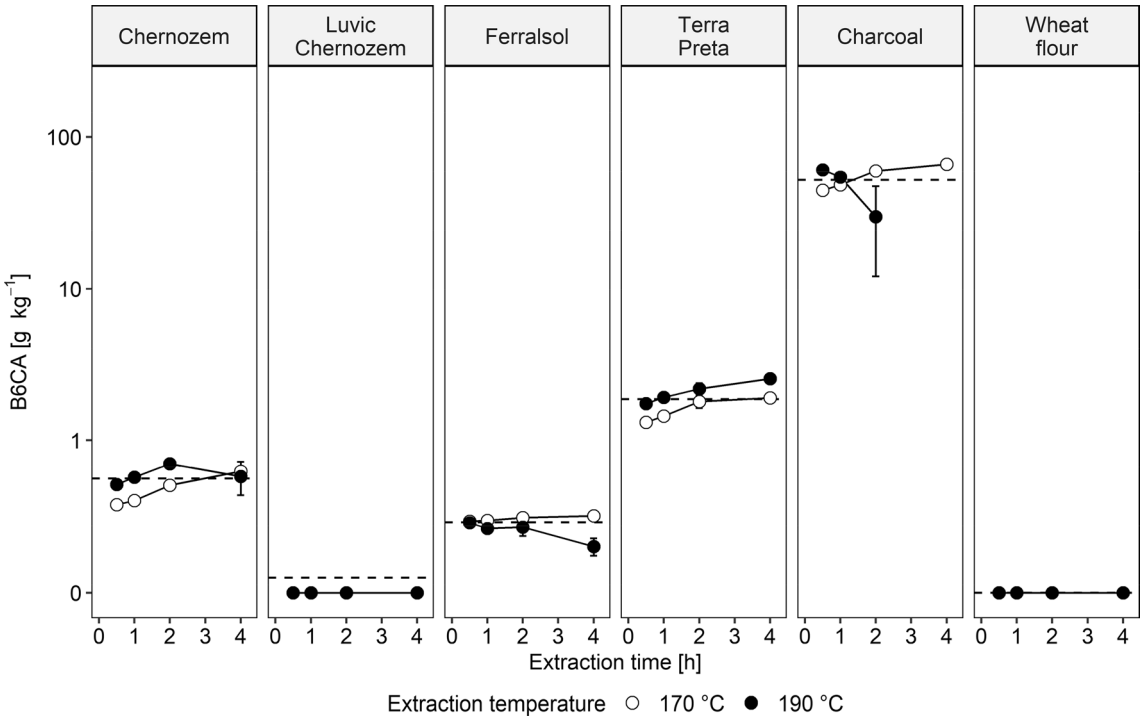

Fig. 6 Comparison of the B6CA content (mellitic acid) in the sample series extracted by conventional nitric acid oxidation (dashed lines) with the B6CA yields of the microwave-assisted digestions at 170 °C (white) and 190 °C (black) for different extraction times. Error bars represent standard errors of replicated digestions ($n = 3$)

Table 6 Correlation analysis for the proportions of B6CA (mellitic acid) in the respective sample series differentiated according to the extraction conditions by using various temperature–time programs in the microwave

	170 °C				190 °C			
	0.5 h	1 h	2 h	4 h	0.5 h	1 h	2 h	4 h
Slope	0.85	0.93	1.15	1.27	1.17	1.04	0.57	n.d.*
Intercept	−0.08	−0.08	−0.11	−0.12	−0.13	−0.03	0.31	n.d
R^2	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9987	n.d
r	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9993	n.d
p	0.001	0.001	0.001	0.001	0.001	0.001	0.001	n.d

*No data (sample decomposed, limit conditions for analytes have been exceeded)

digestion. Accordingly, the microwave settings were aimed at the maximum recovery of the analyzed analytes (BPCA) with a minimum of extraction time. A meaningful evaluation of the data and a comparison of both methods were then possible. In addition to various soil samples, we included a reference soil with known black carbon values as well as wheat flour with no black carbon and a charcoal sample, which should ideally yield 100% black carbon to ensure the functionality of the method. The results of the reference material flour showed that neither for the yield of total black carbon (Fig. 2) nor for the individual BPCA (Figs. 3, 4, 5, 6), any artifacts were generated by the method under the set microwave parameters. In the course of this, we were able to confirm the statement of another study, which assumes the absence of benzene polycarboxylic acids in all blank materials for their determination (Dittmar 2008). Furthermore, the reference material charcoal proved that every microwave-assisted digestion under the conditions tested represented black carbon digestion, with the exception of the microwave digestion at 4 h and 190 °C. Under these microwave parameters, the samples evaporated completely during the digestion and these samples were excluded from further analysis. All further microwave digestions were reproducible in the selected ranges of 0.5, 1, 2, and 4 h at 170 °C, and 0.5, 1, and 2 h at 190 °C. Black carbon data at 170 °C showed a direct proportionality of the increasing yields with increasing extraction time and the values increasingly resembled those of the conventional method. The most ideal result in the microwave test series, however, was provided by

