Relevance of biochar metabolization—evidence from a long-term biochar field experiment

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Biochar soil amendments are a promising strategy for long-term carbon dioxide removal due to their high aromaticity and low decay rate. While complete degradation of biochar was formerly investigated by CO_2 measurements and found to be negligible, we are focusing now at potential biochar metabolites. The major constituent of biochar is black carbon (BC), which can slowly be metabolized by microorganisms into benzene polycarboxylic acids (BPCA), so-called black carbon metabolites (BC_m). The relevance of this mechanism for the total dissipation of biochar under field conditions remains unknown due to a lack of long-term observations and BC_m measurements. This study aimed to quantify BC and BC_m in a long-term biochar field experiment covering 11 years in Bayreuth, Germany. Two variants were tested: biochar alone (31.5 Mg ha^{-1}) and a mixture of 31.5 Mg ha^{-1} biochar with 70 Mg ha^{-1} compost. Irrespective of the variant, the BC stocks increased threefold following biochar addition to 15−20 Mg C ha^{-1}, and decreased slightly after 11 years in both treatments. BC_m stocks were generally low, comprising <3% of total BC. Their content was higher before the application of biochar than afterwards; thus, the addition of biochar did not lead to higher BC_m release, but rather the opposite. This result indicates that microorganisms preferentially consume more labile substances introduced with the biochar rather than BC and that microbial diversity may need time to adjust their diet. Thus, BC_m release could play a larger role over longer timescales. Considering the total amounts of biochar added to the soil, BC_m represents a very small fraction which, for the ecological evaluation of biochar's dissipation, is negligible.

KEYWORDS

biochar, long-term field experiment, biochar dissipation, metabolization, benzene polycarboxylic acids

1 Introduction

Soils are the largest terrestrial carbon (C) sink, storing more C than the combined total found in terrestrial vegetation and the atmosphere (Lehmann and Kleber, 2015). By increasing the global soil organic carbon (SOC) stocks, large amounts of carbon dioxide emissions could be offset, making such soil-based strategies key for carbon dioxide removal (CDR). Soil-based CDR strategies have varying CO_2 reduction rates and potential adoption areas, leading to potential global CO_2 removal rates ranging from 0.01 to 1.8 Gt (10^{15} g) CO_2 per year (Paustian et al., 2016). Thus, many of these strategies need to be implemented in parallel to help closing the gap between C sources and sinks.

Out of many soil-based CDR strategies, biochar offers the greatest potential for CO_2 reduction and the largest potential area for implementation (Paustian et al., 2016). Biochar is the C-enriched, polyaromatic solid product of thermochemical conversion of biomass under limited oxygen supply, i.e., through pyrolysis (Guo et al., 2016). During pyrolysis, organic macromolecules of the original biomass such as hemicelluloses, celluloses and lignin are transformed into highly aromatic black carbon (BC) structures (Lehmann and Joseph, 2024). Black carbon is an umbrella term for polycondensed aromatic moieties, either produced from wildfires or pyrolysis, or fossil C burning. In nature, they mostly result from pyrogenic sources, but polycondensed aromatic moieties can also be produced biologically, e.g., by the fungus *Aspergillus niger* (Vaezzadeh et al., 2023; Glaser and Knorr, 2008). Biochar contains variable amounts of BC, depending on the temperature and duration of pyrolysis (Schimmelpfennig and Glaser, 2012). Besides the highly condensed and aromatic BC being the main fraction of biochar, it also contains aliphatic compounds. These compounds possess higher mineralization rates than BC because microbes preferably target their weaker C-bonds (Rudra et al., 2024). Therefore, they represent the "labile fraction" of biochar. BC is also referred to as the "aromatic backbone," and represents the "stable fraction," which persists in the long-term (Kuzyakov et al., 2014).

Biochar can be used for various purposes, including the improvement of waste management, materials, energy production, and the mitigation of air and soil pollutants (Lehmann and Joseph, 2024). Used as a soil amendment, biochar has proven to have many agronomic benefits, such as improved soil hydraulic properties, nutrient retention and availability, and thus increased plant biomass (Schmidt et al., 2021).

However, once applied to soil, biochar does not remain rigidly in place but dissipates slowly via diverse transformation and translocation processes. Understanding the dissipation pathways of biochar is of high agronomic significance. To ensure the soil health improving effects, biochar particles should ideally remain in the soil rooting zone to facilitate the retention of water and nutrients that are required for plant growth. In addition, a holistic understanding of biochar dissipation is important for the growing carbon credit market. Biochar-based CDR solutions currently dominate the CDR market, generating the highest sales among CDR technologies (CDR.fyi, 2024). Although biochar stocks may decline in certain soil layers due to translocation, persisting particles still contribute to long-term carbon sequestration and should be accounted for accordingly. Based on findings of biochar dissipation studies, efficient soil management solutions can be developed ensuring minimal dissipation, maximizing the soil-improving effects of biochar.

