



Rock-enhanced biochar exhibits similar priming effect as pure biochar application while improving short-term carbon stabilization in agricultural soils

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Abstract

Combined application of biochar and silicate rock powder might have synergistic effects on carbon dioxide removal and soil improvement. However, it remains unknown how their combination affects mineralization and stabilization of soil organic carbon (SOC). We compared pure ¹³C-labeled wheat-straw biochar, pure basanite rock powder, their co-application, and rock-enhanced biochar from co-pyrolysis of wheat and basanite. All amendments were mixed with three agricultural topsoils (temperate silty, temperate sandy, tropical sandy) and incubated for 66 days. The $\delta^{13}\text{C}$ -signal of the respired CO₂ was monitored to determine amendment-induced priming of native SOC. After incubation, a density fractionation was conducted to investigate the potential stabilization of native and biochar-derived SOC as free particulate organic matter (fPOM), occluded POM (oPOM), and mineral-associated organic matter (MAOM). Pure basanite application did not influence short-term SOC dynamics, probably due to limited weathering within the 66-day incubation. In combined applications (co-application and rock-enhanced biochar), the biochar effect was dominant. The effects of biochar-containing amendments (biochar, co-application, and rock-enhanced biochar) were mainly controlled by texture and pH. The sandy soils exhibited positive priming following the application of biochar-containing amendments. In the temperate soils, positive priming occurred in MAOM, while native fPOM was partly shifted to oPOM. Input of biochar-derived SOC compensated losses of native SOC in fPOM and MAOM, resulting in overall SOC gains in most fractions. While priming intensity of all biochar-containing amendments was comparable, rock-enhanced biochar most strongly increased SOC in the density fraction interpreted as MAOM and had a higher short-term stability. These findings demonstrate that short-term effects of combined applications on SOC dynamics were dominated by the biochar component, while highlighting the potential of co-pyrolysis for short-term SOC stabilization.

Keywords Silicate rock powder · Carbon dioxide removal (CDR) · Enhanced rock weathering (ERW) · Mineral-associated organic matter (MAOM) · Particulate organic matter (POM) · Biochar stability

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Introduction

To mitigate climate change and meet long-term climate targets, the removal of atmospheric carbon (C) dioxide plays an important role (Riahi et al. 2022). Soil applications of biochar and silicate rock powder for enhanced rock weathering combine C dioxide removal with soil fertility improvement (Buss et al. 2021; Joseph et al. 2021; Beerling et al. 2025). While most recent research considered both C dioxide removal technologies separately, their combined application could offer synergistic effects (Amann and Hartmann 2019; Joseph et al. 2021). However, effects of combined biochar

and silicate rock powder application on SOC mineralization and stabilization remain unknown.

Biochar is the solid product from biomass pyrolysis and consists mainly of highly aromatic pyrogenic C compounds (Hagemann et al. 2018; Xie et al. 2025). Due to its low degradability, biochar contributes to the persistent SOC pool (Kuzyakov et al. 2014; Wang et al. 2016; Xie et al. 2025) and its application provides a considerable C dioxide removal potential (Lehmann et al. 2021). However, biochar also contains minor amounts of more easily degradable organic compounds (Bruun et al. 2014; Bakshi et al. 2018). This labile C can either stimulate or suppress native SOC mineralization, resulting in a positive or negative priming effect, respectively (Kuzyakov et al. 2000; Yu et al. 2018). Direction and magnitude of biochar-induced priming vary depending on several parameters, such as soil and biochar properties (Zimmerman et al. 2011; Whitman et al. 2015; Joseph et al. 2021) as well as the observed time span after application (Blanco-Canqui et al. 2020). In particular, biochar-induced priming is often positive in sandy soils (Fang et al. 2015; Liu et al. 2015; Wang et al. 2016) with lower pH (Luo et al. 2011; Zhang et al. 2018; Xu et al. 2021) and organic matter content (Zimmerman et al. 2011; Wang et al. 2016). The mineralization of both native and biochar-derived SOC is often highest shortly after biochar application and declines over time (Kuzyakov et al. 2009; Ameloot et al. 2013; Whitman et al. 2015).

Applying silicate rock powder, such as basanite, to agricultural soils can couple C dioxide removal with improvements of soil properties (Swoboda et al. 2022; Dupla et al. 2024; Xu et al. 2025). Weathering of silicate rocks directly enhances soil C content through the reaction between CO₂-derived acidity and silicate minerals, forming bicarbonate and carbonate (Hartmann et al. 2013; Beerling et al. 2025), which can be stored as inorganic C in the soil (Hartmann et al. 2013; Martin 2017). However, the effect of enhanced rock weathering on SOC dynamics, such as SOC mineralization, is less well understood. Even though silicate rock powder does not contain labile C to directly induce a priming effect, it still has the potential to alter SOC mineralization, e.g. via an increase in soil pH (Amann et al. 2020; Lei et al. 2025) or changes in nutrient availability (Harley and Gilkes 2000; Hartmann et al. 2013).

Soil organic C is stabilized through the low bioavailability and biochemical complexity of particulate organic matter (POM), through spatial inaccessibility via occlusion of POM within aggregates (oPOM), or through the association with mineral surfaces (Sollins et al. 1996; von Lützow et al. 2006; Lützow et al. 2008). Aggregate occlusion and mineral-association limit the accessibility of SOC for microbes, thus reducing its biodegradation (von Lützow et al. 2006; Kleber et al. 2015). Both silicate rock powder and biochar have the potential to influence the stabilization of native SOC. The

weathering of silicate rock powder can enhance aggregate formation by releasing bivalent cations, such as Ca and Mg, which promote cation bridging and flocculation of soil particles (Clough and Skjemstad 2000; Buss et al. 2024a; Xu et al. 2025). Additionally, fresh mineral surfaces and reactive secondary minerals, which are formed during weathering, can directly adsorb dissolved organic C (DOC) forming mineral-associated organic matter (MAOM; Reichenbach et al. 2021; Buss et al. 2024a). While most biochar-derived C remains as highly recalcitrant free POM (fPOM; Kalu et al. 2024), it can also be further stabilized by occlusion in aggregates (Yoo et al. 2017; Joseph et al. 2021) or mineral-association (Yang et al. 2016, 2021). Additionally, biochar has the potential to accelerate the formation of aggregates and the stabilization of native SOC as MAOM (Wang et al. 2017; Weng et al. 2017; Xu et al. 2024). The influence of pure biochar and silicate rock powder application on the mineralization of native SOC (amendment-induced priming) and the stabilization of SOC are relatively well understood. However, their combined effects have rarely been investigated. The combination of biochar and silicate rock powder, such as basanite, can comprise their co-application as well as the co-pyrolysis of biomass and rock powder to produce rock-enhanced biochar (Meyer zu Drewer et al. 2025; Vorrath et al. 2025; Ansari et al. 2026). Co-pyrolyzing biomass and minerals can increase the C yield (Buss et al. 2024b) and improve the nutrient content of the pyrolysis product (Meyer zu Drewer et al. 2025). Combining biochar and silicate rock powder can result in the further stabilization of biochar-derived C on rock powder surfaces (Meyer zu Drewer et al. 2025), especially if it is directly bound to mineral surfaces during co-pyrolysis. First studies on the co-application of biochar and silicate rock powder reported divergent effects, including stimulated SOC mineralization due to pH increases (te Pas et al. 2026), inhibition of biochar-induced SOC stabilization by rock powder (Sohng et al. 2025), and additive effects of both amendments on SOC sequestration (Honvault et al. 2024). These divergent first results emphasize the need to understand whether co-application and rock-enhanced biochar exhibit favorable effects on SOC dynamics compared to pure applications.

