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Biochar Reduces CO_2 Emissions Compared to Sugarcane Straw but Induces Short-Term Priming in Tropical Soil

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Keywords: black carbon | carbon sequestration | priming effect | sugarcane straw

ABSTRACT

Biochar is recognised as a feasible carbon dioxide (CO_2) removal technology for achieving net-zero carbon (C) targets to address climate change; however, once applied to the soil, biochar may behave differently compared to fresh biomass. Our study aimed to evaluate the effects of biochar and sugarcane straw on soil CO_2 emission dynamics in a weathered tropical soil. A 56-day incubation experiment was conducted following a completely randomised design, with four replicates and three treatments: soil, soil + straw, and soil + biochar. Gas samples were collected weekly until day 28 to assess the isotope signature of the CO_2 emitted, and until day 56 to determine the total CO_2 emission. Our results demonstrated that biochar was more effective in reducing CO_2 losses as it increased mineralisation rates by 19%, whereas sugarcane straw increased by 126%. Both organic amendments initially induced a positive priming effect; however, for biochar, this response was short-lived. Cumulative, priming effect, and amendment-derived CO_2 emissions had a positive correlation with labile C, oxygen, nitrogen, and C—O, which were mainly seen in sugarcane straw. We conclude that, while biochar may be considered an effective tool for reducing CO_2 losses due to its chemical stability, its initial positive priming effect should be taken into account in future studies assessing its C sequestration potential in tropical environments.

1 | Introduction

Reducing greenhouse gas emissions and implementing carbon dioxide (CO_2) removal strategies that store carbon (C) in nonreactive and persistent forms are valuable tools for achieving net-zero targets aimed at limiting climate change (Babiker et al. 2023). Nevertheless, soil management strategies that promote C accumulation offer benefits beyond environmental impacts, also ensuring food and fibre production. Given their significance, various best management strategies are being studied worldwide. In tropical conditions, many are based on soil conservation practices, such as no-till farming and crop harvesting without burning, which leave large amounts of residual biomass (Menandro et al. 2017). These practices are effective in protecting the soil while still introducing organic compounds (e.g., via root exudates, plant litter, and straw). However, these inputs also have the potential to enhance the mineralisation of soil organic carbon (SOC) by stimulating microbial metabolic processes, a phenomenon known as the positive priming effect (Zhou et al. 2024).

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Summary

- Sugarcane straw induced a 129% higher initial mineralisation of SOC than soils without organic amendment application, whereas biochar increased it by 19%.
- The majority of the C mineralised under straw-amended soils was due to the decomposition of the organic material.
- Biochar positive-priming effect was short-lived, reaching values near zero 28 days after its application.
- Labile- and oxidised-C forms in the material exhibited a positive relationship with CO_2 emissions.

In this scenario, biochar has been considered an alternative once its chemical characteristics contribute to long-term C stability in the soil, which could last up to 1000 years (Woolf et al. 2021). Since the charred material is produced through thermochemical processes (e.g., pyrolysis, hydrolysis, gasification) that convert fresh biomass under elevated temperatures and limited oxygen, several reactions lead to the formation of stable C structures (Ghodake et al. 2021). The presence of these structures ultimately contributes to biochar's persistence in soils, resulting in slower decomposition rates compared to fresh biomass. This resistance to decomposition may represent a key strategy for enhancing C sequestration in environments with higher soil C losses due to the high temperatures, humidity, and biological activity, which are conditions typically found in tropical regions. However, studies that provide information on how biochar may influence C mineralisation patterns remain particularly scarce in tropical soils, especially in South America (Zhang et al. 2020). These results are relevant for calibrating process-based models to particular edaphoclimatic conditions, thereby increasing the reliability of model predictions over the impacts of land management on C fluxes. Moreover, identifying potentially material-related parameters that are related to CO_2 flux responses could help predict the outcomes following the addition of different biomass types based on their characteristics.

We hypothesise that due to its chemical recalcitrancy, biochar will decrease soil CO_2 emissions and induce a negative priming effect. Therefore, this study aimed to: (a) assess the cumulative CO_2 emissions to determine the extent to which the biochar is more stable than fresh material; (b) quantify C losses derived from the priming effect and from the added organic amendments following biochar or straw application; and (c) identify the chemical properties (i.e., labile C content, elemental composition, and surface functional groups) most strongly associated with CO_2 emissions from distinct C sources, in order to better understand the key drivers of the observed responses.

