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Comparison of three quantification methods used to detect biochar carbon migration in a tropical soil: A 4.5-year field experiment in Zambia

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ABSTRACT

Understanding the stability and movement of biochar in soil is pivotal for its effective use in soil improvement and carbon sequestration projects. Building on a previous study that evaluated the migration of three size fractions of maize biochar carbon (BC) after 4.5 years in a Zambian loamy sand soil using $\delta^{13}C$ isotopes, this study compares the results with those using chemothermal oxidation (CTO) and benzene polycarboxylic acid (BPCA) biomarkers. While the $\delta^{13}C$ method registered the most BC in the application layer (0–7 cm), it detected less BC in lower layers (7–30 cm, 3.2–7.9 % downward migration), and with a greater variance, than the other two methods. The BPCA method detected relatively more BC in the lower layers (9.1–20.2 % downward migration), particularly for fine-sized biochar. It also detected the most BC in the control soil plot and outside the experimental block, which suggests either its efficiency in fine biochar detection or an issue with false positive detection. The CTO method, though less sensitive in detecting fine biochar particle BC, was strongly correlated with $\delta^{13}C$ isotope results, thus representing a cost-effective and simpler alternative to the other BC quantification methods. These findings underscore the necessity of methodological consideration in biochar C quantification to ensure accurate assessment of its distribution and stability. This is a pressing need for correct assignment of climate mitigation credits. More field studies should be carried out involving multiple biochar types and quantification methods to refine our understanding of biochar C dynamics in soil.

1. Introduction

Biochar is a solid product produced from the pyrolysis of biomass, i.e combustion in the absence of or with limited oxygen. Biochar has attracted global attention due to its wide range of applications in various areas such as soil improvement (increasing plant productivity and contaminant remediation), waste management via filtration, and climate change mitigation via carbon sequestration. The carbon component of biochar is highly refractory to degradation in soils (Cross and Sohi, 2011), and reviews and *meta*-studies have shown that the stability of biochar ranges from a few decades to thousands of years (Budai et al., 2013; Lehmann and Joseph, 2015; Wang et al., 2016). Biochar not only sequesters the carbon in biomass, but also may reduce native soil organic carbon (SOC) degradation (negative priming), thus further contributing to its climate change mitigating effects (Lehmann and Joseph, 2015; Spokas, 2010; Weng et al., 2017).

Despite its high recalcitrance, many recent studies have shown that biochar carbon (BC) losses do occur via microbial and abiotic oxidation and leaching (Bostick et al., 2018; Spokas, 2010; Zimmerman, 2010). The inherent chemical stability of BC varies along a continuum based on their relative abundance of condensed aromatic components (Singh et al., 2012; Zimmerman and Gao, 2013) which is, itself, directly related primarily to the temperature at which the biochar was produced, and secondarily to its parent biomass type (Hilscher et al., 2009; Jindo and Sonoki, 2019; Nguyen et al., 2010; Zimmerman, 2010). It is critical that accurate predictions of BC stability can be made so that climate mitigation policies can be developed, and correct assignments of carbon offset credits can be made to biochar producers and users. However, at

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present, these formulations are based completely on laboratory incubations of biochar. Recent field studies suggest that in situ biochar stability may be much lower than those derived from laboratory incubations (Pulcher et al., 2022; Ventura et al., 2015). This is likely because the closed and constant conditions of laboratory studies are not optimal for microbial activity and abiotic oxidation of biochar. However, field studies of biochar stability are much more difficult to carry out because 1) changes in two or more sources of organic C, BC and SOC, must be distinguished, and 2) losses of BC to vertical or horizontal migration must be accounted for.

Applied biochar particles may be mobile, both vertically and horizontally. Research has shown that biochar particles can be transported vertically into deeper soil layers, especially in sandy or loosely structured soils, where percolation is more pronounced (Obia et al., 2024; Schiedung et al., 2020). For example, about 50 % of a biochar produced from rice residues was judged to have migrated below the upper 30 cm sandy soil layer in which it was applied over 4 y (Haefele et al., 2011). Horizontal migration, though less studied, can occur through surface runoff, especially on sloped terrains during heavy rainfalls, leading to biochar particles being dispersed across the landscape or into water bodies (Obia et al., 2017). Both of these are influenced by many environmental factors including rainfall, soil porosity, and bioturbation. Additionally, the extent of biochar migration is influenced by its physical properties, such as its particle size, density, and surface area (Ravi et al., 2020). Smaller, lighter particles are more prone to being transported away from the application site (Obia et al., 2024). Both vertical and horizontal biochar migration can complicate efforts to access BC stability in research studies and in C offset verification.

Determination of biochar stability and migration, and thus the climate mitigation potential of biochar, poses the challenge of finding an appropriate BC quantification technique. At present there is no single standard method in use. Different BC quantification methods inevitably lead to varying results because each method quantifies a different window of the BC continuum (Hammes et al., 2007). The methods currently available to distinguish BC from native organic carbon (non-BC) are of three general categories, chemical thermal oxidation (CTO), quantification of aromatic or condensed aromatic carbon analyses, and stable C isotopic methods (Poot et al., 2009). In the first, recalcitrant carbon is isolated by the removal of labile non-BC using thermal treatments and of varying strengths, commonly peroxides, acids, or dichromates. The remaining C, presumed to be pyrogenic, is quantified by standard C analysis.

Biochar of high aromaticity and/or degree of aromatic condensation are more likely to resist biological or thermochemical degradation (Lehmann and Joseph, 2015). This stable component of biochar can be quantified indirectly by spectroscopic methods or directly using molecular markers such as benzene polycarboxylic acids (BPCA). First developed by Glaser et al (1998), BPCA compounds, single-ring aromatic molecules with different numbers of carboxylic acid side-group carboxylation (i.e., B3-, B4-, B5- and B6-CA, representing BPCA with three, four, five and six carboxylic groups, respectively) are the products of the high-pressure oxidation of polycyclic structures and can be quantified by gas or liquid chromatographic analysis. Following quantification, a conversion factor must be used to estimate total BC (e.g., Bostick et al., 2018).