Therefore, the objective of this study was to identify the combined effects of both amendments on short-term SOC (both native SOC and biochar-derived C) dynamics, including short-term amendment-induced priming and stabilization, in contrasting agricultural soils and to assess how these effects differ from those of pure biochar or basanite application. Wheat-derived biochar, basanite rock powder, their co-application, and rock-enhanced biochar from co-pyrolysis of wheat straw and basanite were compared as soil amendments. Incubation experiments were conducted using three agricultural soils from Germany (sandy, silty) and Kenya (sandy), representing temperate and tropical soil properties.

We hypothesized that the combined application of biochar and basanite will (i) affect the mineralization rate of native SOC depending on soil properties, as biochar-induced priming has been shown to be more pronounced in soil with coarser texture, lower pH, and lower SOC content, and (ii) increase the amount of SOC in oPOM and MAOM, as both amendments individually promote aggregate formation and mineral association of SOC, compared to unamended soils.

Materials and methods

Soils and amendments

The study was conducted using three different topsoils from agricultural fields (0–30 cm; Table 1). A temperate silty soil was collected in central Germany (Goldbach, Germany, 49°59'32.4"N 9°09'53.8"E), a temperate sandy soil was collected in northern Germany (Gartow, Germany, 53°01'26.9"N 11°27'12.7"E), and a tropical sandy soil was collected in central Kenya (Machanga, Kenya, 0°27'26.8"S 37°39'37.4"E). The soils were air-dried and stored until used in spring 2024. The soils in the respective areas are classified as Cambisol (LfU 2025), Podzol (LBEG 2017), and Nitisol (FAO and UNESCO 1974) according to the world reference base (IUSS Working Group WRB 2022).

These soils were incubated with four different soil amendments: biochar, basanite rock powder, their co-application, or rock-enhanced biochar. The biochar was produced from a mixture of ^{13}C -labeled and unlabeled wheat straw (*Triticum aestivum* L.). The ^{13}C -labeled wheat straw was derived from field-grown plants exposed to repeated $^{13}\text{CO}_2$ flushing under a hood during the growing season. The homogenized mixture of labeled and unlabeled wheat straw was pelletized to 6 mm without additional binder (WK230 pellet press, Evertec, Dieburg, Germany) and pyrolyzed at 650 °C for 15 min in a continuously operating auger reactor (Hagemann et al. 2020) (Table 2 and further information

Table 2 Initial properties of biochar and rock-enhanced biochar

Properties	Biochar	Rock-enhanced biochar
TC (g kg ⁻¹)	705.5±1.6 ^a	502.7±4.3 ^a
TOC (g kg ⁻¹)	703.7	501.3
TIC (g kg ⁻¹)	1.8±0.1 ^a	1.4±0.3 ^a
N (g kg ⁻¹)	8.9±0.0 ^a	6.3±0.1 ^a
TC: N	79±0.0 ^a	80±0.6 ^a
δ ¹³ C (‰)	140.8±15.9 ^a	146.8±3.4 ^a
H: TC	0.14 ^b	0.14 ^b
O: TC	0.64 ^b	0.70 ^b
pH	9.4 ^b	9.4 ^b
Ash (%)	20.4 ^b	42.2 ^b
Specific surface area (m ² g ⁻¹)	92.6 ^b	48.6 ^b

Further information listed in Table S2

^a Values are the mean of analytical replicates ($n=3$, mean±standard error)

^b values ($n=1$) were measured by Ruhr Lab GmbH according to the analytical guidelines of the European Biochar Certificate (EBC 2024)

TOC is calculated as difference between TC and TIC

TC total carbon, TOC total organic carbon, TIC total inorganic carbon, N total nitrogen

in Table S2). As silicate rock powder, the locally available basanite *Eifelgold* (grain size 0.5–300 µm as described in Vorrath et al. (2025); further information in Table S1) was used. In addition, two combined applications of biochar and basanite were tested – co-application and co-pyrolyzed rock-enhanced biochar. For the co-application, biochar and basanite were mixed before use. To compare the effect of post-pyrolysis mixing to a co-pyrolysis, wheat straw (mix of labeled and unlabeled straw) and basanite were mixed (90:10, w/w), pelletized to 6 mm without additional binder (WK230 pellet press, Evertec, Dieburg, Germany), and pyrolyzed at 650 °C for 15 min to produce rock-enhanced biochar following Meyer zu Drewer et al. (2025) (Table 2 and further information in Table S2). Based on the TC contents of biochar and rock-enhanced biochar (Meyer zu Drewer et al. 2025), rock-enhanced biochar comprised

Table 1 Initial soil properties of temperate silty, temperate sandy, and tropical sandy agricultural topsoil prior to pre-incubation

Properties	Temperate silty soil	Temperate sandy soil	Tropical sandy soil
pH _{CaCl2}	6.9±0.0	4.7±0.0	4.7±0.0
TC (g kg ⁻¹)	18.5±0.0	12.4±0.0	4.7±0.0
TOC (g kg ⁻¹)	13.3±0.1	12.0±0.0	4.6±0.0
TIC (g kg ⁻¹)	5.2±0.1	0.4±0.0	0.1±0.0
N (g kg ⁻¹)	1.3±0.0	1.1±0.0	0.3±0.0
TC: N	13.9±0.0	11.6±0.0	15.0±0.0
δ ¹³ C (‰)	-23.2±0.1	-26.7±0.0	-19.2±0.0
Sand (%)	28.2±1.0	93.8±0.7	68.9±0.6
Silt (%)	58.0±1.0	2.5±0.4	20.4±0.6
Clay (%)	13.8±0.3	3.7±0.3	10.7±0.2

Data is presented as mean±standard error ($n=3$)

TC total carbon, TOC total organic carbon, TIC total inorganic carbon, N total nitrogen

approximately 71% biochar and 29% basanite. Both biochar and rock-enhanced biochar were hand-crushed and sieved to <2 mm.

Experimental design

To assess the amendment effects on mineralization and stabilization of SOC, an aerobic incubation experiment ($n=4$) was set up. For the control treatment, 120 g of air-dried soil (dry-weight equivalent) was used. For the biochar treatment, 4.71 g of biochar (~3.93 wt%) was mixed with the soil. In the rock powder treatment, 2.38 g of basanite rock powder (~1.98 wt%) was added to the soil. For the co-application treatment, 4.71 g of biochar (~3.93 wt%) and 2.38 g of basanite rock powder (~1.98 wt%) were added to the soil. The co-pyrolysis treatment contained a mixture of soil and 6.70 g of rock-enhanced biochar (~5.58 wt% consisting of ~3.98 wt% biochar and ~1.60 wt% basanite rock powder). Amendment rates were adjusted to provide comparable biochar inputs (approximately 3.95 wt% biochar), resulting in comparable C inputs, among the biochar-containing treatments. Minor deviations in final biochar proportions (3.93% and 3.98% for co-application and co-pyrolysis, respectively) occurred due to material availability constraints and were considered negligible relative to treatment levels. Basanite rock powder was applied at rates similar used in previous studies to ensure comparability (Vorrath et al. 2025). After mixing, the samples were adjusted to 40% water holding capacity (WHC) using sterile distilled water and pre-incubated for 7 days at 20 °C to reactivate microorganisms and buffer potential effects from previous drying.

Following the pre-incubation, 60 g of each sample (dry-weight soil equivalent excluding amendment weight) was adjusted to 60% WHC using sterile distilled water. Moist samples were then transferred into glass flasks, sealed with rubber septa, and incubated at 20 °C for 66 days. This incubation duration allows to determine the amendment effects primarily on short-term SOC mineralization and stabilization. The accumulation of CO₂ in the flask headspace was monitored as a proxy for SOC mineralization using gas chromatography (JAS 6890 N GC, Joint Analytical Systems GmbH, Moers, Germany; 7890 A GC, Agilent Technologies, Santa Clara, United States). To maintain a higher pressure within the flasks (~1100 mbar) compared to the surrounding air and to avoid oxygen deficiency due to high CO₂ concentrations (>2% in headspace), flasks were flushed with synthetic air when necessary. Additional gas samples for $\delta^{13}\text{C}$ analysis were taken on selected days (1, 2, 3, 6, 9, 13, 17, 27, 42, 66) throughout the incubation and analyzed using an isotope ratio mass spectrometer (Delta V Plus, Thermo Fisher Scientific, Bremen, Germany).