the nitric acid oxidation digestion at 190 °C and 1 h. In addition, the reference material charcoal showed that the longer the digestion was carried out at 190 °C, the more losses of BPCA were associated with it (Fig. 2). The difference between the results of the digestion at 170 °C and 4 h and the digestion at 190 °C and 1 h became apparent only when the individual BPCA were examined. Figures 4, 5, and 6 in particular show that the BPCA content at 1 h and 190 °C, especially for Chernozem, Ferrasol, Terra Preta, and charcoal samples, provided more favorable results for the data comparison than those of the digestion at 170 °C and 4 h. This was also confirmed by the statistical data analysis shown in Table 2. For the total yield of black carbon, the digestion at 190 °C and 1 h with a slope of 1.03, an intercept of − 0.05, and a coefficient of determination R^2 of 1 represent the values which relate to each other are best suited for method evaluation with the conventional approach. The statistical evaluation of the data from the individual BPCA also always resulted in the most suitable slope and ideal *Y*-axis intercepts for nitric acid oxidation in the microwave in the temperature–time program at 190 °C and 1 h for all BCPA. The recovery for all samples showed satisfactory results and ranged from a minimum of 81% to a maximum of 113%. For the preferred digestion at 190 °C and 1 h, the recovery of the analytes was between 88 and 108%. Furthermore, the average recovery with the conventional method was between 85 and 105%, while the microwave-supported analysis technique represented recovery between 90 and 107% on average (Table 7). Based on the high recovery, we found that a microwave-assisted extraction of black carbon at 190 °C and 1 h is the optimal condition to replace the conventional method. For this reason, we have to contradict the statement of Dittmar (2008) that considerable losses of BPCA can be expected even at oxidation temperatures higher than 180 °C. In addition, this work showed results after only 4 h of digestion at 170 °C, which were comparable to the values of the extraction products of the conventional method. A sample treatment at 170 °C and 9 h in the microwave is therefore questionable, which anyway exceeds the previous extraction time of 8 h (Glaser et al. 1998) and does not improve the conventional digestion process with regard to the extraction time. Because of this, the microwave-supported method is not recommended as a replacement routine procedure in the analysis of molecular markers according to Dittmar (2008). Instead, we propose the microwave-assisted digestion of BPCAs from soil samples under the optimized conditions at 190 °C and 1 h to be able to substitute the old method without quantitative and qualitative deficits. However, one disadvantage of this new method is a higher consumption of sample material and chemicals by a factor of 2.5 compared to the original method. Advantages are reduction of digestion time from 8 to 1 h and use of a commercially available microwave digestion system.

5 Conclusions

This work presents a method comparison between the previously practiced nitric acid oxidation using pressure digestion apparatus and microwave-assisted extraction to simplify the analytical methods in the determination of black carbon degradation products as molecular markers. For both methods, different soil samples were examined for method evaluation and validation, so that a broad spectrum of properties was covered in the analysis. Our results clearly showed that the 8-h conventional method at 170 °C can best be substituted by a microwave digestion with an optimal extraction time of 1 h and an oxidation temperature of 190 °C. In addition, the workload was reduced considerably and the sample throughput was significantly increased thanks to 40 digestion vessels of the microwave reaction system (CEM Mars6) integrated in the rotating vessel carousel. Therefore, the new method contributes to an overall improvement in the analysis procedure. However, the heating technology based on electromagnetic radiation also brings about laboratory-relevant changes compared to the old method. The microwave reaction system

Table 7 Comparison of the methods regarding to the average recovery rates of the total BPCA content in the examined sample series, differentiated according to the conventional extraction conditions and by the microwave parameters set in the temperature–time program

Sample	170 °C	170 °C				190 °C			
	(conv.) (%)	0.5 h (%)	1 h (%)	2 h (%)	4 h (%)	0.5 h (%)	1 h (%)	2 h (%)	4 h (%)
Chernozem	90	89	95	88	87	90	94	88	84
Luvic Chernozem	93	97	95	97	90	95	93	97	93
Ferralsol	86	86	89	88	93	88	90	90	90
Terra Preta	105	104	105	108	110	110	107	104	95
Wheat flour	85	93	89	94	94	95	92	97	n.c.*
Charcoal	90	96	97	89	87	90	90	84	n.c.*

*Not calculated (due to missing values, samples vaporized through the extreme extraction conditions)

CEM Mars6, for example, stipulates a minimum fill quantity of 5 mL for the Teflon reaction vessels, so that the sample and reagent sizes had to be scaled up by a factor of 2.5 to match the concentration ratio of the sample series of the currently used method for an exact method comparison. This also has the consequence that the consumption of solvents and sample material increases in connection with the sample sizes which are adapted to the Teflon vessels.

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Author contributions BG conceived the study, performed the initial literature search, and revised the manuscript. MG prepared the manuscript, HM and MG performed the laboratory analysis and calculation of results, and TB prepared the graphs and revised the manuscript.

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Declarations

Conflicts of interest The authors declare no conflict of interest.

Compliance with ethical standards The authors declare that their study is compliant with ethical standards.

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