When there are significant differences between the $^{13}C/^{12}C$ ratio of native SOC and the BC added, either through isotopic labelling or natural abundance differences such as when one source is from C_3 plants and the other from C_4 plants, measurement of stable isotope ratios ($\delta^{13}C$) can be used to track migration or loss of biochar C added to soil. A general overview of isotopic techniques applied in studies involving biochar was presented by Glaser et al. (2013), highlighting the utility of this method in understanding the dynamics of biochar in soil systems.

Each method for tracking biochar C is associated with a number of assumptions and uncertainties. For example, the CTO method assumes that it removes all non-pyrogenic C. This has been widely questioned based on the observation of strong correlations between SOC and CTO-derived BC across many studies (Zimmerman and Mitra, 2017). Further, CTO methods might oxidize some forms of pyrogenic carbon, thereby underestimating biochar content (Roth et al., 2012). These discrepancies highlight the need for careful calibration and possibly the integration of multiple methods to improve the accuracy of biochar quantification in environmental samples. While BPCAs derive from condensed aromatics that are abundant in pyrogenic materials, some recent studies suggest that condensed aromatics can have non-pyrogenic sources (Goranov et al., 2024) and BPCAs may be produced during sample processing (Kappenberg et al., 2016), though this has been questioned (Di Rauso Simeone et al., 2024). There are also uncertainties associated with the appropriate conversion factor to use when converting BPCA to total BC (Zimmerman and Mitra, 2017). If the $^{13}C/^{12}C$ isotope ratios of biochar C and SOC are similar, or only small amounts of biochar have been added, large uncertainties may be associated with the use of stable isotopic measurements to quantify BC (Chalk and Smith, 2022).

To better understand what portion of BC is detected by the different quantification methods, and how use of different methods might affect our understanding of BC stability and migration, comparative studies carried out in field settings are needed. Studies comparing BC quantification methods are limited (Hammes et al., 2007; Roth et al., 2012; Schmidt et al., 2001) and, to the best of our knowledge, there are no field studies comparing BC mineralization or migration in soils using multiple quantitative methods. Thus, BC was quantified by three methods 4.5 y after the application of corn cob biochar of different particle size fractions to a loamy sand agricultural soil at Mkushi, Zambia. BC recovery from the soil profile, quantified using only a ^{13}C isotopic mixing model, was reported previously (Obia et al., 2024). Significant BC migration was found, with 38–75 % leaving the application layer (upper 7 cm), as well as evidence of lateral dispersion to areas beyond the application block. Biochar size and dose affected its movement, with fine and intermediate-sized BC displaying the greatest downward migration. The presence of biochar had a positive priming effect, with native SOC stocks in each treatment plot reduced by 2.8–24.5 %. For the present work, it is hypothesized that there will be differences in the quantity of BC in soils as determined by different methods. Specifically, the ^{13}C isotope approach, in contrast to BPCAs, will poorly detect small concentrations of BC, thus may be less useful for BC migration studies. The CTO method, while potentially suffering from uncertainties in its inclusion of non-pyrogenic C, may prove to be a good compromise between these two methods. These differences could be used to track different portions of pyrogenic C through the environment and better enable biochar C accounting efforts so that accurate C sequestration credits can be assigned.

2. Material and methods

2.1. Material and field methods

The field experiment was established in April 2013 in Mkushi, Zambia (S13 44.839, E29 05.972) on almost flat terrain. The soil at the site is classified as an Acrisol according to FAO's World Reference Base classification (WRB-FAO, 2015). The experiment was arranged as a block of nine 50 cm × 50 cm plots (Fig. 1a) and was sampled after 4.5 years. The plots were planted with corn and fertilized with NPK fertilizer during each growing season over the 4.5 years. The average annual precipitation and temperature at Mkushi is 1220 mm and 20.4 °C, respectively.

Each plot was enclosed with hard plastic sheets vertically inserted to a depth of 10 cm into the soil and extending 10 cm above the soil. Treatments consisted of three biochar particle sizes (<0.5, 0.5–1 and 1–5 mm, referred to as fine, intermediate and coarse biochar, respectively), prepared by crushing and sieving. These particle size cutoffs were chosen based on prior finding that each represented about one-third of the biochar produced (unpublished data).The fine- and coarse-

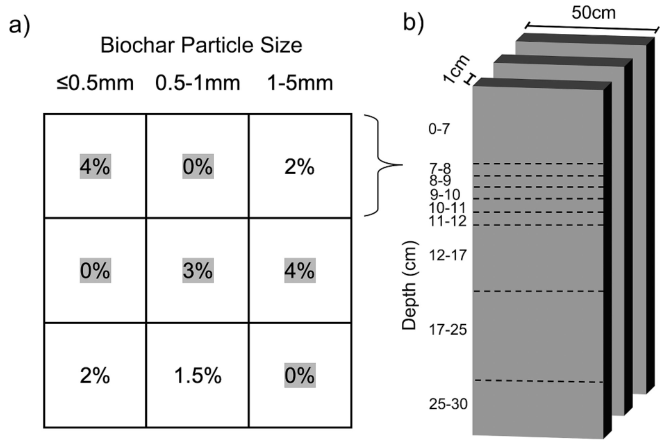

Fig. 1. Schematic of a) experimental block design with 50 × 50 cm treatment plots. Numbers in boxes indicate biochar dose by weight percent and highlighted plots are those that were used in this study, and b) 1 × 50 cm (width × depth) samples collected in each plot from the depth intervals indicated.

size biochar plots used in this study were applied at a rate of 4 % (BC4) by weight, amounting to 0.873 kg per plot. Due to shortage of material, the intermediate-size biochar was applied at slightly lower rates of 3 % (BC3), corresponding to 0.666 kg biochar per plot. Detailed information on the study site and soil and biochar properties, including nutrient content, can be found in Obia et al. (2024).

