



Carbon Mineralization and CO₂ Evolution Dynamics of Wood Biochar Co-Applied with Organic Manures in Inceptisol Soil

Jaisiya Meena^{a++*} and A. B. Gosavi^{a#}

^a Division of Soil Science, Government College of Agriculture, Pune, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Abstract

Biochar–manure co-application has the potential to modify soil carbon turnover and nutrient release, but the magnitude and direction of these changes depend on amendment type, ratio, and incubation time. An experiment was conducted to evaluate carbon mineralisation following the incorporation of wood biochar with farmyard manure (FYM), vermicompost (VC), and poultry manure (PM) in an Inceptisol. A total of 11 treatments were evaluated in a completely randomised design with three replications, with observations recorded at 0, 15, 30, 60, 90, and 120 days of incubation. The treatments included an untreated soil control, wood biochar alone, the three organic manures alone, and manure–biochar combinations at 1:1 and 1:2 ratios. CO₂-C evolution was determined using standard soil analytical procedures. CO₂-C evolution increased from the beginning of incubation to approximately 60 days and subsequently declined or became comparatively stable. Total CO₂-C mineralisation was studied using the discarding method, and the maximum value (209.76 mg CO₂-C 100 g⁻¹ soil) was observed following incubation of soil + vermicompost @ 8 t/ha + biochar @ 16 t/ha (1:2 ratio) over 120 days. Overall, incubation of wood biochar with vermicompost, poultry manure, or FYM at a 1:2 proportion was found suitable for carbon mineralisation during 60–90 days of incubation, compared with 103.74 mg CO₂-C 100 g⁻¹ soil in the control. The soil + vermicompost @ 8 t/ha + wood biochar @ 16 t/ha (1:2 ratio) treatment also produced the highest CO₂-C evolution at 15, 30, 90, and 120 days, whereas soil + FYM + wood biochar at 1:2 produced the highest value at 60 days (47.08 mg CO₂-C 100 g⁻¹ soil). Thus, manure type and biochar proportion may be selected according to the intended soil-management objective.

⁺⁺M.Sc. (Agri.) Soil Science; [#]I/C Professor of Soil Science;

*Corresponding author: E-mail: jaisiyameena212@gmail.com;

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1. Introduction

The term 'biochar', first introduced into the modern scientific lexicon by Lehmann (Kumar *et al.*, 2020), refers to a highly porous, low-density, carbon-rich solid matrix. Biochar is synthesised through thermochemical conversion of diverse biomass feedstocks under strictly anaerobic or oxygen-limited conditions. Pyrolysis is conducted at temperatures ranging between 300°C and 450°C (Cantrell *et al.*, 2012). The primary operational pathways for its production include slow and fast pyrolysis, gasification, carbonisation, torrefaction, and hydrothermal carbonisation (Rosales *et al.*, 2017). The resulting solid fraction is a highly stable, carbon-rich black material characterised by dense polyaromatic networks (Mohan *et al.*, 2006). Woody biomass generally contains high organic carbon levels (48%–54%) alongside low ash levels (0.5%–2%). In contrast, non-woody or herbaceous plants contain slightly lower organic carbon levels (45%–52%) but substantially higher ash concentrations (3%–12%). When subjected to slow pyrolysis, feedstocks typically retain 42%–65% of their initial organic carbon and virtually all of their mineral ash content (Moral *et al.*, 2005). Incorporating 5% biochar into soil has been shown to decrease cumulative soil organic carbon (SOC) mineralisation by 7.95%–10.7%. This reduction occurs because biochar adsorbs SOC and traps organic molecules within its porous structure. Consequently, microbial access to carbon is physically restricted, lowering its availability and slowing overall carbon breakdown. Biochar's capacity for long-term soil carbon sequestration offers a substantial pathway for climate change mitigation (Oliveira *et al.*, 2017). Beyond its agricultural benefits, biochar is increasingly valued for its capacity to increase soil organic carbon (SOC) levels and promote long-term carbon sequestration in cultivated lands (Woolf *et al.*, 2010). A 15% rise in C mineralisation after biochar application has been reported, indicating positive priming within a relatively short period (Maestrini *et al.*, 2014). Furthermore, a 120-day incubation experiment showed that amendments with rice husk, pecan shell, and bamboo biochar resulted in initial negative priming of SOM mineralisation after 15 days, which increased with time (Liu *et al.*, 2018). CO₂ evolved from soil after amendment reflects microbial respiration and the mineralisation of susceptible organic carbon pools. A rapid increase in CO₂ evolution generally indicates active decomposition of readily available substrates, whereas a later decline may indicate exhaustion of the most labile fraction and increasing dominance of more resistant carbon pools. In biochar-amended soil, however, CO₂ evolution should not be interpreted simply as a loss of biochar carbon because the measured signal may include mineralisation of manure carbon and native soil organic matter. The direction and magnitude of this response depend on feedstock and soil characteristics.