Mineralization rates of biochar depend on various factors: biological, physical, and chemical processes occurring in soil, as well as biochar's properties, which are defined by its feedstocks and production parameters (Lehmann et al., 2024). More C-rich feedstocks, such as wood and crop residues have been shown to enhance the stability of the resulting biochar more than biochar made from pyrolysis N-rich feedstocks such as manures (Almutairi et al., 2023). Prolonged pyrolysis durations and higher temperatures produce biochar with a higher degree of aromaticity, along with lower H/C and O/C ratios—both of which are reliable predictors of biochar's stability (Ippolito et al., 2020).

Decomposition by microbial metabolization is an overlooked pathway of biochar field dissipation due to the relatively low rates at which measurable mass losses of biochar and resulting transformation products may not be detected within typical research periods. Most published studies have relied on biochar incubation experiments—mostly in laboratory conditions—and the assessment of its physical and chemical properties, including elemental analysis, industrial analysis, and chemical oxidation resistance, along with modeling approaches such as the dual-pool exponential model. This model, although widely recognized, often overlooks environmental influences (Budai et al., 2013; IPCC, 2019; Luo et al., 2023; Wang et al., 2022). However, environmental conditions can significantly accelerate the mineralization of biochar and reduce its long-term stability in soils. Laboratory experiments often may not accurately estimate biochar stability, as they do not fully replicate the complex, time-dependent processes occurring under field conditions. Biomarker-based analysis using ^{14}C-labeled biochar (produced from ryegrass at a pyrolysis temperature of 400°C resulting in a C/N ratio of 22) over an 8.5-year incubation period has shown an average total mass loss of approximately 0.3% per year. From the remaining 96% total biochar mass, 1.5–2.6% of the ^{14}C could be recovered in microbial phospholipid fatty acids after 1.7 years (Kuzyakov et al., 2014). However, such ^{14}C-labeling studies are cost-intensive and, if performed in a laboratory incubation setting, their transferability into real world conditions is limited. Field ageing alters biochar properties, including increased specific surface area and oxygen-containing functional groups, and decreased carbon content—all of which influence its degradation dynamics. As Luo et al. (2023) emphasized, most current studies are limited to short-term, single-factor laboratory experiments. Meanwhile, the development of robust predictive models—especially those employing machine learning and AI—is constrained by the lack of long-term field data and poor adherence to FAIR (findable, accessible, interoperable and re-usable) data principles. Therefore, well-designed long-term field trials and advanced analytical tools are urgently needed to improve our understanding and prediction of biochar stability under real environmental conditions.

The polycondensed aromatic moieties defining BC produce benzene polycarboxylic acids (BPCA) during natural oxidation processes, mainly driven by microbial activity. In the following manuscript we will refer to them as black carbon metabolites (BC_m). This process is similar to the accelerated oxidation of BC during the analytical BPCA method (Glaser et al., 1998), however under natural oxidation conditions, which are much slower. To avoid confusion in this manuscript, we will refer to BPCA resulting from the natural oxidation of BC as BPCA-BC_m, and to BPCA resulting from analytical oxidation for BC determination as BPCA-BC.

Such BC_m, have been documented to be ubiquitous in soils (Di Rauso Simeone et al., 2018; Haumaier, 2010), similar to their parent compound, BC. The content of BC_m has been found to vary across soil types, ranging from 0 to 0.24 g kg^{-1}, contributing relatively to 0–38% of total BC (Di Rauso Simeone et al., 2018).

Measuring BC_m in biochar-amended soil has not been attempted before. Thus, this is the first study attempting to describe biochar degradation using BC_m in a long-term field experiment. Therefore, the objective of this study was to analyze samples from a long-term biochar field experiment—collected prior to biochar application, and 3 and 11 years thereafter—for BC and BC_m. The results were used to conduct time series analysis, and to evaluate if BC_m are generated after the application of biochar, and how this influences the BC stocks. The goal was to assess whether biochar metabolization to BC_m is a relevant

biochar dissipation pathway in a loamy soil and under temperate climatic conditions.

We hypothesize that:

1 The addition of biochar leads to a significant increase of BC_m in the soil due to partial biochar degradation.
2 The co-addition of compost reduces BC_m formation compared to biochar alone, due to reduced biochar degradation when mixed with compost.

2 Materials and methods

2.1 Sampling area, approach and amendments properties

We utilized a long-term biochar field experiment, which was initiated in 2010 near Bayreuth, Germany (49°56′01.7″, 11°31′17.1″). The mean annual precipitation was 507 mm and the mean annual temperature was 8.2°C during the experimental period. The field experiment was conducted on a cropland site. The soil type was sandy loam Cambisol with a pH at 5.4, and a soil organic carbon content of 16 g kg^{-1}.

The experiment was initiated to analyze the effects of different organic soil amendments (OSA), consisting of biochar mixed with compost on soil properties and crop yields under organic farming conditions. For our investigation, we used two out of the ten treatments of the field experiment: (1) biochar alone applied at a rate of 31.5 Mg ha^{-1}, and (2) a mixture of biochar and compost, consisting of 31.5 Mg ha^{-1} biochar and 70 Mg ha^{-1} compost dry matter. Each treatment was replicated five times independently, arranged in a Latin rectangle, with three of these replicates selected for our study.