To track BC via the stable isotope approach, its ^{13}C signature must be distinct from that of the native soil ^{13}C signature. Because the soil SOC was mainly derived from C_3 plants, a biochar derived from a C_4 plant was made. Thus, biochar was prepared from dry corncob, i.e. cob with grains removed, in a retort kiln at a temperature of approximately 400 to 500 °C with a residence time of 24 h. For biochar application, the uppermost 7 cm of soil from each plot, including the reference plot, was removed before loosening the soil further to 30 cm depth, a common agricultural process in Zambia. The required amount of each biochar was mixed with soil from the upper 7 cm layer and then returned to the plot.

Samples were collected by excavating three 50 × 1 cm slices from each of nine plots in the block (Fig. 1b) in September 2017, 4 y and 5 months (~4.5 years) after biochar application. Three replicate samples were collected from nine soil depth intervals: 0–7 cm (biochar application depth), and 7–8, 8–9, 9–10, 10–11, 11–12, 12–17, 17–25 and 25–30 cm. Further, to assess the lateral transport of biochar, triplicate 50 × 1 cm soil samples were also collected from the 0–7 cm depth interval of soils outside the experimental block at distances of 0.5, 1, 2 and 5 m to the north and south (the most level orientation). Each depth interval sampled was homogenized by hand mixing, dried, ground, and stored until further analyses.

2.2. Analytical methods

Biochar carbon (BC) quantities determined by stable C isotopes, the CTO method of Kurth, Mackenzie and Deluca (KMD), and BPCA biomarkers, were designated as BC_I, BC_K, and BC_B, respectively.

δ^{13}C Stable Isotope Method – The δ^{13}C signature of the original soil, biochar, and collected sample mixtures was measured on a Thermo-Finnigan DeltaPlus XL isotope ratio mass spectrometer with a GasBench II universal online gas preparation device at the University of Florida, Florida, U.S. Values were calibrated relative to a CO_2 reference gas and all isotopic ratios are expressed in standard delta notation as $d^{13}CO_2$ in units of per mil (‰) relative to the Vienna Pee Dee Belemnite (VPDB) standard.

The difference in δ^{13}C isotope signature between the SOC and the BC (δ^{13}C of −19.5 and −12.3, respectively) allowed the calculation of the proportion of C from each mixture using a two-component isotope mixing ratio equation:

$$BC\ Fraction(f) = \frac{\delta^{13}C_{mixture} - \delta^{13}C_{ref\ soil}}{\delta^{13}C_{biochar} - \delta^{13}C_{ref\ soil}} \tag{1}$$

where $\delta^{13}C_{mixture}$ is the δ^{13}C signature of a given soil sample at the time of collection. The BC_I (g) in a sample was calculated as:

$$BC_I = TOC \times f \tag{2}$$

The total TOC stock for each depth interval was calculated as:

$$TOC\ stock = Plot\ area\ \left(cm^2\right) \times Depth\ range(cm) \times bulk\ density\ \left(g\ cm^{-3}\right) \times \frac{C\%}{100} \tag{3}$$

KMD Method – Biochar carbon contents of pre- and post-emplacement soil-biochar mixtures and control samples were analyzed by the Kurth-Mackenzie-Deluca (KMD) method following Kurth et al. (2006). About 1 g of sample was ground to < 0.76 μm, then 20 ml of 30 % H_2O_2, and 10 ml of 1 M HNO_3, were added into 250 ml glass beakers. The mixtures were then heated to 100 °C on a heating plate for 16 h with periodic stirring. After complete digestion (no observation of further effervescence), samples were filtered through Whatman #2 filter paper, after which, the sediment-laden filters were oven-dried at 60 °C overnight. Samples were carefully scraped from the filters, weighed, and analyzed for C content ($C_{post\ digestion}$) on a Carlo-Erba NA-1500 CHS Elemental Analyzer.

Biochar carbon content measured via *KMD method* was calculated as:

$$BC_K = \left(C_{post-digestion}\right)\left(\frac{mg}{g}\right) \times \left(\frac{m_{post-digestion}}{m_{pre-digestion}}\right) \times Soil\ mass\ (kg) \tag{4}$$

where $m_{pre\text{-}digestion}$ and $m_{post\text{-}digestion}$ are the soil weights before and after digestion, respectively.

BPCA Method – The BPCA analysis of soil and sediment samples followed the methods established by Brodowski et al. (2005) and Dittmar (2008), with modification. Briefly, samples were first pretreated with 4 M trifluoroacetic acid (TFA) at 105 °C for 4 h to remove Fe and Al. After drying in an oven at 50 °C and rinsing with distilled water, the TFA-pretreated samples of about 100 mg were oxidized in 1 ml sealed ampoules with 1 ml nitric acid at 170 °C for 9 h. Next, samples were blown to dryness under N_2 gas, dissolved in DI water, and filtered through 2 μm nylon syringe filters. The filtrate of each sample was cleaned by passing through a glass column packed with Dowex 50x ion exchange resin and then eluted with DI water. The eluate was freeze-dried and was finally dissolved in methanol.

BPCA compounds in these extracts were detected using high performance liquid chromatography (HPLC, Shimadzu LC-20 Prominence Series) equipped with a C18 column (3.5 μm, 2.1 × 150 mm, Waters Sunfire) and a diode array detector (DAD) (Surveyor, Thermo-Scientific, SPD-M20A) at 230 nm wavelength with a binary gradient program as described in Bostick et al. (2018). BPCA concentrations were quantified using peak area curves generated from external BPCA standards.

Because of destruction during the harsh acid treatment, only ~25 % of the C present in condensed aromatics can be recovered as BPCA monomers (Schmidt et al., 2017). Therefore, the sum of the B5CA and B6CA molecules was multiplied by a scaling factor of 7.04, determined using BPCA analysis of pure graphite (Bostick et al., 2018), to calculate total PyC as determined by BPCA analysis (referred to as BC_B).

2.3. Replication and data treatment

In each experimental treatment plot, three unique samples were collected. Each was analyzed once via stable C isotopes, and twice via KMD method. Because of its cost and time consumption, BPCA was only determined in the three samples of each application layer (0–7 cm) treatment plot. Single analyses were conducted for samples of lower soil depths. All the data are presented as means of these replicate analyses. Means, standard deviations and regression correlation coefficients were computed using Microsoft Excel software. BC recovery% was calculated as BC content of a soil sample divided by the product of the added biochar mass and the C content of the biochar.