Chemical analysis of soil samples

The remaining material of each sample after the pre-incubation was further analyzed, determining initial sample properties at the beginning of the incubation. Soil pH was determined in 0.01 M CaCl₂ solution (soil : CaCl₂ solution=1:2.5) using a pH meter (MP230, Mettler-Toledo GmbH, Gießen, Germany). The electric conductivity (EC) was measured in deionized water (soil : water=1:2.5) using a conductivity meter (Cond 330i, WTW GmbH, Weilheim, Germany).

After pre-incubation and incubation, a subsample was ground and dried at 105 °C to determine total C and N contents using an elemental analyzer (vario MAX cube, Elementar Analysensysteme GmbH, Langenselbold, Germany). The total inorganic C (TIC) content was determined using a TOC analyzer (solis TOC[®] cube, Elementar Analysensysteme GmbH, Langenselbold, Germany). The TOC content was calculated as the difference between total C and TIC. Subsamples frozen (-20 °C) after pre-incubation and incubation were used to determine microbial biomass C (MBC) by chloroform fumigation extraction following DIN (2011), adapted from Vance et al. (1987). Therefore, two subsamples of 12.5 g (dry weight equivalent) were weighed into glass flasks. One of the subsamples was directly extracted with 50 ml of 0.05 M K₂SO₄ solution to quantify DOC. The second subsample was fumigated with chloroform to lyse microbial cell walls and was afterwards extracted using K₂SO₄ solution. To determine C contents, the extracts were measured using a TOC analyzer (TOC-L, Shimadzu Corporation, Kyoto, Japan). The difference between the fumigated and non-fumigated extracts is interpreted as MBC, using an extraction factor k_{EC} of 0.45 (Joergensen 1996). The 0.05 M K₂SO₄ solution was used to improve $\delta^{13}\text{C}$ determination of MBC using an isotope ratio mass spectrometer (Werth and Kuzyakov 2010) with a TOC analyzer for liquid injection (OI Analytical Aurora 1030, Xylem Inc., Washington, United States).

Density fractionation

To separate SOC into differently stabilized fractions, a density fractionation was conducted after the incubation following Golchin et al. (1994) and Viret and Grand (2019). We used a sodium polytungstate ($3 \text{ Na}_2\text{WO}_4 \cdot 9 \text{ WO}_3 \cdot \text{H}_2\text{O}$) solution with a density of 1.8 g cm^{-3} following Beer et al. (2022). Approximately 10 g of air-dried sample was weighed into a centrifuge tube and 40 ml of sodium polytungstate solution was slowly added. The suspension was carefully stirred and after 30 min sedimentation time was centrifuged at 2500g for 60 min. The supernatant, containing the free light fraction (fLF; considered as fPOM), was filtered (cellulose acetate filter, 0.45 μm) using vacuum

where necessary. The fLF was washed with distilled water to remove the remaining sodium polytungstate solution until the supernatant had an EC of $<50 \mu\text{S cm}^{-1}$. Afterwards, the fLF was dried at 65°C and weighed. To disrupt aggregates and separate the occluded light fraction (oLF; considered as oPOM), 40 ml of sodium polytungstate were added to the remaining sample in the centrifuge tube and the sample was treated with an ultrasonication probe (280 J ml^{-1} ; Bandelin HD-200 with probe UW2070, Bandelin electronic GmbH & Co.KG, Berlin, Germany) for 12 min. Afterwards the oLF was extracted, washed, and dried in the same way as the fLF. The remaining heavy fraction (HF; containing MAOM) was also washed with distilled water until the EC of the supernatant was $<50 \mu\text{S cm}^{-1}$, dried at 105°C , and weighed. The recovery rate of the initial sample weight was in a range of 94–100%, the recovery rate of the initial SOC was $98.5\% \pm 1.7\%$ (ranging from 81 to 138%). The dried fractions were ground and measured at an elemental analyzer (Euro EA 3000, EuroVector, Pavia, Italy) to determine C and N content, and at an isotope ratio mass spectrometer to determine the $\delta^{13}\text{C}$ value of the density fractions.

Calculation of C mineralization and priming effect

The CO_2 concentration in the flask headspace was corrected for gas pressure (measured during the incubation using a manometer) and volume (based on flask volume and gas pressure), and used to calculate the cumulative CO_2 production per initial sample mass ($\text{mg CO}_2\text{-C g}^{-1}$ dry weight excluding amendment mass) and per initial TOC ($\text{mg CO}_2\text{-C g}^{-1}$ TOC including biochar-derived C).

The use of ^{13}C -labeled amendments enabled the distinction between amendment-derived SOC and native SOC (Kuzaykov 2011). To trace their fate and to investigate the priming effect of the amendments, the $\delta^{13}\text{C}$ values (‰) of the amendment-C prior to incubation, of the released CO_2 during incubation, of the MBC and of density fractions after incubation were determined using an isotope ratio mass spectrometer (Delta V Plus, Thermo Fisher Scientific, Bremen, Germany). The abundance of ^{13}C is expressed as $\delta^{13}\text{C}$ representing the ratios of $^{13}\text{C}:^{12}\text{C}$ relative to the Vienna-PeeDee-Belemnite-Standard, defined as $[(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000$. The $\delta^{13}\text{C}$ values of the ^{13}C -labeled amendments and native SOC were used to determine the contributions of amendment-C and native SOC to CO_2 , MBC, and density fractions by using a two-pool mixing model (Eqs. 1 and 2) following Bamminger et al. (2014) and Fang et al. (2015):

$$X = \frac{(\delta_{\text{treatment}} - \delta_{\text{control}})}{(\delta_{\text{amendment}} - \delta_{\text{control}})} \times 100 \quad (1)$$

$$Y = 100 - X \quad (2)$$

where X is the proportion of SOC derived from the amendments (%), $\delta_{\text{treatment}}$ is the $\delta^{13}\text{C}$ value from the sample including soil and amendment (‰), δ_{control} is the $\delta^{13}\text{C}$ value from the control soils (‰) containing only native SOC, $\delta_{\text{amendment}}$ is the $\delta^{13}\text{C}$ value of the amendment (biochar or rock-enhanced biochar) (‰), and Y is the proportion of SOC derived from native SOC (%). Both X and Y were calculated for produced CO_2 , MBC, and density fractions.

The mineralized amendment-C ($\text{mg mineralized amendment-C g}^{-1}$ amendment-C) was calculated by multiplying the cumulative CO_2 production ($\text{mg CO}_2\text{-C g}^{-1}$ TOC) with the proportion of SOC derived from the amendments X and by normalizing to one gram of initial amendment-C. The mineralized native SOC ($\text{mg mineralized native SOC g}^{-1}$ native SOC) was calculated by multiplying the cumulative CO_2 production ($\text{mg CO}_2\text{-C g}^{-1}$ TOC) with Y and by normalizing to one gram of initial native SOC (Fang et al. 2015).

Priming effects of the amendments were calculated from the difference in the native SOC mineralization between amended and unamended samples (control):

$$PE_{\text{absolute}} = \text{mineralized SOC}_{\text{treatment}} - \text{mineralized SOC}_{\text{control}} \quad (3)$$

$$PE_{\text{relative}} = \frac{(\text{mineralized SOC}_{\text{treatment}} - \text{mineralized SOC}_{\text{control}})}{\text{mineralized SOC}_{\text{control}}} \times 100 \quad (4)$$

where PE_{absolute} is the absolute priming effect ($\text{mg mineralized native SOC g}^{-1}$ native SOC), $\text{mineralized SOC}_{\text{treatment}}$ is the native SOC mineralized in the amended treatments ($\text{mg mineralized native SOC g}^{-1}$ native SOC), $\text{mineralized SOC}_{\text{control}}$ is native SOC mineralized in the control ($\text{mg mineralized native SOC g}^{-1}$ native SOC), and PE_{relative} is the priming effect (%) relative to the amount of mineralized SOC in the control at the end of the incubation. For control and basanite treatments, the total CO_2 production was assumed to result from the release of native C, since no amendment-C was added.