From 2012 onwards, all plots were treated equally with organic fertilizers, either maize biogas digestate applied with a liquid manure spreader or cow/horse slurry broadcasted at rates of 15–30 Mg ha^{-1} annually. Further details on the field experiment can be found in Cooper et al. (2020) and Gross et al. (2024).

The biochar, produced by CarbonTerra (Wallerstein, Germany), was made from pine wood chips through a two-step pyrolysis process: slow pyrolysis at 550°C for 36 h followed by high-temperature pyrolysis at 800°C for 2 h. The compost, produced by BKE Bio-Kompost and Entsorgung GmbH & Co. (Bindlach, Germany), was derived from green waste.

Table 1 summarizes the key properties of the biochar and compost used. The organic materials were manually distributed over the plots and then incorporated into the soil to a depth of 10 cm using a rotary tiller. Biochar was applied only once at the start of the experiment in 2010, while compost was applied annually until 2012.

2.2 Soil sampling and preparation

Soil sampling was carried out in 2010 (prior to the application of soil amendments), as well as in 2013 and 2021. In 2010 and 2013, samples were collected from two depths: 0–10 cm and 10–30 cm. In 2021, samples were taken from a depth of 0–30 cm. At the center of each plot, five samples were collected using an auger and combined into a single composite sample. The soil samples were oven-dried at

TABLE 1 Main biochar and compost properties of the field experiment in Bayreuth.

Amendment properties	Biochar	Compost
Total carbon (g kg^{-1})	843	186
Total nitrogen (g kg^{-1})	4	10
C/N	239.2	18.8
H/C (atomic ratio)	0.11	1.41
pH (CaCl$_2$)	8.8	7.0
Ash (g kg^{-1})	90.3	66.1
CEC (mmol$_c$ kg^{-1})	72.7	304.3
Black carbon (g kg^{-1} C)	795	n.d.[a]

[a]n.d., not defined.

40°C for 48 h. For subsequent analysis, the samples were sieved to <2 mm and ground using a vibratory disc mill.

2.3 Soil analysis

2.3.1 Black carbon and black carbon metabolite analysis

Soil organic carbon (SOC) was measured using dry combustion with a CN elemental analyzer (Elementar Vario EL, Heraeus, Hanau, Germany). Each sample was treated with diluted hydrochloric acid to remove inorganic carbon. To ensure consistency, samples were analyzed as complete time series for each treatment, minimizing systematic errors over time.

The BC content was determined as benzene polycarboxylic acids (BPCA) following the method of Glaser et al. (1998), with modifications by Brodowski et al. (2005). In brief, samples were hydrolyzed using trifluoroacetic acid to remove polyvalent cations. Following oxidation of the filtered hydrolysates with nitric acid in a high-pressure digestion apparatus, residual polyvalent cations were removed by passing diluted aliquots through a cation exchange resin. BPCA were quantified after derivatization using a Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector and an HP5 column (30 m × 0.25 mm × 0.25 μm). The aromaticity of the samples was assessed by calculating the relative contributions of hemimellitic, trimellitic, and trimesic acids (B3CA-BC); pyromellitic, melophanic, and prehnitic acids (B4CA-BC); benzene pentacarboxylic acid (B5CA-BC); and mellitic acid (B6CA-BC). The sums of individual BPCA-BC amounts were multiplied by 2.27, to convert them into charcoal equivalents (Glaser et al., 1998).

To determine the content of BC_m, we applied a modified method of Di Rauso Simeone et al. (2018), a variation of the aforementioned method originally developed by Haumaier (2010). Deviating from the original protocol, the extraction was performed over 24 h instead of 48 h, and centrifugation was conducted at 3,700g. Only 20 mL of extract, rather than 25 mL, was passed through the cation exchange column. For chromatographic analysis, we used an ICS 3000 system equipped with a Dionex IonPac AS19-HC column (4 × 250 mm) and employed potassium hydroxide (KOH) as the eluent, instead of sodium hydroxide (NaOH), at a constant flow rate of 1 mL min^{-1} and 30°C column temperature. The elution gradient was programmed as follows: 0–1 min 35 mM KOH, 1–5 min 40 mM KOH, 5–20 min 80 mM KOH, 20–30 min 100 mM KOH, 30–35 min hold on 100 mM

KOH, 35–40 min 50 mM KOH and 40–50 min hold on 50 mM. Detection was performed using a UV detector set at 254 nm. The sums of all measured individual BPCA-BC_m amounts were multiplied by 2.27 to convert them into BC_m equivalents, enabling comparability with the BC values reported in this study. Individual BPCA-BC_m included hemimellitic, trimellitic, and trimesic acids (B3CA-BC_m); pyromellitic, melophanic, and prehnitic acids (B4CA-BC_m); benzene pentacarboxylic acid (B5CA-BC_m); and mellitic acid (B6CA-BC_m).