2 | Materials and Methods

An incubation experiment with Ferralsol was performed in controlled conditions. Soil sampling was conducted in a native tropical forest in São Paulo state, Brazil (S 22°48′ W 47°03′), at a depth of 0.2 m. For the experimental setup preparation, 30 g of soil (total C: 26.6 g kg^{-1}; $\delta^{13}C$ (‰): −26.70) was dried, sieved (2 mm), and added to 250 mL Erlenmeyer flasks. Soil chemical (Raij et al. 2001) and physical (Dane and Topp 2020) properties are available in Table S1. The biochar utilised was obtained by slow pyrolysis at 450°C of air-dried, milled sugarcane straw (~10% moisture).

The experiment followed a completely randomised design, with four replicates and three treatments: soil (control), soil + straw (straw), and soil + biochar (biochar). The soil-containing experimental units were pre-incubated for 5 days at 22°C and 65% of the soil water holding capacity (WHC), with these conditions maintained throughout the experimental period. Subsequently, except for the control treatment, each experimental unit received a C input of 1 g as sugarcane straw (total C: 485 g kg^{-1}; total nitrogen (N): 5 g kg^{-1}; C/N ratio: 97; $\delta^{13}C$ (‰): −12.52) or biochar (total C = 519 g kg^{-1}; total N: 5 g kg^{-1}; C/N ratio: 104; $\delta^{13}C$ (‰): −14.67). The straw was dried and milled into smaller particles, whereas the biochar was sieved through a 0.149 mm mesh to ensure uniform particle size. Both materials were then evenly homogenised with the soil. The experimental units remained closed during the experimental period with rubber stops, where CO_2 emissions were quantified using the soda lime adsorption method (Edwards 1982). Soda lime traps were used to determine weekly the CO_2 emissions accumulated over a seven-day interval until Day 56 after the beginning of the experiment.

Gas samples (~12 mL) were collected weekly from each flask until Day 28 to assess the isotopic signature of the emitted CO_2. Since the soda lime method provided CO_2 fluxes over seven-day intervals, the CO_2 concentration in the samples was also analysed using a gas chromatograph (Autosampler XL, PerkinElmer, Cambridge, UK) to calculate isotopic ratios. An Isotope Ratio Mass Spectrometer (ThermoFinnigan, Bremen, Germany) was used to quantify the $\delta^{13}C$ of the gas samples. These values were then used to distinguish the proportion of CO_2 derived from the organic amendment C by a simple two-part mixing model following the equations proposed by Cross and Sohi (Cross and Sohi 2011). The absolute priming effect (or primed soil CO_2) was calculated using the following equation (Guenet et al. 2012):

$$\mathrm{PE}\left(\mathrm{mg\ C\ kg\ dry\ soil}^{-1}\right) = \alpha \times Q_{\mathrm{sample}} - Q_{\mathrm{control}}$$

where, PE is priming effect; Q_{sample} is the total CO_2 emission from the soils with biochar or straw, and Q_{sample} is the emissions of soil only. The α was obtained by $\alpha = \left(\delta_{\mathrm{biochar/straw}} - \delta_{\mathrm{sample}}\right) / \left(\delta_{\mathrm{biochar/straw}} - \delta_{\mathrm{control}}\right)$, with the isotopic signature from biochar or straw ($\delta_{\mathrm{biochar/straw}}$), from CO_2 emissions of the sample (δ_{sample}), and the control treatment ($\delta_{\mathrm{control}}$).

Sugarcane straw and biochar were evaluated by the X-ray photoelectron spectroscopy (XPS) analysis to chemically characterise the organic materials outermost layer in terms of elemental composition and C bonds (Figure 1). Peak assignments for C-1 s spectra identification followed the parameters detailed in (Singh