The TOC (mg C g^{-1} soil) present in the density fractions ($\text{normalized TOC}_{\text{fraction}}$) was calculated to determine the fractions' contributions to the overall SOC pool following Eq. 5:

$$\text{normalized TOC}_{\text{fraction}} = \frac{\text{mass}_{\text{fraction}} \times \text{relative TOC}_{\text{fraction}}}{\text{mass}_{\text{soil}}} \quad (5)$$

where $\text{mass}_{\text{fraction}}$ and $\text{relative TOC}_{\text{fraction}}$ are mass (mg) and the TOC content (%) of the fraction, respectively, and $\text{mass}_{\text{soil}}$ is the soil mass (g soil excluding the weight of the amendments).

Additionally, to the overall priming effect, the priming effect (%) of the amendments for the density fractions was calculated. Therefore, the SOC in each density fraction was distinguished in native and amendment-derived as described

in Eqs. 1 and 2, and calculated the fraction-specific priming ($PE_{fraction}$) following Eq. 6:

$$PE_{fraction} = \frac{(SOC_{treatment} - SOC_{control})}{SOC_{control}} * 100 \quad (6)$$

$SOC_{treatment}$ is the native SOC in the density fractions of the amended treatments (mg native SOC g⁻¹ soil) and $SOC_{control}$ is native SOC in the density fractions of the control (mg native SOC g⁻¹ soil) at the end of the incubation.

Differences (%) of amended treatments compared to control, e.g. in CO₂ production, were calculated using Eq. 7:

$$difference = \frac{value_{amended} - value_{control}}{value_{control}} * 100 \quad (7)$$

where $value_{control}$ is the measured or calculated value of the control treatment and $value_{amended}$ is the measured or calculated value of the amended treatments.

Statistical analysis

To test for significant differences ($P < 0.05$) between treatments within one soil for repeated measurements, such as CO₂ production, we used a linear mixed-effects model with the sample as a random factor as well as treatment, incubation day, and their interaction as fixed factors. For C mineralization, MBC, and density fractions, a one-way analysis of variance (ANOVA) was conducted. Both statistical analyses were conducted separately for each soil, since the large baseline differences among soil types masked treatment effects, which were the primary objective of this study. Both linear mixed effects model and ANOVA were followed by a Tukey HSD post-hoc test for pairwise comparison. For all analyses, normality and variance homogeneity of model residues were checked visually using qqplots. All calculations and statistical tests were conducted in RStudio (version 4.4.2) using ‘dplyr’ package (Wickham et al. 2023) for data handling, ‘lme4’ package (Bates et al. 2015) for linear mixed effects model computing, ‘multcomp’ package (Hothorn et al. 2008) for compact letter display, ‘ggplot2’ package (Wickham 2016) and ‘gridExtra’ (Auguie and Antonov 2017) for data visualization.

Results

Carbon mineralization and priming effect

The relative priming effect following amendment application compared to control was soil dependent and varied during the incubation (Fig. 1; Table S3). In the temperate

silty soil, during the course of the incubation the biochar-containing amendments (pure biochar, co-application with basanite rock powder, rock-enhanced biochar) initially induced a negative priming, which switched to a cumulative positive priming latest at day 42 (Fig. S1). Overall, only the co-pyrolysis treatment increased the native SOC mineralization in the temperate silty soil ($P = 0.03$), while biochar and co-application did not differ from control (Fig. 1a). In both sandy soils, the application of the three biochar-containing amendments increased the mineralization of native SOC (Fig. 1b, c) and induced cumulative positive priming throughout the entire incubation period (Fig. S1). This effect was more pronounced in the temperate sandy soil with priming up to ~109% compared to control (Fig. 1b). In contrast to the biochar-containing amendments, the application of rock powder did not affect native SOC mineralization within the 66 days of incubation in all three soils (Fig. 1).

Amendment-C mineralization ranged from 0.0345% ± 0.0008% to 0.0843% ± 0.0011% within the 66-day incubation, with the highest proportion mineralized in the temperate sandy soil (Fig. 2). In both sandy soils, both pure biochar and co-application had higher mineralization of amendment-C than the co-pyrolysis treatment ($P < 0.01$) (Fig. 2b, c). In the temperate silty soil, co-application treatment showed higher amendment-C mineralization than the co-pyrolysis treatment ($P = 0.014$) (Fig. 2a).

Carbon in density fractions: amount and origin

The incorporation of SOC in the density fractions of the unamended controls differed strongly between soils (Fig. 3). In the temperate silty and the tropical sandy soil, most of the SOC was recovered in the HF. In the temperate sandy soil, the oLF contained most of the SOC. The application of biochar-containing amendments increased the overall SOC stored in all density fractions in all three soils compared to the unamended control except the HF in the temperate sandy soil. In the biochar-containing treatments, the SOC stored in the fLF increased most strongly compared to control, with fLF containing the largest proportion of SOC in all three soils. The increase in SOC stored in the oLF following the application of biochar-containing amendments was most pronounced in the tropical sandy soil. Biochar-containing amendments increased SOC storage in the HF by 11–33% in temperate soils, and by more than 300% in the tropical sandy soil. The co-pyrolysis treatment led to larger SOC incorporation into HF across all soils, compared to biochar and co-application. In contrast, pure rock powder application alone did not significantly alter the SOC stored in any of the three density fractions.

The proportion of native SOC in the density fractions was influenced by the biochar-containing amendments

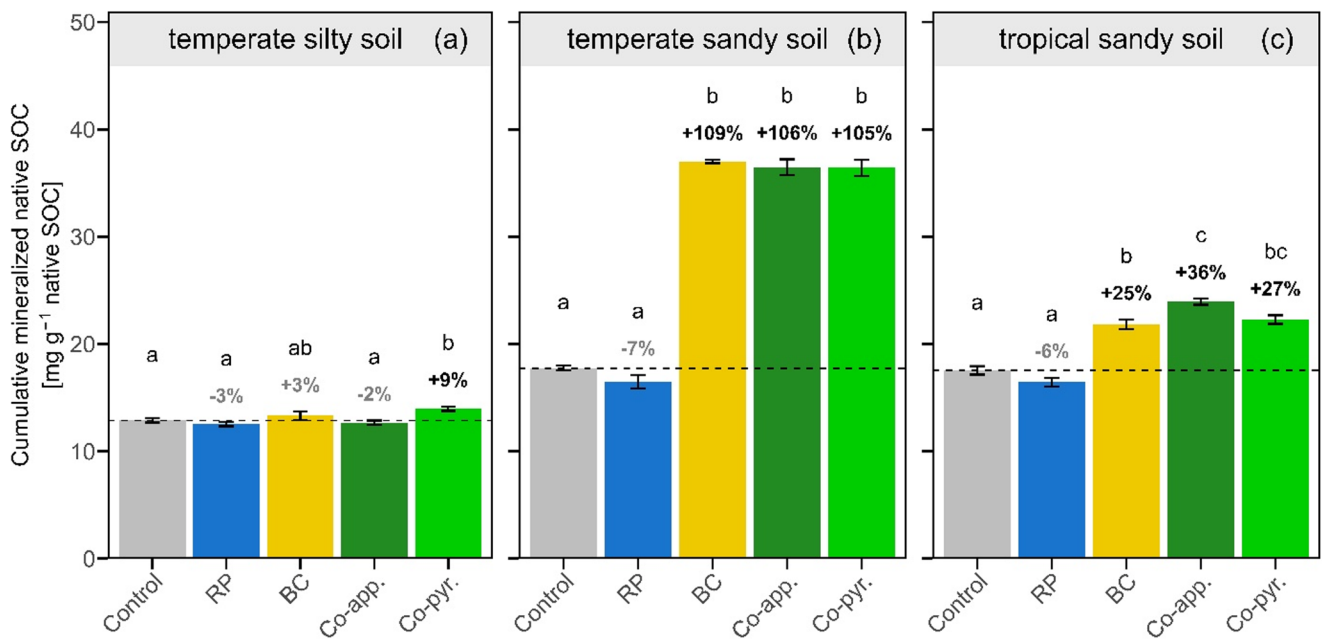


Fig. 1 Cumulative mineralized native SOC (mg mineralized native SOC g⁻¹ native SOC) of temperate silty (a), temperate sandy (b), and tropical sandy soil (c) in five treatments ($n=4$, mean and standard error) after 66 days of incubation (temporal cumulative mineralized native SOC in Fig. S2). The dashed black line shows the cumulative mineralized native SOC in the control. Numbers above the bars are the relative priming effect (%) of the respective treatment compared to control (black=significant, grey=insignificant; mean amendment-