Accuracy and sensitivity have been published elsewhere (Glaser et al., 1998 for BC, Di Rauso Simeone et al., 2018 for BC_m) and are applicable to this study, as we followed their analytical procedure as closely as possible. BPCA-BC_m analytical method QA/QC parameters are described in detail in Di Rauso Simeone et al. (2018). In brief, recovery of BPCA-BC_m spiked to soil samples covering a wide range of texture, pH, and TOC was 81 ± 19%. The practical quantification limits (PQL) were: B3CA = 0.30 mg C kg^{-1}; B4CA = 0.27 mg C kg^{-1}; B5CA = 0.26 mg C kg^{-1}, B6CA = 0.26 mg C kg^{-1}. BPCA analytical method QA/QC parameters are provided in Glaser et al. (1998). Recoveries of BPCA from soil samples were between 95 and 105%. PQL for this method were: B3CA = 54 mg C kg^{-1}; B4CA = 47 mg C kg^{-1}; B5CA = 44 mg C kg^{-1}, B6CA = 42 mg C kg^{-1}.

2.3.2 Soil texture, bulk density, and stock calculation

Soil texture estimation was based on particle-size distribution, analyzed through laser diffractometry. We applied the pedotransfer function of Men et al. (2008) to calculate bulk densities for each treatment plot and sampling date (Equation 1), as bulk density (BD) data were not consistently available for all sampling dates. To ensure accuracy, the estimated BD was compared with field measurements whenever available.

$$\text{Bulk density} = 1.386 - 0.078 \times \text{SOC} + 0.001 \times \text{Silt} + 0.001 \times \text{Clay} \quad (1)$$

The BD is expressed in g cm^{-3} and the SOC, silt, and clay content in %.

The estimated BD values were used to calculate BC and BC_m as stocks. This step was crucial for comparing data from soil depths of 0–10 cm and 10–30 cm (collected before 2021) with the data from 2021, which was gathered from a unified soil depth of 0–30 cm. To create a comparable basis for our measured data between the years, we decided to express the results in unified stocks covering 0–30 cm. Stocks were quantified in accordance with FAO (2019).

2.4 Statistical analysis

Statistical analysis was carried out using R version 4.1.2 (R Core Team, 2021) and the R package lme4 (Bates et al., 2015). The differences of BC or BC_m stocks among different sampling dates in time were analyzed using a linear mixed-effects model. Since the response variable (BC or BC_m) becomes dependent due to repeated measurements on the same plots, both fixed effects (treatment structure) and random effects (time) influence our model results, thus a mixed-effects model was used (Piepho et al., 2003). Stepwise model simplification was applied, eliminating first non-significant interaction effects, then non-significant main effects (Crawley, 2012). Variance
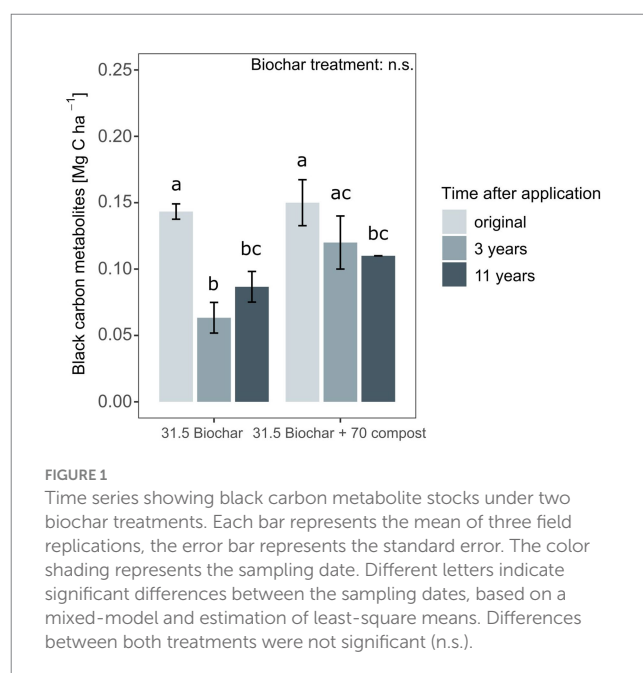
components were estimated with the residual maximum likelihood (REML) method (Kenward and Roger, 1997). Normality of model residuals was checked using the Shapiro–Wilk test. To meet the assumptions of parametric modeling, a Box-Cox transformation was applied in cases where residuals were non-normal distributed. Significant effects were determined using least-squares means estimation with the R package emmeans (Lenth et al., 2023). *Post-hoc* test results are provided in Supplementary Tables S1, S2.
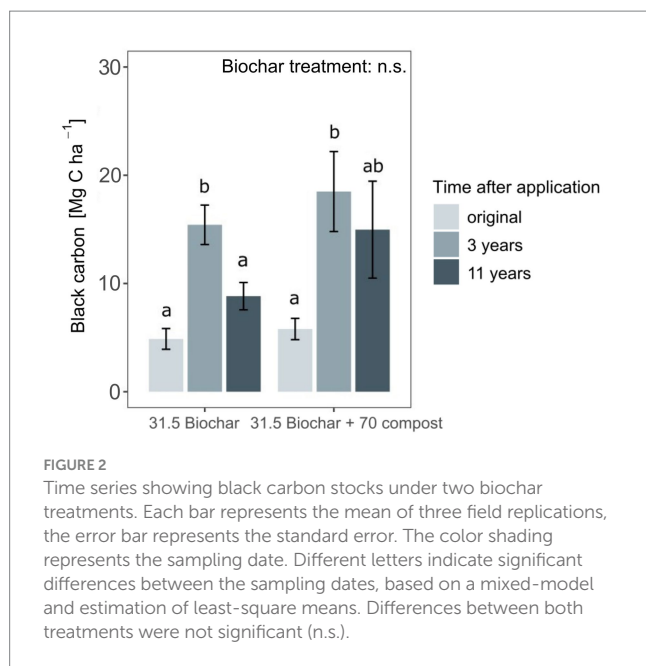
3 Results