The interaction between biochar and fresh organic amendments is particularly relevant to carbon mineralisation because the two materials contribute carbon pools of contrasting decomposability. Organic manures generally supply readily metabolised substrates and nutrients that can stimulate microbial respiration, whereas wood-derived biochar is dominated by comparatively persistent aromatic carbon and may also adsorb dissolved organic compounds. Consequently, co-application can alter both the quantity of carbon available to microorganisms and the accessibility of native soil organic matter. A synthesis of incubation studies showed that the magnitude and direction of biochar-induced priming vary with feedstock, pyrolysis conditions, soil properties, incubation temperature, and microbial biomass, indicating that a single response cannot be generalised across soils (Zhang *et al.*, 2022). Mechanistic reviews similarly emphasise that biochar can modify mineralisation through changes in substrate sorption, nutrient availability, microbial habitat, and microbial community activity (Rasul *et al.*, 2022). In a 120-day incubation, co-application of biochar with non-pyrolysed organic materials increased total carbon mineralisation relative to an unamended control while retaining a substantial proportion of the added carbon, illustrating that increased respiration and carbon accrual can occur concurrently (Dey & Mavi, 2022). These interactions provide a basis for evaluating the temporal pattern of CO₂-C evolution from wood biochar applied with FYM, vermicompost, and poultry manure in the present Inceptisol. Recent field evidence indicates that biochar and organic manures can produce contrasting short-term responses in soil organic carbon and nutrient dynamics, reinforcing the need to evaluate amendment-specific effects when interpreting soil carbon turnover (Afridi *et al.*, 2026).

Incubation of compost amended with different proportions of biochar has likewise shown that mixture composition can alter the temporal pattern of soil CO₂ emissions, while combined biochar–manure application can modify microbial activity and manure-induced CO₂ release (Koomson *et al.*, 2026; Lebrun *et al.*, 2024). Recent studies further demonstrate that biochar source and the relative contributions of plant- and manure-derived materials can influence soil carbon stability, carbon mineralisation kinetics, and CO₂ responses over time (Sapkota *et al.*, 2025; Harisa *et al.*, 2025).

A direct, treatment-wise comparison of wood biochar co-applied with FYM, vermicompost, and poultry manure at 1:1 and 1:2 ratios across a common 120-day incubation period in an Inceptisol is therefore needed to clarify how amendment type, ratio, and incubation time shape CO₂-C evolution within this experimental system.

Therefore, the objective of the present study was to evaluate the temporal dynamics of CO₂-C evolution and cumulative carbon mineralisation in an Inceptisol amended with wood biochar alone or co-applied with FYM, vermicompost, and poultry manure at 1:1 and 1:2 ratios over 120 days of incubation.

2. Materials and Method

An experiment was conducted at the Division of Soil Science, Government College of Agriculture, Pune, during the winter of 2025–26 to evaluate carbon mineralisation in an Inceptisol amended with wood biochar and selected organic manures. The study comprised a controlled laboratory-incubation component followed by soil analysis. The experiment was laid out in a completely randomised design (CRD) with 11 treatments and three replications. For the carbon-mineralisation study, 100 g of soil was used for each incubation unit. The treatment structure enabled comparison of an untreated soil control, wood biochar applied alone, individual organic manures applied alone, and manure–biochar combinations at defined proportions.

The treatment combinations are presented in Table 1. T1 consisted of unamended soil and served as the control, while T2 received wood biochar @ 10 t/ha. The sole organic-manure treatments comprised FYM @ 12 t/ha (T3), vermicompost @ 8 t/ha (T4), and poultry manure @ 4 t/ha (T5). For the combined treatments, FYM @ 12 t/ha was co-applied with wood biochar @ 12 and 24 t/ha to obtain 1:1 and 1:2 ratios, respectively (T6 and T7). Vermicompost @ 8 t/ha was combined with wood biochar @ 8 and 16 t/ha (T8 and T9), and poultry manure @ 4 t/ha was combined with wood biochar @ 4 and 8 t/ha (T10 and T11), corresponding to 1:1 and 1:2 ratios, respectively.