induced priming effect $\pm$ standard error in Table S3; temporal cumulative priming effect in Fig. S1). Lower case letters indicate significant differences in cumulative mineralized native SOC between treatments within one soil ($P<0.05$). RP=rock powder treatment containing basanite, BC=biochar treatment, Co-app. = co-application treatment containing biochar and basanite, Co-pyr. = co-pyrolysis treatment containing rock-enhanced biochar

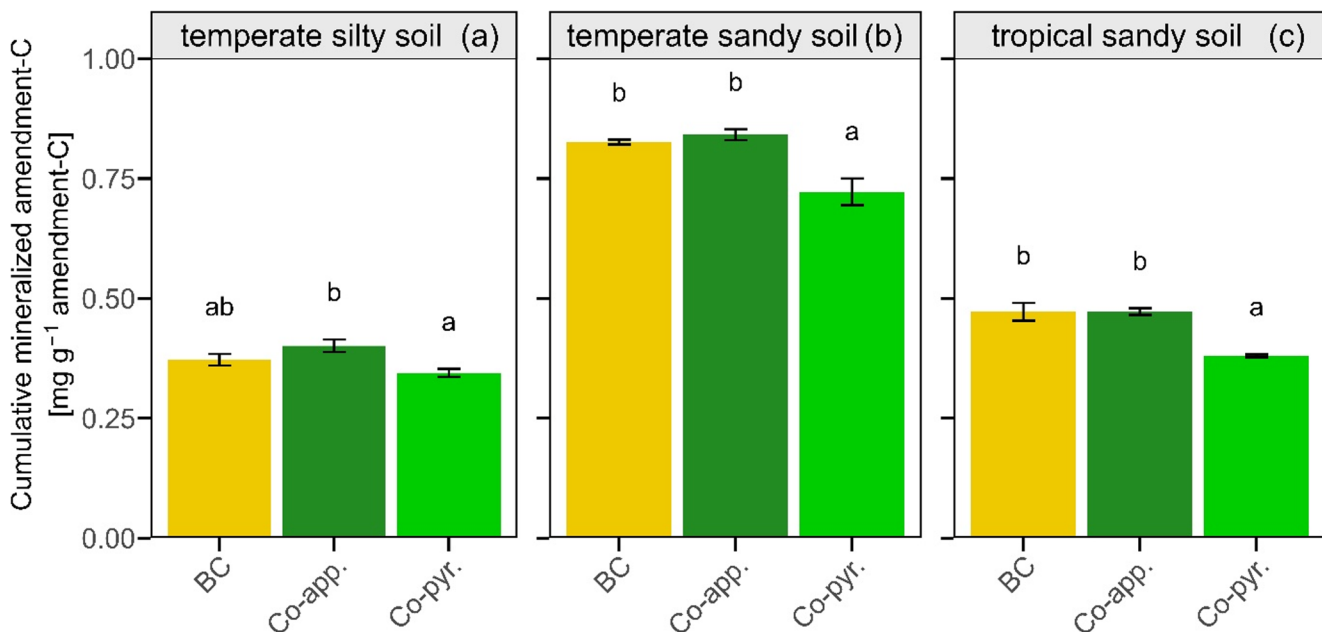


Fig. 2 Cumulative mineralized amendment-C (mg mineralized amendment-C g⁻¹ amendment-C) of temperate silty (a), temperate sandy (b), and tropical sandy soil (c) in three treatments ($n=4$, mean and standard error) after 66 days of incubation (temporal cumulative mineralized amendment-C in Fig. S2). Lower case letters indicate significant

differences in cumulative mineralized amendment-C between treatments within one soil ($P<0.05$). BC=biochar treatment, Co-app. = co-application treatment containing biochar and basanite, Co-pyr. = co-pyrolysis treatment containing rock-enhanced biochar

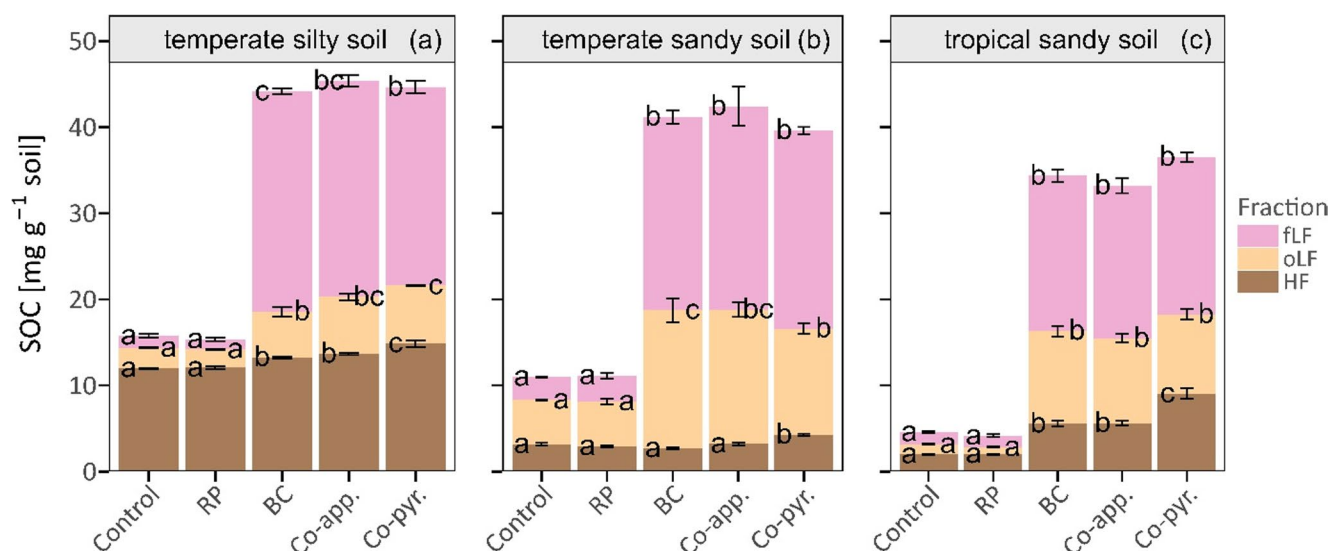


Fig. 3 Soil organic carbon (SOC) content (mg g^{-1} soil excluding amendment weight) of the free light fraction (fLF, similar to fPOM), occluded light fraction (oLF, similar to oPOM), and heavy fraction (HF, similar to MAOM) of temperate silty (a), temperate sandy (b), and tropical sandy soil (c) in five treatments ($n=4$, mean and standard error) after 66 days of incubation. Lower case letters indicate significant differences between treatments within one density fraction and one soil ($P<0.05$).