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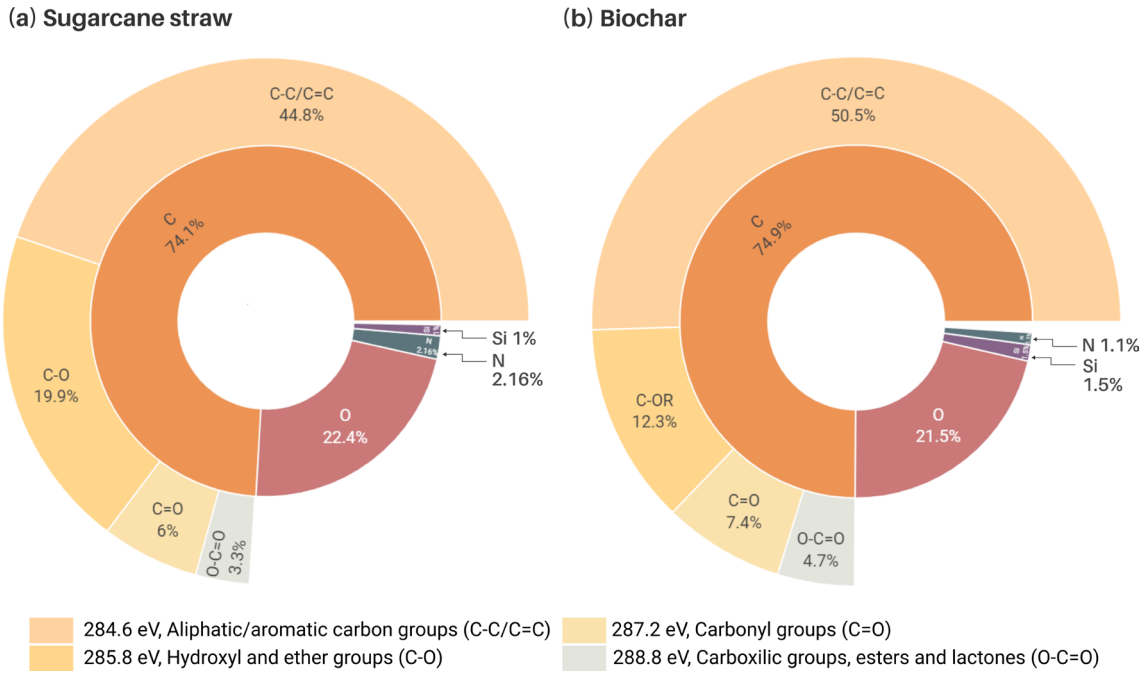

FIGURE 1 | Elemental composition (inner circle) and C chemical bond composition (%) (outer circle) of the surface of straw (a) and biochar (b) obtained by XPS analysis ($n = 3$). The figure legend indicates the energy range (eV) employed in peak assignments for the C-1s spectra.

et al. 2014). Micromorphology images were obtained by scanning electron microscopy (SEM) to compare the physical properties of the materials (Figure S1).

We conducted an additional protocol to assess the labile C content (i.e., the amount of C evolved from a material) from both biochar and sugarcane straw, as this parameter provides insight into the fraction of C readily available for microbial decomposition. A sample of each material (2 g) was added to a 250 mL Erlenmeyer flask containing 20 g of sterilised quartz sand, with four repetitions in a completely randomised design ($n = 4$). Throughout the experiment, moisture was maintained at 65% of the WHC using distilled water. The CO_2 evolution throughout the experiment was quantified gravimetrically using soda lime (Edwards 1982), where the soda lime mass gain is converted to mineralised C using the conversion factor of [1.69 × (mass gain) × 12/44] (Cross and Sohi 2011). Labile C was calculated as the ratio of mineralised C (evolved CO_2) to total C content (i.e., soil C + biochar/straw C). Control was represented by an empty flask, which was used to enable the correction of the CO_2 gained during the preparation of the vials, the flask headspace at closure, and the re-drying of the soda lime prior to weighing.

Statistical analyses were obtained using the R software (R Core Team 2022). One-way analysis of variance (ANOVA) was employed to assess the effects of cumulative CO_2 emissions at the end of the experiment, and a repeated-measures ANOVA (Pinheiro et al. 2022) was used to evaluate parameters that were compared over time. Log transformations were performed to improve model fit and guarantee that statistical assumptions were satisfied. Significance levels were set at 0.05, and statistical differences were evaluated by the Tukey test using the *emmeans* package (Lenth 2022). The Principal Component Analysis (PCA) was performed to assess the biochar properties that exhibited