3. Results

The mean quantity of total BC (g), i.e. stock, and fraction recovered for each soil depth after 4.5 years, determined by different methods, is provided in Tables 1 and 2, respectively. We focus here mostly on BC recovery because different amounts of biochar were added to different plots. The recovery of BC showed notable differences between biochar types and with analytical method used. There was also variation in the precision of BC determination (instrumental plus sample-to-sample variation) with significant variability in relative standard deviation (RSD) between different soil depths and with different methods. In general, BC_I had much higher RSD (92 % on average) than BC_K (26 % on average) and BC_B (19 % on average). Some RSD in measurement of BC_I reached as high as 173 %. Generally, BC RSD values were the lowest in the upper layer, where BC concentrations were the greatest, and increased with depth.

All methods detected BC in the reference plot (Table 1). The BC_B analysis indicated the greatest total BC stock in the reference soil (94.1 g), followed by BC_K (80.7 g), and then BC_I (49.1 g). While the BPCA method quantified the least BC in the surface layer of the reference plot ($BC_B = 24$ g), it detected the most in underlaying soil layers ($BC_B = 70.1$ g) where the stable C isotope method detected the least ($BC_I = 7.2$ g).

3.1. *Biochar carbon recovery variation with soil depth and laterally*

All biochar treatment plots had average BC stocks greater than those of the reference plots in the whole soil profile and within the application layer. This was also true of BC stocks in lower layers of the soil profiles (7–30 cm) as determined by isotopic measurements ($BC_I = 15.0$, 22.9, and 36.9 g in fine, medium and coarse plots, respectively, compared to 7.2 g in the reference plot). However, some treatment plots had BC stocks in lower layers that were less than that of the reference plot, as determined by KMD and BPCAs. For example, in the BC stock in the 7–30 cm layers of the fine treatment plot, $BC_K = 31.7$ g versus 34.0 g in the reference plot. In these layers of the coarse treatment plot, $BC_B = 60.2$ g versus 70.1 g in the reference plot.

Average total BC recovery in the treatment plots, as a fraction of that applied, ranged from 31 to 49 %, 26 to 46 %, and 30 to 39 % as determined by stable C isotopes, KMD, and BPCA methods, respectively (Table 2). While these recoveries are of similar range, the distribution of these recovery values differed with BC detection method. There was always more BC_I recovery in the application layer, compared to BC_K and BC_B, but it detected the least BC in the lower layers of all treatment types. In contrast, the BPCA method detected less BC (BC_B) in the application layer (same or less than BC_K), particularly in the coarse biochar treatment, but the most BC in most layers below the application layer, particularly in the fine-sized biochar-amended soil.

In general, there was greater total recovery of coarse BC (38.8–48.6 %) than medium (29.5–31.6 %) or fine size BC (25.7–39.4 %), across all methods. This was also true within the application layer (Fig. 2). Below the application layer, BC_I and BC_K recovery was greatest from the coarse biochar treatment, followed by the medium size treatment, but the BPCA method recovered more BC in the fine biochar treatment. These trends were similar at every soil depth interval sampled (Fig. 3).

All methods detected BC in soils outside the experimental block (Table 3). The BC_I values were lowest or nil in many samples outside the experimental block, whereas the KMD and BPCA method detected BC in all these samples, with BPCA detecting the most. Other than possibly the BC_K South transect, there was no clear trend of BC decrease with distance from the experimental block.

3.2. *Quantified BC method relationships*

Because of the large differences between BC concentration in the upper and lower layers of the soil profiles, correlations between BC concentrations determined by the different methods can only be examined using total organic carbon-normalized BC data (BC/TOC) data. All

Table 1

Mean total organic carbon stock (g), biochar carbon (BC) recovery (g) and relative standard deviation (RSD, %) after 4.5 years in the field as determined by stable C isotopes, KMD and BPCA methods (BC_I, BC_K, BC_B, respectively).

Soil Depths (cm)	**Reference**							**Fine Biochar**						
	TOC stock	**BC_I**	**RSD**	**BC_K**	**RSD**	**BC_B**	**RSD**	**TOC stock**	**BC_I**	**RSD**	**BC_K**	**RSD**	**BC_B**	**RSD**
0–7	157.3	41.9	41	46.7	30	24.0	*	231.4	130.0	9	89.1	13	89.90	21
7–8	17.1	1.8	124	2.3	59	4.7	*	18.5	3.8	35	2.9	24	7.50	*
8–9	15.9	1.7	37	2.8	37	3.9	*	17.7	2.1	33	2.2	20	7.87	*
9–10	16.2	0.2	172	2.2	30	4.0	*	16.1	1.0	58	2.5	26	7.87	*
10–11	15.9	0.8	159	1.9	28	4.5	*	17.1	0.6	84	1.8	25	3.60	*
11–12	16.2	0.1	173	1.4	23	4.5	*	16.4	0.4	173	1.5	14	3.40	*
12–17	77.9	1.2	173	6.7	17	22.1	*	69.3	3.7	86	8.6	45	18.87	*
17–25	98.0	0.0	173	9.9	10	20.7	*	113.5	2.4	173	8.6	46	34.53	*
25–30	60.7	1.3	128	6.9	23	5.9	*	59.1	1.0	173	3.7	11	11.32	*
Total (g)	475.4	49.1		80.7		94.1		559.2	145.0		120.9		184.9	