3.1 Temporal dynamics of black carbon metabolites and black carbon

Overall, BC_m stocks were very low. In both treatments, BC_m stocks were higher before the application of biochar than 3 years afterwards (Figure 1). The addition of 31.5 Mg ha^{-1} biochar significantly decreased the BC_m stocks by 53% 3 years after application. Adding 70 Mg ha^{-1} compost to 31.5 Mg ha^{-1} biochar resulted in a 12% decrease, although this change was not statistically significant (n.s.) during the same period. In the long term, between 3 and 11 years after biochar and compost application, there was no significant change of BC_m stocks in either treatment. However, the pristine biochar treatment showed a slight, non-significant increase of 39%, whereas the biochar + compost treatment showed a slight, non-significant decrease of 8% (Figure 1).

The addition of 31.5 Mg ha^{-1} biochar, with or without 70 Mg ha^{-1} of compost, led to approximately threefold higher BC stocks 3 years after the application (Figure 2). After an additional 8 years, BC stocks declined significantly in the pristine biochar treatment, whereas the biochar + compost treatment did not show a significant change. Overall, BC stocks in the pristine biochar treatment dropped more pronounced in the long-term (−43%) than the biochar + compost

FIGURE 1
Time series showing black carbon metabolite stocks under two biochar treatments. Each bar represents the mean of three field replications, the error bar represents the standard error. The color shading represents the sampling date. Different letters indicate significant differences between the sampling dates, based on a mixed-model and estimation of least-square means. Differences between both treatments were not significant (n.s.).

FIGURE 2
Time series showing black carbon stocks under two biochar treatments. Each bar represents the mean of three field replications, the error bar represents the standard error. The color shading represents the sampling date. Different letters indicate significant differences between the sampling dates, based on a mixed-model and estimation of least-square means. Differences between both treatments were not significant (n.s.).

treatment (−19%), although no statistically significant treatment effect was observed.

3.2 Relative contribution of individual BPCA to black carbon metabolites and black carbon

Lower aromatic B3CA-BC$_m$ dominated the BC$_m$ stocks, irrespective of sampling date, the presence of biochar in the soil, or the type of biochar treatment (Figure 3). The relative contribution of B3CA-BC$_m$ to total BC$_m$ ranged between 70 and 75% across both treatments and all time points, while higher aromatic BC$_m$ played a minor role. However, in both treatments, the relative amount of B3CA-BC$_m$ was largest 11 years after biochar application, at the expense of higher aromatic B6CA-BC$_m$.

The relative contribution of higher aromatic BPCA-BC to total BC stocks increased following biochar application compared to the original soil (Figure 4). The combined contribution of B5CA-BC and B6CA-BC accounted for over 75% of total BC stocks 3 years after biochar had been applied, regardless of the biochar treatment. In the long-term, the contribution pattern remained largely unchanged, although the proportion of higher aromatic BPCA-BC continued to increased slightly.

3.3 Relative contribution of BPCA-BC$_m$ to BPCA-BC

The relative contribution of BPCA-BC$_m$ to BPCA-BC was higher prior to biochar application, with values of 2.4 and 3.4% in the 31.5 Mg ha^{-1} biochar and 31.5 Mg ha^{-1} Biochar + 70 Mg ha^{-1} compost treatment plots, respectively (Table 2). After biochar addition, the relative proportion of BPCA-BC$_m$ dropped to ≤1%, regardless of the treatment. Among individual BPCA-BC$_m$, the lower aromatic B3CA-BC$_m$ had the highest contribution. In the original soil, approximately

10% of B3CA-BC in the soil represented B3CA-BC$_m$. After an initial decline in the relative contribution of B3CA-BC$_m$ to B3CA-BC 3 years after biochar application, the contribution increased to 20 and 26% 11 years after application in the pristine biochar and biochar-compost mix treatments, respectively (Table 2).