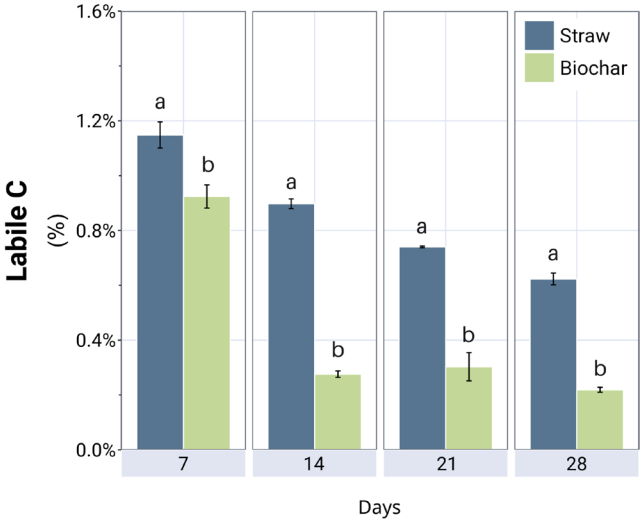

FIGURE 2 | Labile C content from sugarcane straw and biochar during a 28-day incubation period. Data (mean ± SE, $n = 4$) followed by different letters denote significant differences among treatments at each period by Tukey's test ($p < 0.05$).

significant relationships with soil CO_2 emissions from different soil C sources by day 28.

3 | Results and Discussion

Biochar is primarily known as a highly recalcitrant material due to the thermochemical conditions of pyrolysis, which transform the more labile C from fresh biomass into more aromatic/condensed stable structures (Li et al. 2023). Our findings are aligned with this statement, as sugarcane straw demonstrated higher labile C

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content in comparison to its derived biochar (Figure 2). We could also observe that biochar exhibited a more pronounced initial loss of labile C, decreasing 77% in the period between days 7 and 14. This initial loss is attributed to the release of water-soluble organic compounds from the biochar structure, thereby increasing the dissolved organic C pool in soils. As this fraction is highly available to be metabolised by soil biota, it is rapidly mineralised in soil (Han et al. 2020), which most likely caused the high CO_2 emission in the first week following biochar application. Conversely, sugarcane straw mineralisation was more uniform, presumably reflecting its organic structure undergoing biotic breakdown over time as straw comprises a myriad of C-containing structures in varying degrees of lability (Menandro et al. 2017), serving as a constant energy source for microorganisms.

In addition to the lower labile C content, biochar application was a more efficient approach for mitigating CO_2 emissions while providing a direct C input. This was evidenced by a 19% increase in soil mineralisation when biochar was applied compared to the control, whereas straw addition resulted in a 126% increase (Figure 3a). Given these results, biochar induced an initial mineralisation 2-fold lower compared to the addition of fresh biomass. These findings are consistent with previous research indicating that biochar application induces lower cumulative CO_2 emissions than soils with straw addition (Zhou et al. 2024; Liu et al. 2020). Temporal CO_2 emissions, measured using both gas chromatography and the soda lime method, can be found in Tables S2 and S3, respectively.

Straw-amended soil consistently showed a higher amount of CO_2 derived from the straw throughout the experiment compared to biochar treatments, with emissions 70% higher by day 28 ($p < 0.05$) (Figure 3b). This result might be explained by the higher amount of labile C components in the fresh biomass (Figure 2), which serves as an energy source for decomposing microorganisms. Although straw addition increased total CO_2 emissions, it did not initially induce a significant positive priming effect (i.e., extra mineralisation of SOC) (Figure 3c). In contrast to our findings, Zhou et al. (2024) showed that maize straw triggered an initial positive priming effect in a Ferrasol in subtropical monsoon climate conditions. A possible explanation for the discrepancy in these responses can be attributed to the different types of fresh biomass used. Specifically, the maize straw evaluated in the study exhibited a lower C/N ratio (C/N ratio of ~64) compared to the sugarcane straw. When using a more easily degradable material, the soil microbiome may rapidly metabolise the C found in the biomass, potentially utilising SOC to sustain microbial growth. This implies that different types of biomass will lead to distinct mineralisation dynamics.