Soil Depths (cm)	**Medium Biochar**							**Coarse Biochar**						
	TOC stock	**BC_I**	**RSD**	**BC_K**	**RSD**	**BC_B**	**RSD**	**TOC stock**	**BC_I**	**RSD**	**BC_K**	**RSD**	**BC_B**	**RSD**
0–7	186.8	90.3	13	72.8	18	73.09	8	274.7	191.5	44	162.5	24	121.8	20
7–8	16.2	2.8	91	3.3	40	3.05	*	16.2	5.5	52	6.3	27	7.22	*
8–9	17.4	1.9	99	3.0	20	0.99	*	15.7	3.7	53	4.1	29	8.17	*
9–10	14.1	1.2	13	1.9	10	2.19	*	13.2	1.9	24	3.4	20	4.32	*
10–11	13.8	0.6	89	1.6	15	1.19	*	12.9	1.1	56	1.8	17	3.30	*
11–12	13.7	0.3	173	1.8	40	1.65	*	13.3	0.3	172	2.4	39	3.02	*
12–17	62.9	5.4	88	8.8	20	9.85	*	63.4	5.8	53	8.5	34	10.16	*
17–25	82.6	8.9	29	12.7	10	8.50	*	94.6	13.8	65	17.3	26	18.09	*
25–30	38.2	1.9	87	5.3	15	5.16	*	46.2	4.7	89	10.3	38	5.92	*
Total (g)	445.5	113.3		111.2		105.7		550.4	228.4		216.6		182.0	

* = no RSD because only single analyses were carried out.

Table 2

Mean biochar carbon (BC) recovery (%) and relative standard deviation (RSD, %) after 4.5 years in the field as determined by stable C isotopes, KMD and BPCA methods (BC_I, BC_K, BC_B, respectively).

Soil Depths (cm)	Fine Biochar						Medium Biochar					
	BC_I	RSD	BC_K	RSD	BC_B	RSD	BC_I	RSD	BC_K	RSD	BC_B	RSD
0–7	27.7	9	19.0	13	19.1	21	25.2	13	20.3	18	20.4	8
7–8	0.8	35	0.6	24	1.6	*	0.8	91	0.9	40	0.9	*
8–9	0.5	33	0.5	20	1.7	*	0.5	99	0.8	20	0.3	*
9–10	0.2	58	0.5	26	1.7	*	0.3	13	0.5	10	0.6	*
10–11	0.1	84	0.4	25	0.8	*	0.2	89	0.5	15	0.3	*
11–12	0.1	173	0.3	14	0.7	*	0.1	173	0.5	40	0.5	*
12–17	0.8	86	1.8	45	4.0	*	1.5	88	2.5	20	2.7	*
17–25	0.5	173	1.8	46	7.4	*	2.5	29	3.5	10	2.4	*
25–30	0.2	173	0.8	11	2.4	*	0.5	87	1.5	15	1.4	*
Total recovery %	30.9		25.7		39.4		31.6		31.0		29.5	

Soil Depths (cm)	Coarse Biochar					
	BC_I	RSD	BC_K	RSD	BC_B	RSD
0–7	40.8	44	34.6	24	25.9	20
7–8	1.2	52	1.3	27	1.5	*
8–9	0.8	53	0.9	29	1.7	*
9–10	0.4	24	0.7	20	0.9	*
10–11	0.2	56	0.4	17	0.7	*
11–12	0.1	172	0.5	39	0.6	*
12–17	1.2	53	1.8	34	2.2	*
17–25	2.9	65	3.7	26	3.9	*
25–30	1.0	89	2.2	38	1.3	*
Total recovery %	48.6		46.1		38.8	

* = no RSD because only single analyses were carried out.

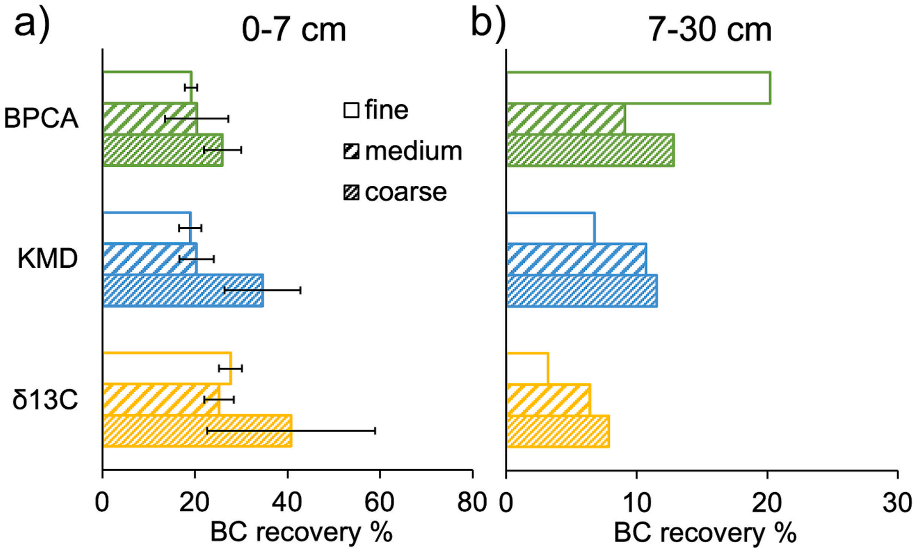

Fig. 2. Biochar carbon recovery (BC%) in the a) 0–7 cm biochar addition layer, and b) 7–30 cm depth interval below the addition layer, as determined by different methods after 4.5 y field emplacement. Open, single and double hatch to indicate fine (<0.5 mm), medium (0.5–1 mm), coarse (1–5 mm) sized biochar, respectively.

the BC markers were significantly directly intercorrelated within the whole data set (Table 4). However, BC_I and BC_K were the most strongly correlated (r = 0.95) and were also significantly correlated in the lower depth samples (7–30 cm, r = 0.86) and within each of individual soil profile (i.e. fine, medium, and coarse, r = 0.97–0.99).

In contrast, BPCA-derived values (BC_B) were only strongly correlated with the BC of the other two methods in the medium sized biochar-amended soil plot. In fact, BC_B was weakly negatively correlated with BC_I and BC_K in the reference plot, which was caused by the lower BC_B recovery at the top of the soil profile (0–7 cm) and the relatively higher BC_B recovery in the lowest portion of the profile (17–30 cm) compared with the other two methods.