In summary, we observed a 53% decrease of BC$_m$ stocks 3 years after biochar addition, but no significant change in the long term. During the same period, BC stocks tripled, 3 years after biochar application. Over 11 years, we have observed a more pronounced decrease in the pristine biochar treatment compared to the biochar + compost treatment. The co-addition of compost did not significantly affect BC$_m$ stocks but slightly mitigated the long-term decline in BC stocks. Lower-aromatic B3CA-BC$_m$ dominated total BC$_m$ stocks throughout the experimental period, while the contribution of higher aromatic BPCA-BC to the BC stocks increased over time following biochar application.

4 Discussion

4.1 Effect of biochar application on the stock dynamic of black carbon metabolites and black carbon

The addition of pure biochar or the mix of biochar and compost did not lead to elevated BC$_m$ generation compared to the original unamended soil (Figure 1). Simultaneously, BC stocks increased upon biochar addition, and the elevated stocks showed slight dissipation effects in the long term (n.s. for the biochar-compost mix). Against this background, biochar degradation to BC$_m$ may not have relevance for this biochar dissipation. First, because the addition of biochar to soil did not lead to a measurable increased release of BC$_m$; and second, because the BC$_m$ stocks are very low (<0.3 Mg C ha^{-1}) and therefore represent a negligible pathway of biochar loss. More likely, biochar dissipation at this location is related to lateral and vertical transport driven by rainfall, tillage, and soil faunal activity, as the findings of Gross et al. (2024) indicate.

The finding that biochar addition significantly reduced BC$_m$ stocks was unexpected, as we initially hypothesized that more BC compounds in the soil would trigger more biochar metabolization products to be produced by microbial breakdown and to be traceable. This clearly indicates a preferential microbial utilization of more labile organic compounds co-introduced with the biochar, rather than BC itself. Due to the relatively high amount of activation energy that microorganisms need to invest for using BC compounds for metabolization, they generally prefer different more labile carbon sources (Lehmann et al., 2024). However, microbial communities may adapt and begin to degrade BC, particularly once their diversity has increased in its presence (Haumaier, 2010; Di Rauso Simeone et al., 2018). Such a microbial dietary adjustment could explain higher BC$_m$ stocks 11 years after addition of pure biochar (Figure 1), potentially indicating higher BC$_m$ release in the longer term—an aspect that warrants further attention.

Another possible explanation is that biochar sorbed the BC$_m$ in the soil onto its surface. Such an affinity of numerous organic molecules to the surface of biochar has been observed elsewhere (Gęca et al., 2022; Kah et al., 2017; Luo et al., 2022; Smernik, 2012). The biochar used in this study has H/C 0.11, indicating presence of

FIGURE 3
Relative contribution of the sum of hemimellitic, trimellitic, and trimesic acids (B3CA-BC$_m$); pyromellitic, melophanic, and prehnitic acids (B4CA-BC$_m$); benzene pentacarboxylic acid (B5CA-BC$_m$); and mellitic acid (B6CA-BC$_m$) to the sum of all BPCA-BC$_m$ of each of the two investigated treatments.
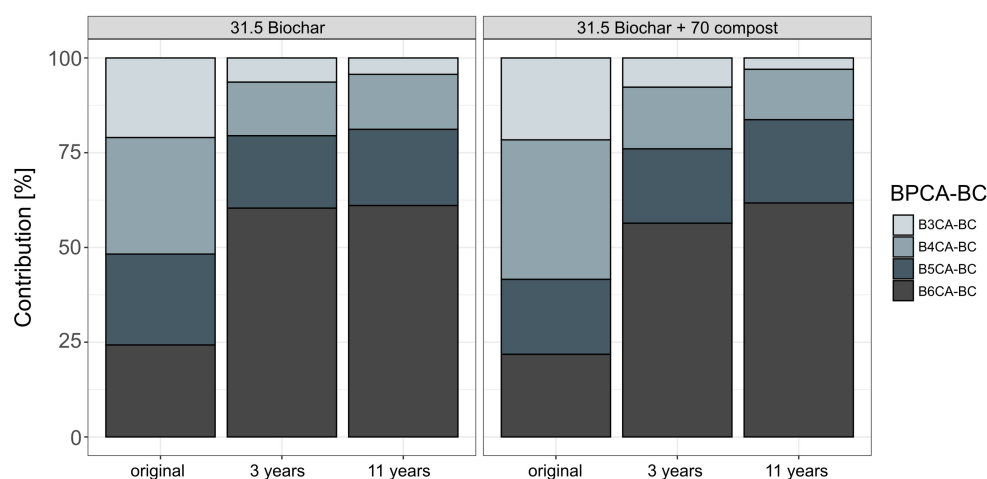

FIGURE 4
Relative contribution of the sum of hemimellitic, trimellitic and trimesic acids (B3CA-BCs), the sum of pyromellitic, melophanic and prehnitic acids (B4CA-BCs), benzene pentacarboxylic acid (B5CA-BC) and mellitic acid (B6CA-BC) to the sum of all BPCA-BC of each of the two investigated treatments.