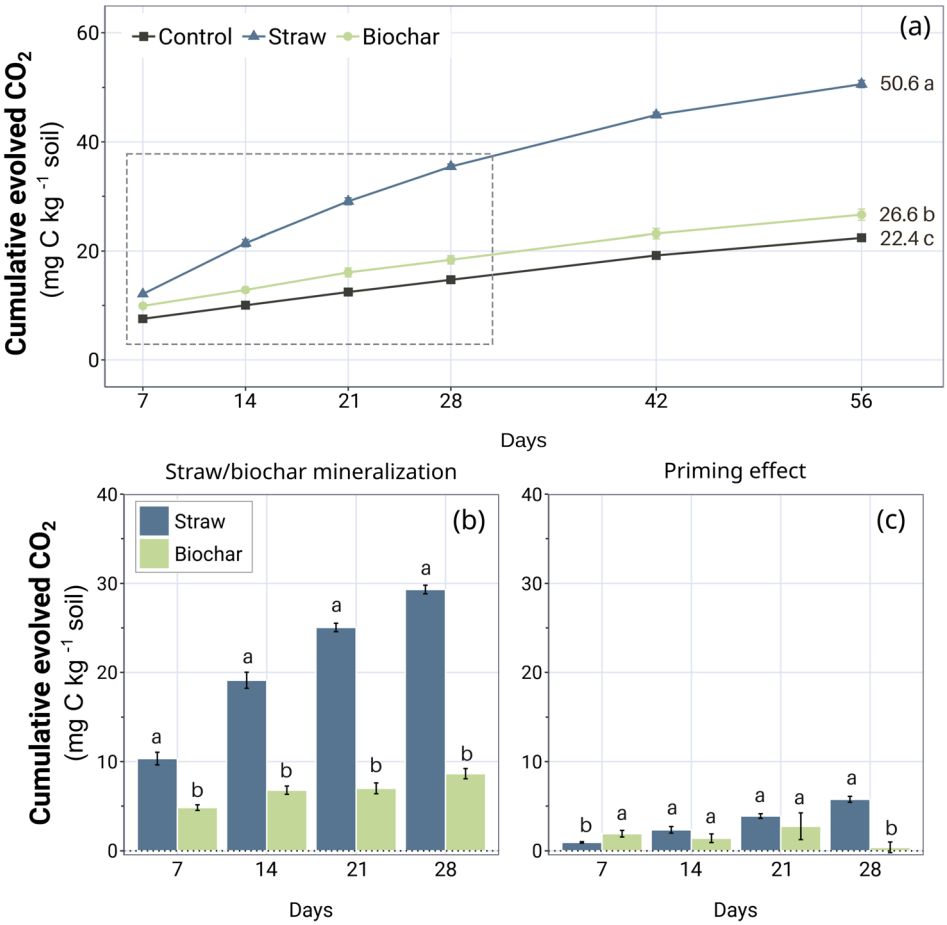

FIGURE 3 | Cumulative soil CO_2 emissions over a 56-day incubation experiment (a), and the respective contribution of biochar or mineralisation (b), and absolute priming effect (c) to total cumulative soil CO_2 emissions by the first 28 days of the incubation. Panels b and c share the same *y*-axis. Data (mean ± SE, $n = 4$) followed by different letters denote significant differences among treatments within each time period by Tukey's test ($p < 0.05$).

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Consequently, the initial benefits of using biochar instead of fresh biomass for SOC conservation may vary depending on the type of biomass in each scenario.

Since biochar has been recognised for its potential to induce a negative priming effect (Zhang et al. 2024), we expected this result in the treatment with biochar. Conversely, biochar induced a higher positive priming effect than sugarcane straw by day 7 (Figure 3c). The absence of an early negative priming effect may be attributed to the specific characteristics of the soil used in this study. Soils commonly found in tropical regions, such as Ferrasols, are typically characterised by high acidity, low fertility, and a relatively low concentration of C (Gomes et al. 2019). These characteristics represent chemical and C constraints for microbes, which may be temporarily alleviated by the C input from biochar addition. As a result, the amount of low-molecular-weight, readily degradable organic compounds present in biochar could initially stimulate microbial activity and enhance SOC mineralisation in tropical soils (Yu et al. 2023).

The same effect was not observed under straw application at day 7, despite its higher proportion of labile C compared to biochar. This likely reflects the continuous release of labile compounds during straw decomposition, in contrast to the short-lived release from biochar (Figure 2). By the end of the first week, the energy provided by biochar may have been insufficient to sustain microbial activity, whereas straw continued to supply labile C molecules, evidenced by the higher proportion of straw-derived CO_2 (Figure 3b). However, this pattern progressively changed, with the positive priming effect induced by biochar tending toward zero by day 28. In contrast, the CO_2 emissions from the priming effect caused by straw application continued to increase throughout the incubation. This pattern may reflect the straw's capacity to stimulate SOC losses through co-metabolism (i.e., losses in the SOC that were enabled by the addition of labile C compounds) as the material decomposes (Guttières et al. 2021). Conversely, since the readily available C in biochar is rapidly depleted (Figure 2), losses by mineralisation are expected to diminish over time (Whitman et al. 2024).