The incubation was continued for 120 days, and observations were recorded at 0, 15, 30, 60, 90, and 120 days. This observation schedule was used to characterise the temporal pattern of carbon mineralisation throughout the incubation period. CO₂-C evolution was used as the response measure for periodical carbon mineralisation, and cumulative CO₂-C over the 120-day incubation period was used to summarise the overall mineralisation response of each treatment. Treatment-wise CO₂-C evolution was therefore compared across the successive incubation intervals as well as on a cumulative basis.

Statistical interpretation was based on the completely randomised design with three replications. For each observation time, the results were accompanied by the standard error of the mean [SE(m)±] and the critical difference (CD) at the 5% level. Where treatment differences were not significant, the result was reported as NS. These statistics were used to identify significant treatment differences and treatments that were statistically at par for periodical CO₂-C evolution.

Table 1. Treatment Details

Tr No.	Details
T ₁	Soil (Control)
T ₂	Incubation of soil + wood biochar @ 10 t/ha
T ₃	Incubation of soil + FYM @ 12 t/ha
T ₄	Incubation of soil + vermicompost @ 8 t/ha
T ₅	Soil + poultry manure @ 4 t/ha
T ₆	Soil + Incubation of FYM @ 12 t/ha + wood biochar @ 12 t/ha (1:1 ratio)
T ₇	Soil + Incubation of FYM @ 12 t/ha + wood biochar @ 24 t/ha (1:2 ratio)
T ₈	Soil + Incubation of vermicompost @ 8 t/ha + wood biochar @ 8 t/ha (1:1 ratio)
T ₉	Soil + Incubation of vermicompost @ 8 t/ha + wood biochar @ 16 t/ha (1:2 ratio)
T ₁₀	Soil + Incubation of poultry manure @ 4 t/ha + wood biochar @ 4 t/ha (1:1 ratio)
T ₁₁	Soil + Incubation of poultry manure @ 4 t/ha + wood biochar @ 8 t/ha (1:2 ratio)

3. Results and Discussion

3.1 Periodical Carbon Mineralisation

The study of CO₂-C mineralisation revealed an increasing trend up to 60 days, after which the rate of CO₂-C mineralisation became comparatively stable up to 120 days. On day 0, higher CO₂ evolution was recorded for soil + vermicompost @ 8 t/ha (19.23 mg CO₂ 100 g⁻¹ soil). At 15 days, the soil + vermicompost @ 8 t/ha + biochar @ 16 t/ha (1:2 ratio) treatment recorded significantly higher CO₂ evolution (32.56 mg CO₂ 100 g⁻¹ soil) and was statistically at par with soil + vermicompost + biochar (1:1) (31.18 mg CO₂ 100 g⁻¹ soil).

At 30 days of incubation, significantly higher CO₂ evolution was recorded for soil + vermicompost @ 8 t/ha + biochar @ 16 t/ha (1:2 ratio) (39.48 mg CO₂ 100 g⁻¹ soil), which was statistically at par with soil + vermicompost @ 8 t/ha (38.40 mg CO₂ 100 g⁻¹ soil), soil + poultry manure @ 4 t/ha (37.44 mg CO₂ 100 g⁻¹ soil), soil + FYM @ 12 t/ha + biochar @ 24 t/ha (1:2 ratio) (34.56 mg CO₂ 100 g⁻¹ soil), soil + vermicompost @ 8 t/ha + biochar @ 8 t/ha (1:1 ratio) (32.81 mg CO₂ 100 g⁻¹ soil), and soil + poultry manure @ 4 t/ha + biochar @ 8 t/ha (1:2 ratio) (32.15 mg CO₂ 100 g⁻¹ soil).

At 60 days, soil + FYM @ 12 t/ha + biochar @ 24 t/ha (1:2 ratio) recorded the highest CO₂ evolution (47.08 mg CO₂ 100 g⁻¹ soil) and was statistically at par with soil + vermicompost @ 8 t/ha + biochar @ 16 t/ha (1:2 ratio) (45.27 mg CO₂ 100 g⁻¹ soil).

At 90 days, soil + vermicompost @ 8 t/ha + biochar @ 16 t/ha (1:2 ratio) recorded the highest CO₂ evolution (41.28 mg CO₂ 100 g⁻¹ soil), whereas the control treatment recorded the lowest value (13.63 mg CO₂ 100 g⁻¹ soil).

Similarly, at 120 days of incubation, soil + vermicompost @ 8 t/ha + biochar @ 16 t/ha (1:2 ratio) recorded the highest CO₂ evolution (33.84 mg CO₂ 100 g⁻¹ soil) and was significantly higher than all other treatments, while the control (T1) recorded the lowest CO₂ evolution (12.20 mg CO₂ 100 g⁻¹ soil).