predominately aromatic structure. The aromatic structures within biochar create electron-dense zones that can attract and stabilize the π-electrons in the aromatic rings of BPCA-BC$_m$. This interaction can be visualized as the stacking of two smooth, planar structures, where the alignment of aromatic rings creates a stable attraction, enhancing the adsorption of these compounds onto the biochar surface (Grgić et al., 2019; Isakovski et al., 2024). Also, planar molecules tend to penetrate to the nanopores of biochar and form π-π-bonds with both walls of the pores which results in an irreversible binding of compound to the biochar. Other opposite mechanisms are related to electrostatic interactions and pH influence. Haumaier (2010) demonstrated that sorption of BC$_m$ to soil mineral components is strongly related to soil pH. Acid soils with high hydrous oxide availability have shown to accumulate BC$_m$ stronger than medium pH or alkaline soils. Higher

soil pH, e.g., induced through input of pyrogenic residues as described by Novotny et al. (2007) can lead to increased mobilization of BC$_m$ and export into groundwater. The input of biochar into our experiment soil initially led to an increase of pH, but this liming effect diminished over time due to biochar aging (Apostolović et al., 2024). The soil pH remained above 7, exceeding the pKa values of all BC$_m$ compounds, indicating that they predominantly exist in their deprotonated form. This increased deprotonation of BPCA-BC$_m$ carboxyl groups can lead to electrostatic repulsion from the negatively charged biochar surfaces, thereby reducing sorption efficiency. Therefore, microbial degradation may have occurred, but the increased pH through biochar's liming effect could have facilitated vertical translocation of the BC$_m$. Biochar aging in soil can also lead to strong alterations of its initial surface properties, including increased ion exchange, higher specific surface

TABLE 2 Relative proportion of metabolized benzene polycarboxylic acids (BPCA-BC$_m$) in the soil to BPCA stored in black carbon (BPCA-BC).

Treatment	Time after application	B3CA-BC$_m$/ B3CA-BC	B4CA-BC$_m$/ B4CA-BC	B5CA-BC$_m$/ B5CA-BC	B6CA-BC$_m$/ B6CA-BC	BPCA-BC$_m$/ BPCA-BC
		(%)				
31.5 Biochar	Initial	11.1 ± 5.1	0.8 ± 0.4	1.5 ± 0.9	1.7 ± 0.9	3.4 ± 2.4
	3 years	4.5 ± 1.2	0.3 ± 0.1	0.3 ± 0.1	0.0 ± 0.1	0.4 ± 0.2
	11 years	20.0 ± 1.9	0.7 ± 0.2	0.7 ± 0.3	0.1 ± 0.1	1.0 ± 0.4
31.5 Biochar + 70 compost	Initial	9.4 ± 5.8	0.5 ± 0.3	1.0 ± 0.1	1.0 ± 0.1	2.4 ± 0.9
	3 years	7.6 ± 1.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.8 ± 0.2
	11 years	25.6 ± 5.1	0.5 ± 0.1	0.5 ± 0.2	0.1 ± 0.0	1.0 ± 0.4

area, and increased polarity (Pignatello et al., 2024). These shifting properties affect the role of biochar in sorption reactions over time and could have also affected the sorption of BC$_m$ to the surface of biochar.

It is noteworthy that this study represents a single observation under humid climatic and loamy soil conditions and the findings might therefore only be transferable into similar regional contexts. As mentioned earlier, biochar degradation rates vary depending on the climate and soil conditions. For example, biochar metabolization rates may be higher in tropical soil, which on average have shown higher BC$_m$ contents among the different soil types and regions studied (Haumaier, 2010; Di Rauso Simeone et al., 2018). Haumaier (2010) and Di Rauso Simeone et al. (2018) both identified the soil pH being a critical influencing factor. Higher pH in Chernozems and Cambisols correlated with lower BC$_m$ contents, possibly due to greater leaching or lower degradation rates. Fe/Al oxide-rich soils (e.g., in Andosols, Ferralsols) likely enhanced BC$_m$ retention through sorption.

Moreover, biochar properties themselves may influence metabolization. In sum, more observations from varying agroecosystem settings, using varying biochars and at varying points in time after the biochar application are needed to validate our findings and to draw clear conclusions about the ecological relevance of biochar metabolization.

4.2 Stability of the measured black carbon

During aging of biochar in soil, higher condensed aromatic moieties relatively increased in the remaining biochar, at the expense of lower aromatic B3CA-BC and B4CA-BC (Figure 4). Larger proportion of B5CA-BC and B6CA-BC reflect higher degrees of aromatic condensation (Glaser et al., 1998), greater oxidative resistance, and are often associated with higher pyrolysis temperatures (Chang et al., 2019), and higher stability. These more condensed B5CA-BC and B6CA-BC remained stable over the long term, persisting for more than 10 years. The sum of them even further increased with progressing time. This suggests that BC stocks may be heading towards a long-term steady state, as the more stable compounds tend to persist. This corroborates with recent estimates about the environmental persistence of biochar, correlating with its H/C ratio, which is a valid proxy for the aromaticity of biochar (Lehmann et al., 2024; Wang et al., 2013). The biochar used in the Bayreuth field experiment was highly aromatic, with a H/C ratio of 0.11 (Table 1). Based on this ratio, the expected

BC$_{100}$ (% of initial C remaining after 100 years) is over 90%, thus long-term stable (Azzi et al., 2024).