RP=rock powder treatment containing basanite, BC=biochar treatment, Co-app. = co-application treatment containing biochar and basanite, Co-pyr. = co-pyrolysis treatment containing rock-enhanced biochar

differing between the soils and the amendments at the end of the 66-day incubation (Fig. 4). The native SOC in fLF was reduced in the temperate silty soil following biochar and co-application and in the temperate sandy soil in the co-application treatment (Fig. 4a, b). The co-pyrolysis treatment did not alter the native SOC content in the fLF of both temperate soils and even increased the native SOC in fLF of the tropical sandy soil (Fig. 4a, b, c). Compared to the control, native SOC contents in the oLF increased (negative priming) or remained constant following the application of biochar-containing amendments (Fig. 4d, e, f). The negative priming was most pronounced in the co-application treatment of the temperate silty soil (-81%; Fig. 4e). Native SOC in the HF of both temperate soils was often reduced following the application of biochar-containing amendments (Fig. 4g, h). This positive priming of HF was most pronounced in the biochar treatment of the temperate sandy soil (+41%). In the tropical sandy soil, only the co-pyrolysis treatment altered the native SOC content in the HF and led to a negative priming (-45%) (Fig. 4i). In contrast to the biochar-containing treatments, rock powder application did not significantly alter the amount of native SOC in the three density fractions.

The application of biochar-containing amendments changed the composition of the SOC stored in the three density fractions by incorporating amendment-C (Fig. 4). Biochar-derived SOC from all three amendments was incorporated to a similar extent. In all soils, the co-pyrolysis treatment led to the highest incorporation of amended-C into the HF fraction (Fig. 4g, h, i).

Microbial biomass C: amount and origin

The effect of the amendments on MBC at the end of the 66-day incubation differed between soils. In the temperate silty soil, both combined applications reduced the MBC compared to control ($P<0.001$), while pure biochar application had no reducing effect (Fig. 5a). In the temperate sandy soil, only the co-pyrolysis treatment showed significantly higher MBC compared to control ($P=0.03$) (Fig. 5b). In the tropical sandy soil, rock powder, biochar, and co-pyrolysis treatments significantly increased MBC compared to control ($P<0.01$), while co-application had no increasing effect (Fig. 5c).

In both temperate soils, amendment-C was not incorporated in the MBC (measurement accuracy for used standards $\pm 0.1\%$; Fig. 5a, b). In contrast, an incorporation of amendment-C in MBC was visible in the tropical sandy soil, accounting for $\sim 0.3\%$ (co-pyrolysis treatment) up to $\sim 1.2\%$ (co-application treatment) of the overall MBC (Fig. 5c).

Discussion

Effects of biochar and basanite rock powder application on SOC dynamics differ with soil type

The effects of biochar-containing amendments (pure biochar, co-application with basanite rock powder, rock-enhanced biochar) on SOC dynamics differed between the

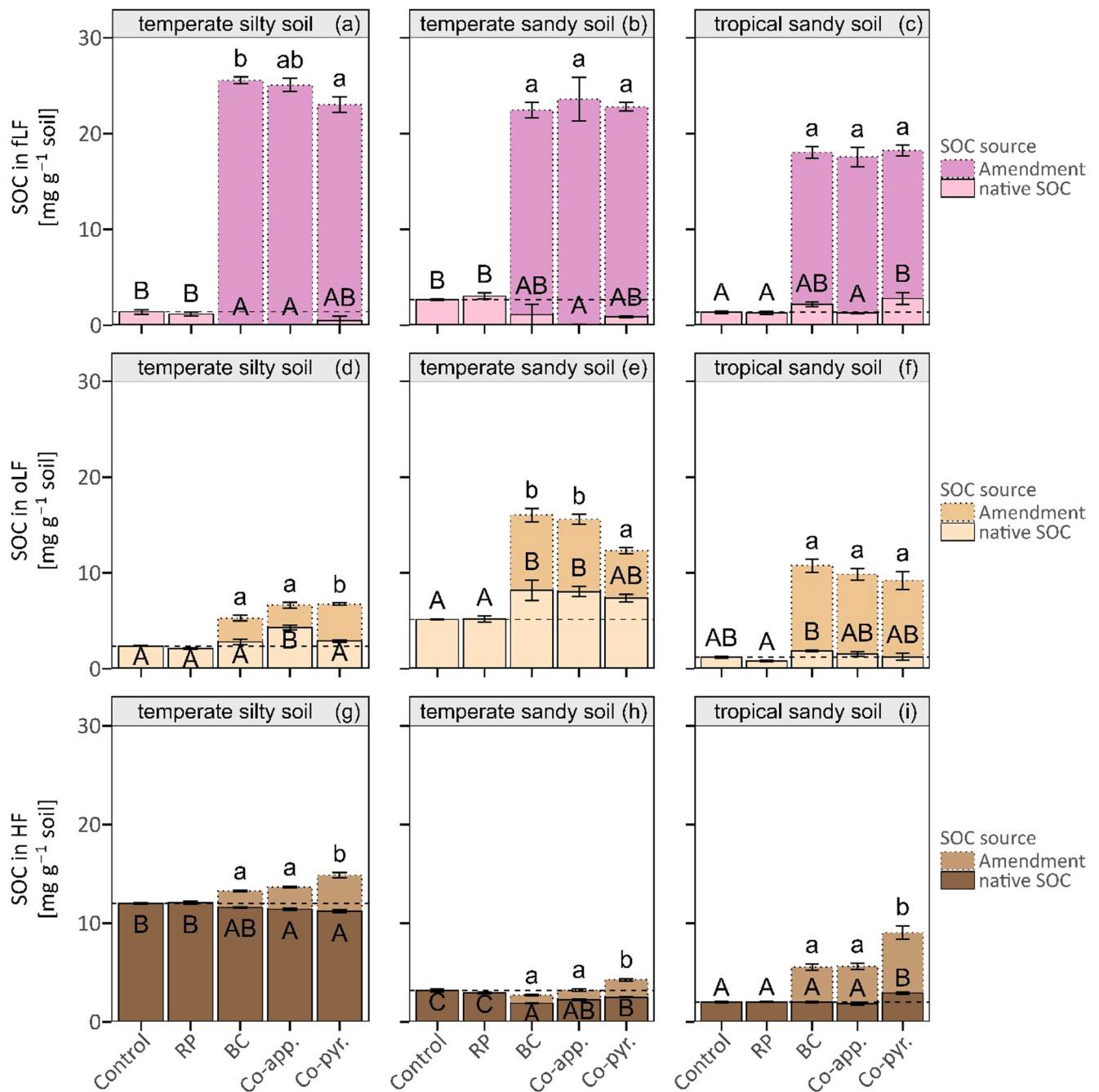


Fig. 4 Source of the soil organic carbon (SOC) content (mg g⁻¹ soil excluding amendment weight) in the free light fraction (fLF, similar to fPOM), occluded light fraction (oLF, similar to oPOM), and heavy fraction (HF, similar to MAOM) of temperate silty (a, d, g), temperate sandy (b, e, h), and tropical sandy (c, f, i) soil in five treatments ($n=4$, mean and standard error) after 66 days of incubation. The black dashed line shows the content of native SOC in the respective control treatment. Lower case letters indicate significant differences in amend-

ment-C content between treatments within one fraction and one soil ($P<0.05$). Capital letters indicate significant differences in native SOC content between treatments within one fraction and one soil ($P<0.05$). RP=rock powder treatment containing basanite, BC=biochar treatment, Co-app. = co-application treatment containing biochar and basanite, Co-pyr. = co-pyrolysis treatment containing rock-enhanced biochar

three agricultural soils used in this study, suggesting that soil type was the dominant factor controlling how biochar-containing amendments affected native SOC mineralization and stabilization. In line with the first hypothesis, this soil effect

was especially visible in the short-term amendment-induced priming (Fig. 1). Clay-rich soils often show negative priming following biochar application (Bruun et al. 2014; Herath et al. 2015; Yu et al. 2020), whereas clay-poor or sandy soils