4. Discussion

4.1. Biochar mobility in soil

4.1.1. Vertical mobility

Using only the ^{13}C isotopic method, Obia et al. (2024) previously found 3.2–7.9 % of the BC that was initially applied to the 0–7 cm layer in lower soil layers (7–30 cm) after 4.5 y in the soil profiles used in this study. Although fine and coarse biochar displayed the greatest downward migration at lower doses, for the higher biochar dosages used in this study, coarse biochar demonstrated greater downward migration (Table 2). These results may be reevaluated in light of the higher RSD of this method compared to the other two, especially for soil samples collected at depths below 7 cm, and the different sensitivities of each

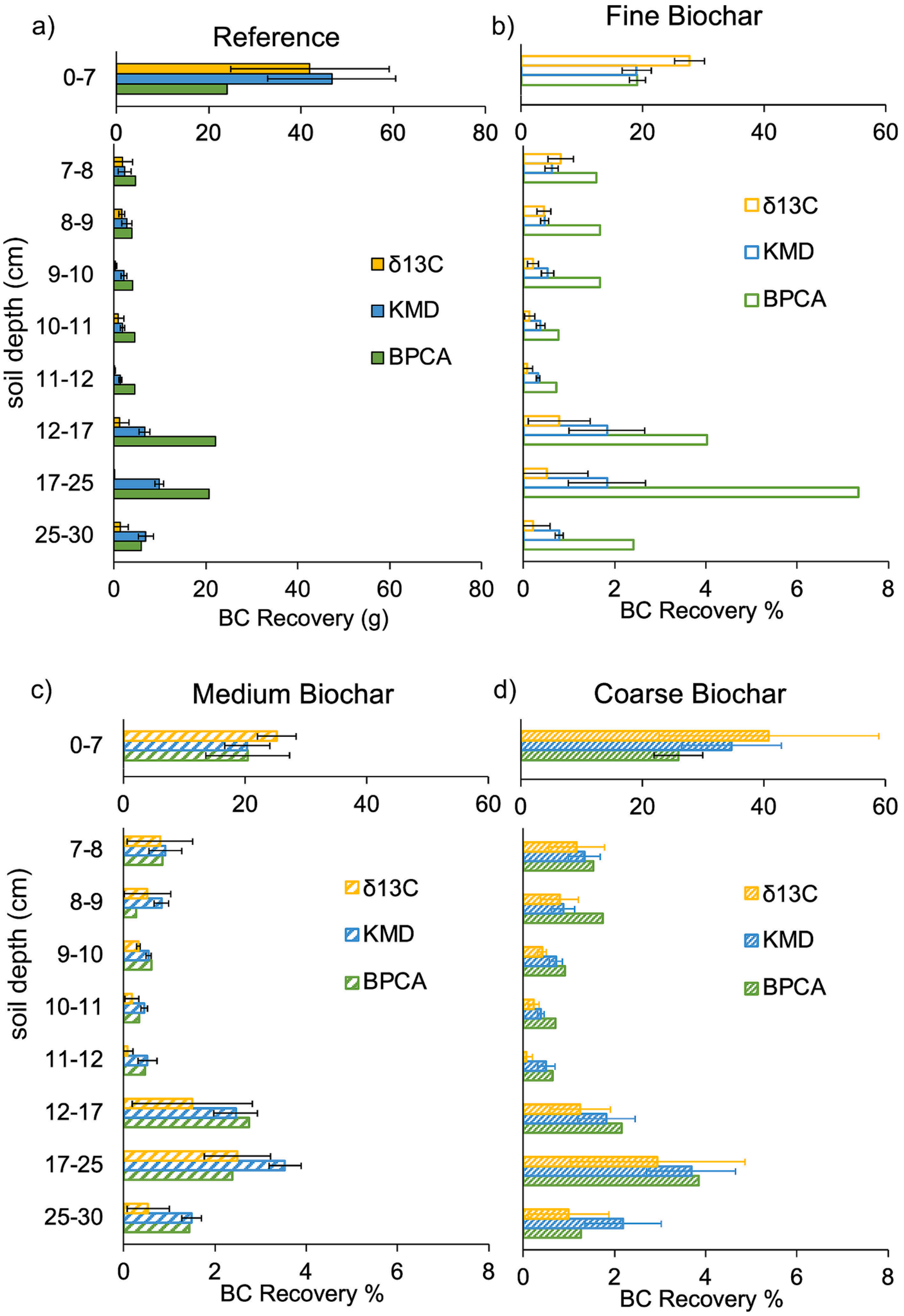

Fig. 3. Biochar carbon (BC) recovery through the soil profile in, a) the reference plot by weight (g) and as a percent of BC added in b) fine, c) medium, and d) coarse-sized biochar addition treatments, as determined by different methods after 4.5 y field emplacement.

method to biochar of different particle sizes. This is in agreement with several previous studies that found smaller biochar particles were more readily transported downward through porous media (Wang et al., 2013; Zhang et al., 2010).

As the other two methods (KMD and BPCAs) registered more BC in lower soil levels, they indicated greater downward BC migration. According to the results of the KMD method, 6.8–11.5 % of the applied BC had migrated to lower soil layers. BC_K values also indicated less downward migration for fine size biochar and more downward migration for medium and coarse size biochar. In contrast, the BPCA method indicated 9.1–20.2 % of the applied BC had migrated to lower soil layers and fine size biochar showed greater downward migration compared to medium and coarse size biochar.

Previous laboratory studies have found that rates of mineralization of larger-sized biochar particles are lower than those of smaller-sized particles (Chen et al., 2017; Sigua et al., 2014; Zimmerman, 2010). This is likely because of the greater amount of surface exposure of fine particles to dissolution, oxidation, and microbial exoenzyme attack. Thus, the lower BC_I and BC_K total recovery of fine biochar may be explained by greater loss of fine biochar through mineralization.

4.1.2. Lateral mobility

The detection of BC, both in the reference plot where no biochar was applied, and in samples collected from soils outside the plot area, strongly suggests that there was significant lateral transport of BC due to a ‘floating’ effect of the lighter, porous BC particles during heavy precipitation or wind events, followed by lateral spreading to outside the application areas. Previously, Obia et al. (2024), on the basis of ^{13}C-

Table 3
Mean BC recovery (g) in the 0–7 cm depth interval of soils outside the experimental block at distances of 0.5, 1, 2 and 5 m to the north and south 4.5 years after biochar addition, as determined by stable C isotopes, KMD and BPCA methods (BC_I, BC_K, BC_B, respectively).