4.3 Effect of biochar on individual BPCA-BC$_m$

The contents of BPCA-BC$_m$ measured in our experiment soil were generally very low (<0.03 g kg^{-1}). Among the individual BPCA-BC$_m$, B3CA-BC$_m$ was by far the most abundant BPCA-BC$_m$ in soil, before and after the addition of biochar, and irrespective of biochar treatment. Soils in temperate climate region like the experiment soil near Bayreuth generally contain more B3CA-BC$_m$ and B4CA-BC$_m$ than higher condensed aromatic moieties such as B5CA-BC$_m$ and B6CA-BC$_m$ (Di Rauso Simeone et al., 2018). Higher condensed BPCA-BC$_m$ are more abundant in tropical soils with higher microbial activity, which can accelerate the degradation of BC into higher condensed aromatic moieties (Möller et al., 2000). Moreover, the acidic conditions in these soils reduce the mobility of BC$_m$. Higher pH in the temperate cropland soil in Bayreuth could have in turn accelerated the mobility of BPCA-BC$_m$ and its vertical translocation. It has previously been shown that BPCA-BC$_m$ are mobile in the soil profile and thus could be identified in soil depths of 100 cm and deeper (Haumaier, 2010; Möller et al., 2000).

The ratio between BPCA-BC$_m$ and BPCA-BC (particularly B3CA-BC$_m$), increased over time following biochar application (Table 2), indicating that B3CA-BC$_m$ may gain relevance in the long term. This finding suggests that BC metabolization mainly leads to B3CA-BC$_m$ formation, while higher-condensed moieties remain stable in the biochar.

5 Conclusion

This study was the first to analyze the temporal dynamics of BC$_m$ released into soil and their relevance for biochar dissipation. A long-term biochar field experiment on loamy soil under humid climate conditions in Bayreuth, Germany was revisited and resampled. Samples taken before and after biochar application were analyzed for BC and BC$_m$, molecular markers for the biochar stability and metabolization, respectively.

We hypothesized that the addition of biochar would lead to a significant increase of BC$_m$ in the experimental soil. However, this

hypothesis must be rejected, as BC_m stocks were higher in unamended soil than in the biochar-treated plots. The second hypothesis, that the co-addition of compost reduces BC_m generation, was supported. BC_m dynamics differed between treatments: the decline in BC_m following biochar addition was less pronounced in the biochar-compost mix treatment, and the pristine biochar treatment showed a slight increase of BC_m after 11 years, suggesting a possible microbial adjustment towards BC utilization.

Based on our findings, metabolization to BPCA of highly aromatic biochar in a loamy soil and under humid conditions is a negligible dissipation pathway over an 11-year period. To maintain efficient carbon sequestration and minimize biochar degradation, it is therefore recommended to use highly aromatic biochar with an H/C ratio of below 0.2. For ensuring biochar stability, it was irrelevant whether pristine biochar was applied alone or mixed with compost. Given the co-addition of compost to biochar have already proven to have beneficial effects for soil health and plant nutrition over pristine biochar, the combined application should be preferred.

While our observations provide valuable insights into BC_m behavior, several questions remain unanswered. It remained unresolved why BC_m stocks decreased after biochar was introduced to the soil, and whether this effect is limited to the first few years or if the BC_m release increases in the long term, as the pristine biochar treatment indicates. Similarly, the role of biochar surfaces in facilitating sorption through π–π interactions, or promoting desorption through electrostatic repulsion of BC_m, remains unclear. Given that this is a single observation under humid climate and loamy soil conditions, the findings might only be transferable into similar regional contexts. Biochar degradation rates strongly differ depending on the climate and soil conditions, thus, biochar metabolization rates could be higher, for example, in tropical soils. Moreover, biochar properties themselves may also influence the occurrence of metabolization.

Overall, the occurrence of BC_m in biochar-amended soils was environmentally insignificant, with concentrations barely detectable using currently available analytical methods. Given the extremely low BC_m stocks (<1% of BC), biochar metabolization appears to play a minor role in long-term dissipation. Instead, vertical and lateral transport remain the major pathways of biochar loss and should receive greater attention in future studies aimed at improving the long-term stability and CDR potential of biochar systems.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://doi.org/10.5281/zenodo.15263448.

Author contributions

AG: Data curation, Formal analysis, Investigation, Validation, Visualization, Writing – original draft. MŠ: Formal analysis,

Investigation, Writing – review & editing. BG: Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Writing – review & editing. TB: Formal analysis, Methodology, Supervision, Writing – review & editing. SM: Formal analysis, Methodology, Resources, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2025.1578363/full#supplementary-material

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