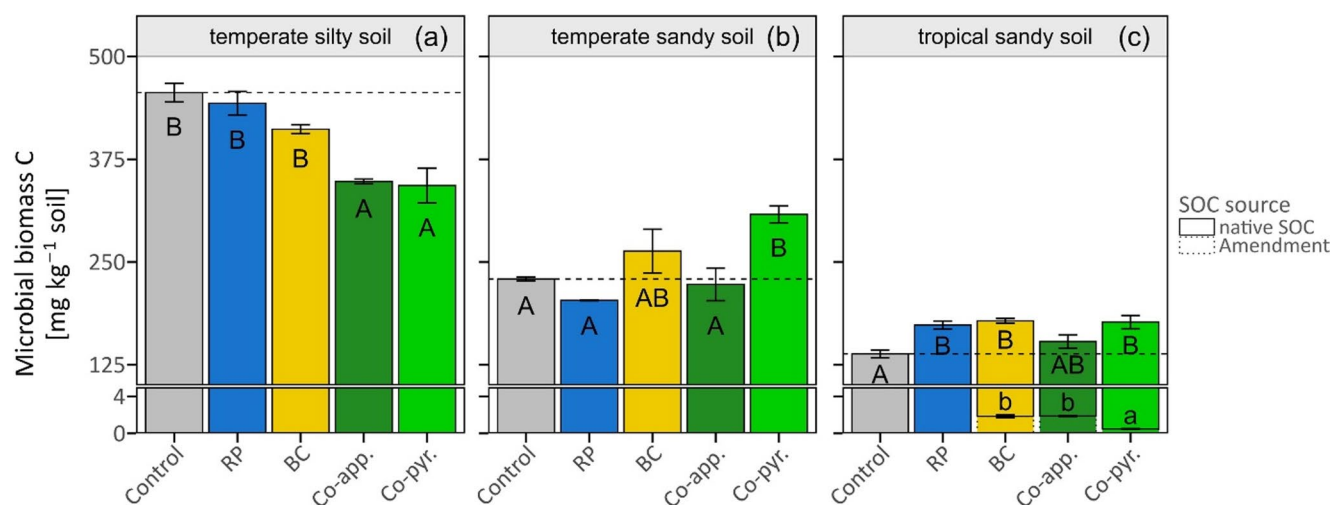


Fig. 5 Content and source of the microbial biomass C (MBC; mg kg⁻¹ soil weight) of the temperate silty (a), temperate sandy (b), and tropical sandy soil (c) in five treatments ($n=4$, mean and standard error) after 66 days of incubation. Lower case letters indicate significant differences in amendment-C content in MBC between biochar-containing treatments within one soil ($P<0.05$). Capital letters indicate significant

differences in native SOC content in MBC between treatments within one soil ($P<0.05$). RP=rock powder treatment containing basanite, BC=biochar treatment, Co-app. = co-application treatment containing biochar and basanite, Co-pyr. = co-pyrolysis treatment containing rock-enhanced biochar

commonly experience positive priming (Fang et al. 2015; Liu et al. 2015; Wang et al. 2016). Consistent with this pattern occurring in incubations between 28 days (Yu et al. 2020) and 2 years (Fang et al. 2015), we observed minimal priming in the finer-textured temperate silty soil but positive amendment-induced priming in both sandy soils during the 66-day incubation. In the three soils investigated in this study, the texture coincided with the soils' pH response to the application of biochar-containing amendments, making it difficult to attribute the observed priming effect to either factor independently. The temperate silty soil showed a weak pH increase (6.9 to 7.5 during pre-incubation) and almost no priming, while both sandy soils experienced a strong pH increase (4.7 to 7.2–7.4 during pre-incubation), coinciding with positive priming. Both pH increase (Luo et al. 2011; Zhang et al. 2018; Xu et al. 2021) and the input of labile biochar compounds, especially to sandy soils poor in organic matter (Zimmerman et al. 2011; Wang et al. 2016), can enhance microbial activity and modify the microbial community composition (Li et al. 2026). Thus, the application of biochar-containing amendments can potentially enhance native SOC mineralization, resulting in positive priming, depending on soil type.

In the temperate sandy soil, where positive priming was most pronounced, the observed losses probably mainly originated from native MAOM (up to 45%). Even though the fraction-specific priming could not be measured over the course of the incubation and the exact mechanisms causing the amendment-induced priming could not be observed, the losses of native MAOM during the 66-day incubation were likely a consequence of the amendment-induced priming

since this SOC fraction showed the biggest losses of native SOC in the temperate sandy soil (Fig. 4). The typically stable native MAOM may respond to released labile biochar-derived C (Kleber et al. 2015; Jilling et al. 2021) and could be mobilized due to the high sorption affinity of biochar-derived C, particularly in sandy soils with limited sorption capacity, which potentially leads to the mineralization of native MAOM (Schiedung et al. 2020).

Soil texture not only affected the response of native SOC to biochar-containing amendments, but also the stability of those amendments (Fig. 2). Biochar generally contains a wide range of C compounds, including readily available compounds, such as aliphatic compounds, as well as highly stable aromatic clusters (Keiluweit et al. 2010). During a 66-day incubation, only readily available C compounds can be mineralized. Thus, the set-up of this study does not allow conclusions about the behavior of the persistent majority of biochar-derived C (Azzi et al. 2024; Hagemann et al. 2025; Sanei et al. 2025). However, the highest mineralization of biochar-derived C occurred in the temperate sandy soil, which might indicate a lower stability of labile biochar components in clay-poor soils and shows the importance of soil texture for biochars' persistence (Bruun et al. 2014; Yang et al. 2022; Schmidt et al. 2025). After the labile compounds are mineralized, amendment mineralization is expected to decline (Ameloot et al. 2013; Wang et al. 2016; Azzi et al. 2024), indicating the long-term SOC storage potential of biochar-containing amendments.

Regarding both native SOC and biochar-derived C, their stabilization in oPOM and MAOM was more pronounced in the SOC-poor tropical sandy soil compared to

both temperate soils (Fig. 4). This indicates a connection between initial SOC content and SOC stabilization potential following biochar-containing amendment application. Overall, the results of this study demonstrate that soil type, specifically soil texture, pH, and SOC content, regulated both the response of native SOC and the stability of labile biochar components, and should therefore be explicitly considered when predicting the effect of biochar-containing amendments on SOC dynamics.

Effects of combined applications on SOC dynamics were driven by biochar

Both combined applications (co-application of biochar and basanite as well as rock-enhanced biochar) followed the trends observed following pure biochar application for most analyzed parameters. Biochar-induced priming often shifts from positive to negative over time (Maestrini et al. 2015; Ding et al. 2018; Joseph et al. 2021), as the release of labile biochar-derived C declines within days or weeks (Bruun et al. 2008, 2014). In line with this, the biochar-containing amendments induced strong positive priming in both sandy soils during the first days of incubation (Fig. S1). Afterwards, cumulative priming slowed down and even declined in the tropical sandy soil, indicating potential negative priming in the long-term (Zimmerman et al. 2011). The amendment-induced priming likely originated from a loss of native MAOM, especially in the temperate sandy soil (Fig. 4). The observed positive priming of native fPOM in both sandy soils turned out to be, at least partly, a shift of native SOC from fPOM to oPOM caused by the application of biochar-containing amendments, increasing the amount of native SOC stabilized in aggregates in those investigated sandy soils. While the priming effect of pure biochar application is well researched, that of combined biochar and silicate rock powder application remains poorly understood. The findings of this study add to the already inconsistent results reported in previous studies (Honvault et al. 2024; Vienne et al. 2026), underlining the importance of further research into the underlying mechanisms of a combined biochar and rock powder application depending on soil and amendment properties.

Supporting the second hypothesis, the application of biochar-containing amendments led to either constant or often increased SOC stored in all three density fractions (Fig. 3). This increase in fraction SOC often resulted from the incorporation of biochar-derived C (Fig. 4). The partial loss of native SOC in fPOM and MAOM was similar or smaller than the biochar-derived C input, often resulting in an overall increase of SOC in those pools. As previously observed (Herath et al. 2014; Singh et al. 2014; Paetsch et al. 2017; Schiedung et al. 2020; Kalu et al. 2024), the main part of

the biochar-derived C remained in the fPOM. This alters the properties of the otherwise rather unstable fPOM, which should be kept in mind when discussing SOC fractions following biochar application.