Soil plots	BC_I	BC_K	BC_B
Lateral 0.5 m North	0.0	7.7	17.5
Lateral 0.5 m South	11.0	11.1	
Lateral 1 m North	0.0	9.8	20.4
Lateral 1 m South	0.0	11.2	
Lateral 2 m North	7.0	17.6	22.8
Lateral 2 m South	0.0	7.4	
Lateral 3 m North	0.0	7.7	23.2
Lateral 3 m South	0.0	6.7	
Lateral 5 m North	0.0	12.6	17.5
Lateral 5 m South	0.0	4.0	

Table 4
Correlation coefficients for linear relationships between TOC-normalized BC concentrations as determined by C isotopes, KMD and BPCA methods (BC_I, BC_K, BC_B, respectively) in the whole data set and in 30 cm soil profiles of each particle size, 4.5 years after biochar addition. Underlines indicate significant coefficient values ($p < 0.05$).

Reference			All 0–30 cm			All 7–30 cm		
	BC_K	BC_B		BC_K	BC_B		BC_K	BC_B
BC_I	0.96	−0.30	BC_I	0.95	0.51	BC_I	0.86	0.47
BC_B	−0.42		BC_B	0.46		BC_B	0.34	
Fine			**Medium**			**Coarse**		
	BC_K	BC_B		BC_K	BC_B		BC_K	BC_B
BC_I	0.97	0.42	BC_I	0.99	0.91	BC_I	0.97	0.66
BC_B	0.46		BC_B	0.91		BC_B	0.67	

analysis only, estimated BC mineralization to be no more than 8 %. Thus, about 25–60 % of the added BC was unaccounted for using the ^{13}C isotopic method and presumed to be transported laterally out of the experimental plots. These erosional losses are on the order of those reported previously (Major et al., 2010; Rumpel et al., 2009). Because somewhat less BC was detected using the KMD and BPCA methods through the whole profile, more BC was unaccounted for, 53.9–74.3 % and 60.6–70.5 % of the BC added, respectively, and can be presumed to have been transported laterally. This conclusion is supported by the greater BC found in the reference plot and outside the experimental block, according to the KMD and BPCA methods. This may be explained by the greater ability of the KMD and BPCA methods to detect fine particle-associated BC, which is also most likely to have migrated laterally. On the other hand, BC_K and BC_B may have included pre-existing BC content (as evidenced by the large amount found in the reference plot) which was not detected by BC_I since it registers only C3-derived biochar.

4.2. Evaluation of δ^{13}C stable isotope method

The δ^{13}C isotope method detected more BC in the biochar application soil layer than other methods but detected less BC at lower depths to which biochar migrated. In addition, because there were some samples with δ^{13}C values greater than or equal to the native soil δ^{13}C value, their BC_I content was calculated as zero, which contrasts with the KMD and BPCA methods that always measured BC content. We have found that it is common for these methods to register BC in soil of all regions, despite their lack of fire history (Lyu et al., 2023). The poorer detection of low amounts of BC by the isotope method may have resulted in lower BC_I content of soils at depths below the application layer.

The use of the δ^{13}C isotope method has been widely claimed to provide specificity and enhanced sensitivity in differentiating between soil- and biochar-derived C (Chalk et al., 2021; Cross and Sohi, 2011; Luo et al., 2011; Zimmerman et al., 2011). However, the ^{13}C natural abundance approach has limitations. Isotopic techniques are much less effective at differentiating C sources if the δ^{13}C of a second source such as biochar is in the same range as that of the native SOC (Zimmerman et al., 2011). Although biochar is predominantly an organic material, it may also contain inorganic carbon in the form of carbonates. If not accounted for, these may lead to inaccuracies in BC stability using isotopic methods (Chalk and Smith, 2022). In this study, the low amounts of BC in deeper soil layers 4.5 years after field emplacement may have contributed to the loss of sensitivity due to isotope dilution. That is, δ^{13}C may also become less sensitive at the deeper soil because the contribution of biochar-derived C is much smaller when compared with that of SOC. Sensitivity loss of δ^{13}C method has been reported in previous studies (Leng et al., 2019; Major et al., 2010; Singh et al., 2015). In addition, the use of dual-source isotope mixing models to distinguish carbon sources in biochar-treated soils is challenging if there is plant or root contribution of a third carbon source, especially when BC contents are low (Whitman et al., 2015).

Although the δ^{13}C method has been widely used to enhance the understanding of biochar C dynamics in soil systems under laboratory conditions, field conditions involve a variety of complex factors such as biochar degradation, biochar migration and plant inputs. The δ^{13}C natural abundance method may be less sensitive in some cases and results should be viewed with caution.

4.3. Evaluation of KMD method

Although BC quantified by the KMD method was in most cases well-correlated with that of the δ^{13}C method, unlike the isotope method, the KMD method always detected some BC and mean BC_K values were of relatively lower RSD (Table 2). The KMD method recovered the most BC from coarse biochar treatments, then medium biochar, and least in the fine biochar-amended soil profiles. The KMD method indicated less BC recovery than BC_I in the 0–7 cm biochar application layer for all biochar treatments but had more recovery in the 7–30 cm layers.

The lower BC_K recovery in the application layer, especially for the fine biochar treatments, may be due to shortcomings of the KMD method. The thermal and chemical oxidation pretreatment aims to remove as much non-BC organic matter as possible. But some BC may be destroyed in the process (Zimmerman and Mitra, 2017). This risk of losing small BC particles through the harsh treatment procedures is likely greatest, causing the greatest underestimation of fine-sized BC (Poot et al., 2009). The obvious advantages of the KMD method are its simplicity and low cost, and much smaller analytical variation compared to δ^{13}C isotope methods, according to our results. Because BC quantified by the KMD method correlated well with that of the commonly used δ^{13}C isotope methods, however, it may be a viable option for many studies in which fine biochar is not a major portion of the biochar added.