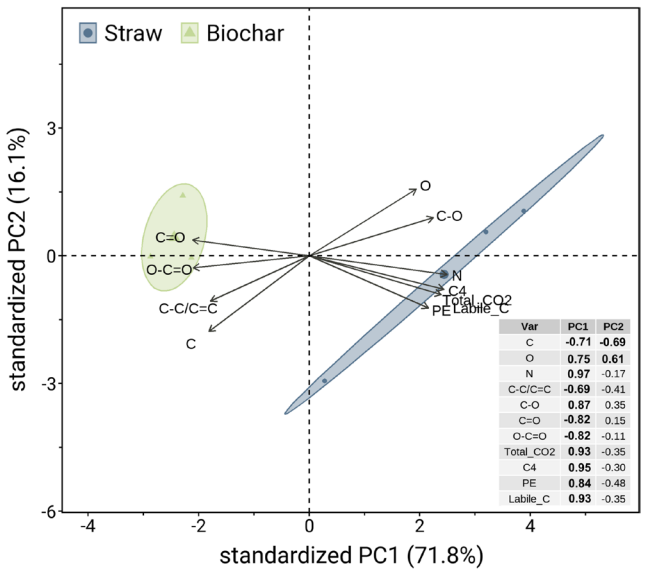

FIGURE 4 | Results of the principal component analyses (PCA) of the organic amendments properties and CO_2 emissions originating from different soil C pools. Different ellipses indicate the grouping of different PCA means for treatments, and the response variables are represented by arrows. Loading values higher than 0.5 in absolute values were considered for factor interpretation (bold numbers). Total CO_2: CO_2 fluxes, C_4, amendment-derived CO_2 emissions; PE, priming effect.

Once the lower concentration of aromatic C forms and N content in the organic input dictates the enzymatic decomposition by microbial activity, its chemical composition will ultimately influence the mineralisation patterns of SOC pools. In this study, total CO_2 emissions exhibited a positive correlation with both amendment-derived CO_2 and the priming effect, as these parameters were primarily higher under straw than biochar at day 28 (Figure 4). This effect was linked with the high levels of labile- and oxidised-C forms in the straw. In contrast, parameters that contribute to the recalcitrance of the material, including aromatic groups (C—C/C═C), had a negative correlation with the priming effect. These findings indicate that the presence of more condensed and stable C-structures in biochar, which are more resistant to microbial decomposition, contributed to the lower CO_2 emissions in biochar-containing soil. However, this was only observed due to the lower priming effect induced by biochar on day 28 compared to straw, suggesting that the recalcitrant nature of biochar contributes more significantly to its long-term stability once its labile C fraction is depleted.

4 | Conclusions

Our findings reinforce biochar's ability to represent a C input that induces lower CO_2 emissions by soils compared with fresh biomass. However, in contrast with our hypothesis, biochar did not induce a negative priming effect when added to tropical soil with low pH and C content. Despite the initial mineralisation of native SOC following biochar addition, this positive priming effect is expected to be short-lived, approaching values close to zero by the end of the first month. By this time, the higher C content and presence of aromatic C groups (C—C/C═C) in biochar will contribute to a reduced priming effect and lower CO_2 emissions. Conversely, properties that enhance microbial decomposition, such as labile C, N, and oxygen content, had a positive link with soil CO_2 fluxes. Our findings highlight that biochar will have a distinct impact on soil CO_2 fluxes compared with fresh biomass, with chemical properties of organic amendments playing a key role in shaping this response. We recommend that future studies account for these factors when calibrating biotic-based models to improve long-term predictions of C stocks in soils with biochar application under tropical conditions.

Author Contributions

Fernanda Palmeira Gabetto: writing – review and editing, writing – original draft, formal analysis, visualization, investigation. **Bernardo Melo Montes Nogueira Borges:** conceptualization, investigation, writing – review and editing, methodology, formal analysis. **João Luís Nunes Carvalho:** methodology, resources, project administration, supervision, funding acquisition, writing – review and editing.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** ejss70186-sup-0001-Supinfo.

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