Biochar-derived C was recovered in both oPOM and MAOM (Fig. 4), as observed in previous studies (Brodowski et al. 2006; Singh et al. 2014; Paetsch et al. 2017; Schiedung et al. 2020). The association to mineral surfaces can either occur through the direct sorption of labile biochar-derived DOC (Yang et al. 2016, 2021) or via an in-vivo microbial turnover pathway (Sokol et al. 2018; Manzoni and Cotrufo 2024). For the tropical sandy soil, where also an incorporation of biochar-derived C into MBC was observed (Fig. 5), the latter pathway might contribute to the stabilization of biochar-derived C as MAOM. The absence of biochar-derived C in MBC of both sandy soils could be explained by the detection limit of the used TOC analyzer of 1‰, since the microbial incorporation of biochar-derived C into MAOM and thus also in MBC is likely during the 66-day incubation.

The observed incorporation of biochar-derived C in MAOM may partly reflect the density-based definition of MAOM. Since biochar can reach particle densities of up to 2 g cm^{-3} (Brewer et al. 2014), biochar particles with a density $> 1.8 \text{ g cm}^{-3}$ may be recovered in the HF fraction (representing MAOM) even in the absence of mineral associations. This is particularly relevant for rock-enhanced biochar, since it has a higher ash content and mineral associations can already occur during pyrolysis, so certain carbonaceous fractions can have a particle density $> 2.5 \text{ g cm}^{-3}$ (Meyer zu Drewer et al. 2025). Although density fractionation is commonly used to assess SOC stabilization after biochar application (e.g. Grunwald et al. 2017; Kalu et al. 2024), this methodological effect could contribute to the occurrence of biochar-derived C in MAOM. Nonetheless, this effect cannot fully explain the results of this study, since clear differences in the amount of biochar-derived C in MAOM among soils was observed (Fig. 4). This association of biochar-derived C with mineral surfaces might also result from a re-adsorption of DOC released from biochar-containing amendments onto soil mineral surfaces or rock particles (Manzoni and Cotrufo 2024), a mechanism that may be particularly relevant following the application of rock-enhanced biochar. In line with this, te Pas et al. (2026) observed the formation of secondary minerals during weathering and suggests that this might be beneficial for the long-term stabilization of co-applied biochar. Moreover, even though not conducted in this study, the association of biochar-derived C to minerals has been observed rather directly using thermal analysis (Burgeon et al. 2021; Fouché et al. 2023), supporting the association of biochar-derived C to mineral surfaces. While Fang et al. (2018) and Schiedung

et al. (2020) identified fine soil texture as the main driver of biochar-C stabilization in field and column experiments, strong interactions between biochar-derived C and soil minerals unaffected by texture were observed in this incubation study. The magnitude of those interactions in this relatively short period of 66 days underlines the potential for a further stabilization of biochar-derived C in soils. Long-term studies are needed to assess whether these interactions persist over time and improve long-term SOC stabilization.

Co-pyrolysis of biomass and basanite enhanced SOC stability of rock-enhanced biochar

Basanite rock powder application had no short-term effect on most soil properties, neither pure nor in both combined applications, within 66 days in the investigated setting. The potential effects of silicate rock powder application on SOC dynamics depend on several factors, including the observed time span following application (Xu et al. 2025). Previous studies found effects of silicate rock powder application on both SOC mineralization (Buss et al. 2024a; Anthony et al. 2025) and stabilization (Buss et al. 2024a; Costanzo et al. 2025) mainly on longer time scales. Thus, even though this was not confirmed by measured pH or alkalinity release after the incubation, the missing effect in this study probably results from low basanite weathering rates during the 66-day incubation (Sokol et al. 2024; Xu et al. 2025). While also positive priming (Lei et al. 2025) and reduced SOC accrual (Sokol et al. 2024) have been reported, most previous studies show the potential of enhanced rock weathering to improve SOC dynamics in the long-term.

Differences between biochar-containing amendments might have been caused by application rates or compositional differences. When co-pyrolyzed with wheat straw to rock-enhanced biochar, basanite rock powder had a pronounced positive effect on SOC stabilization. The application of rock-enhanced biochar led to an overall higher content of SOC in the HF, interpreted as MAOM, compared to pure and co-applied biochar, thus more strongly increasing the stable SOC pool (Fig. 3). This short-term increase in MAOM originated from a more pronounced occurrence of rock-enhanced biochar-C in the HF, defined as mineral-associations, compared to biochar-derived C from pure and co-application (Fig. 4). This indicates either a density-driven recovery of rock-enhanced biochar in the HF interpreted as MAOM, a higher affinity of rock-enhanced biochar-C to form MAOM, or direct formation of mineral-associations during co-pyrolysis of biomass and basanite rock powder. Such mineral-associations could include coating of rock particles with biochar-derived C, as observed in Meyer zu Drewer et al. (2025). The pronounced stabilization of rock-enhanced biochar-C was reflected in a significantly lower

mineralization and thus higher stability of its labile compounds compared to pure and co-applied biochar within the 66-day incubation (Fig. 2). This is potentially supported by the initially greater stability of rock-enhanced biochar (determined via hydropyrolysis from Meyer zu Drewer et al. 2025) and the lower specific surface area compared to biochar (Table 2), probably reducing interactions between soil and rock-enhanced biochar. This difference in the stability of the labile rock-enhanced biochar components highlights the potential of co-pyrolyzing biomass and basanite to further improve the persistence of biochar-derived C. These findings should be verified under field conditions to assess the actual potential of co-pyrolysis for amendment stability and stabilization in soil-plant-systems.

Conclusions

Basanite rock powder, applied pure or combined with biochar, had negligible effects on the short-term mineralization and stabilization of native SOC, likely due to limited rock weathering during this 66-day incubation. Therefore, the changes in SOC dynamics following the application of both combined amendments was controlled by biochar rather than basanite rock powder. All biochar-containing amendments (pure biochar, co-application, rock-enhanced biochar) had strong effects on short-term SOC dynamics, although responses varied across soils, depending on texture, soil pH, and initial SOC content. Across treatments and soils, the incorporation of biochar-derived C into fPOM, oPOM, and MAOM offset native SOC losses in fPOM and MAOM and supported the redistribution of native fPOM to occlusion in oPOM within 66 days. Rock-enhanced biochar resulted in the greatest increase in SOC in the HF, defined as the stable MAOM fraction, suggesting an enhanced short-term SOC stabilization potential compared to pure and co-applied biochar in the investigated setting. This pattern hints at the formation of organo-mineral complexes during co-pyrolysis of biomass and basanite, which is reflected in a higher short-term stability of the labile rock-enhanced biochar compounds that would otherwise be mineralized. These findings indicate the short-term potential of co-pyrolysis as a promising strategy to enhance the stabilization of labile biochar-derived C in agricultural topsoils, beyond the effect of co-application. However, confirming these mechanisms on long-term field scales requires further studies, including soil-plant-interactions, different application rates, and characterizing organo-mineral binding mechanisms as a next step in understanding the interactions between amendments and SOC dynamics.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00374-026-02030-7>.

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Author contributions Conceptualization was carried out by M.A., A.E., and J.N.B. Formal analysis and visualization were performed by M.A. Investigation was conducted by M.A. and A.L. Funding acquisition was carried out by N.H., A.E., and J.N.B. Methodology was developed by M.A. and J.N.B. Resources were provided by J.M.z.D., N.H., A.E., and J.N.B. Supervision was provided by A.E. and J.N.B. The original draft of the manuscript was written by M.A. Review and editing of the manuscript were carried out by J.M.z.D., N.H., A.E., and J.N.B. All authors read and approved the final manuscript.

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Data availability The datasets is available under <http://doi.org/10.25592/uhhfdm.18194>.

Declarations

Competing interests The authors declare no competing interests.

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