4.4. Evaluation of BPCA method

In the soils of this study, BC determined by the BPCA method showed weaker correlations with that determined by the δ^{13}C and KMD methods, and the variations in measurements of replicates were lower. Compared to the other methods, BC_B values were lower in the application soil layer and greater in deeper layers (12–30 cm) of the soil profile (Fig. 3). This was most obvious in the fine size biochar-amended soils. Only the BPCA method measured similar BC recovery in the fine and coarse sized biochar treatments, which also led to more BC recovered in fine size biochar-amended soil than other methods and lower BC recoveries in coarse size biochar-amended soil. These results may be related to the assumptions and analytical procedures of the BPCA method.

The BPCA method was evaluated in previous comparative laboratory studies and was found to be suitable for quantifying BC in soils and sediments (Hammes et al., 2007). The precision and accuracy of the BPCA method was evaluated by applying it to 21 biochar types (Schmidt et al., 2017), and the values of BPCA concentrations and patterns were found to be highly reproducible. However, the BPCA method also has drawbacks. Biochar contains non-aromatic components that cannot be converted to BPCAs, and some non-pyrogenic organic materials may also yield BPCAs (Brodowski et al., 2005; Goranov et al., 2024). For example, pine wood exposed to iron nails under natural weathering conditions for a dozen years resulted in the creation of a material rich in condensed aromatic structures that yield BPCAs (Brodowski et al., 2005; Goranov et al., 2024). Furthermore, laboratory experiments have successfully generated condensed aromatic carbon, which can produce BPCAs, through the oxidation of various organic materials, including pine wood and needles, maple, brown-rotted oak wood, algae, corn roots, aspergillin and tree bark (Di Rauso Simeone et al., 2024; Goranov et al., 2024). The presence of these non-pyrogenic sources of aromatic C could have led to an overestimation of BC in the soils through the BPCA method of this study, and an underestimate of that lost laterally to other parts of the system.

To calculate BC_B values, a conversion factor of 7.04 was applied to B5CA + B6CA concentrations in the samples following Bostick et al. (2018), derived from the BPCA yield of pure graphene oxide. However, this conversion factor is likely not accurate when applied to the BC content of the corn biochar used in this study. Because the aromatic components of biochar are likely less condensed than graphene, and because there are non-aromatic components of biochar, BC_B values could have all been underestimated through the BPCA method, leading to an overestimate of that lost to the system. Had we used the conversion factor of 2.27 proposed earlier by Glasser et al. (1998), this underestimate would have been even greater. We hypothesize that BC_K was overestimated predominantly in the lower soil layers of the soil where there was relatively less BC compared to SOC and underestimated in the application layer where the added biochar contained abundant non-condensed BC. This is supported by our finding of $BC_I > BC_K$ in lower layers and $BC_I < BC_K$ in application layers.

Previous studies that considered biochar transport as a function of particle size found smaller particles to be more readily transported downward through porous media (Wang et al., 2013; Zhang et al., 2010). Some fine biochar particles may have attained colloid size (<53 μm) during crushing and sieving, which further increases its downward mobility (Yang et al., 2020). Smaller particles would be most likely to have been ‘floated’ by surface runoff and transported laterally as well. Thus, the finding that fine-sized BC_B was of greater relative content at the deeper soil depth (12–30 cm), in the reference plots, and in soils outside of the experimental blocks (Fig. 3 and Table 3) suggests greater accuracy of the BPCA method in detecting BC migration. However, this will have to be tested by further studies.

5. Conclusion

In this study, BC was quantified in experimental treatment plots 4.5 years after biochar field emplacement using $\delta^{13}C$ isotopes, KMD (CTO), and BPCA biomarkers. All methods showed that biochar of different sizes migrated both vertically and laterally, but there were significant method-dependent differences in the details of the results. The $\delta^{13}C$ isotope method indicated higher BC recovery in the application layer compared to the lower layers, while the BPCA method detected more BC in the lower layers, especially in the fine biochar treatments. The KMD method revealed moderate BC recovery rates across most layers and showed less distinction between biochar sizes compared to the other methods. These differences highlight the importance of choosing appropriate methods for accurately assessing biochar distribution and persistence in soil environments as each method was found to have advantages and disadvantages.

The $\delta^{13}C$ isotope method was shown to be most effective in detecting large quantities of BC while the CTO approach is recommended when BC quantities are low or when BC particles are very fine. However, its major disadvantage is that it may under- or overestimate BC quantities in different circumstances. Its poor correlations with the results of the other two methods warrant caution in its use in field studies such as this one. However, this can be dealt with through the use of control plots and it may be a useful tool if targeting of the more refractory portion of BC is required.

Based on its strong correlation with $\delta^{13}C$ isotope method results, a CTO method like KMD may serve as an excellent alterative soil BC quantification method. The KMD method is simple and relatively inexpensive, highly replicable even with low BC concentrations, and has the potential to successfully cope with the presence of different sizes of biochar. The potential for inclusion of non-BC by KMD analysis can be dealt with through the use of control (i.e., non-biochar addition) plots, though this may be challenging due to the potential for BC migration into adjacent reference plots as observed in the present study.

The proper quantification and tracking of BC in soil will become increasingly important as biochar sees greater use in agriculture. Reliable methods will be needed for certification of negative emissions for the purpose of C offset imbursement and mitigation pledge validation. In order to obtain accurate estimates of BC movement and stability, it is essential to understand the limitations and proper application of different BC quantification methods. The results of this study demonstrate that complex factors may bias BC quantitative information provided by different methods. More comparative field studies using multiple BC quantification methods and a range of biochar types should be carried out.

CRediT authorship contribution statement

Jing Lyu: Writing – original draft, Formal analysis. **Alfred Obia:** Investigation, Formal analysis. **Gerard Cornelissen:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Jan Mulder:** Writing – review & editing, Investigation, Conceptualization. **Andreas Botnen Smebye:** Methodology, Conceptualization. **Andrew R. Zimmerman:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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