Overall, the highest cumulative CO₂-C evolution was recorded for soil + vermicompost @ 8 t/ha + biochar @ 16 t/ha (1:2 ratio) (209.76 mg CO₂-C 100 g⁻¹ soil), followed by soil + vermicompost @ 8 t/ha + biochar @ 8 t/ha (1:1 ratio) (186.46 mg CO₂-C 100 g⁻¹ soil) and soil + vermicompost @ 8 t/ha (184.76 mg CO₂-C 100 g⁻¹ soil), whereas the lowest cumulative CO₂-C evolution was observed in the control treatment (103.74 mg CO₂-C 100 g⁻¹ soil), indicating greater microbial activity and carbon mineralisation under vermicompost combined with wood biochar, particularly at the 1:2 ratio. Similar studies reported that biochar decreased CO₂ emissions from soil by 30–37.2% (Chen *et al.*, 2014; Mukherjee *et al.*, 2014), while biochar application to soil may be a suitable management practice for maintaining soil C (Yin *et al.*, 2014; Zhang *et al.*, 2015). Dainy (2015) also reported similar results. A comparison of CO₂ evolution from coconut-husk biochar and FYM showed that FYM released higher amounts of CO₂ from soil

because of a faster rate of carbon mineralisation than the biochar treatment (Vasu *et al.*, 2015). Comparable C-mineralisation patterns have also been reported by Desalegn *et al.* (2019).

The temporal response observed in the present incubation is consistent with a transition from rapid utilisation of relatively labile substrates to progressively greater control by less readily decomposable carbon pools. The higher CO₂-C evolution under manure-containing treatments during the early and middle incubation stages can reasonably be associated with greater availability of soluble and microbially accessible carbon, although the present measurements do not partition CO₂ among manure carbon, native soil organic carbon, and biochar carbon. Field and incubation evidence has shown that manure can increase soil respiration through enlargement of labile carbon pools and stimulation of carbon-cycling microbial functions, whereas biochar alone may exert a smaller or more variable effect (Han *et al.*, 2023). The subsequent decline after the 60-day maximum is therefore compatible with depletion of the most readily mineralisable substrates and a growing contribution from more resistant pools. This interpretation also agrees with evidence that biochar effects on soil organic carbon mineralisation may change with incubation duration rather than remaining constant over time (Zhang *et al.*, 2022). Moreover, biochar can reduce mineralisation of native or freshly added organic carbon under some conditions by increasing microbial carbon-use efficiency and promoting physical or chemical protection of carbon (Kalu *et al.*, 2024). Accordingly, the comparatively high CO₂-C values of the vermicompost–biochar treatments indicate greater net respiratory activity of the amended soil system during this incubation, but they should not be interpreted as direct evidence that the biochar itself was preferentially decomposed.

3.2 Cumulative Carbon Mineralisation

Soil incubated with wood biochar and organic manures showed a higher amount of C mineralisation throughout the incubation period than the untreated control. The rate of CO₂-C release was relatively low at the initial stage, reached its highest level at 60 days, and then gradually declined during the remainder of the incubation period.

Table 2 Periodical influence of wood biochar incubated with organic manures on CO₂ evolution

Treatment	Days of incubation						Cumulative total
	0	15	30	60	90	120	
	CO ₂ evolution (mg CO ₂ -C 100 g ⁻¹)						
T ₁ : Soil (Control)	16.96	15.76	18.99	20.20	19.63	12.2	103.74
T ₂ : Soil + Wood Biochar @ 10 t/ha	16.30	19.48	24.27	24.15	22.40	17.5	124.10
T ₃ : Soil + FYM @ 12 t/ha	17.80	24.15	30.84	38.64	22.37	16.74	150.54
T ₄ : Soil + Vermicompost @ 8 t/ha	19.23	26.80	38.40	41.59	35.60	23.14	184.76
T ₅ : Soil + Poultry Manure @ 4 t/ha	17.40	29.82	37.44	39.48	29.10	22.41	175.65
T ₆ : Soil + FYM @ 12 t/ha + Biochar @ 12 t/ha (1:1 ratio)	17.00	26.10	29.19	38.83	22.24	17.8	151.16
T ₇ : Soil + FYM @ 12 t/ha + Biochar @ 24 t/ha (1:1 ratio)	18.73	25.55	34.56	47.08	26.30	16.4	168.62
T ₈ : Soil + VC @ 8 t/ha + Biochar @ 8 t/ha (1:1 ratio)	17.20	31.18	32.81	40.20	36.57	28.5	186.46
T ₉ : Soil + VC @ 8 t/ha + Biochar @ 16 t/ha (1:2 ratio)	17.33	32.56	39.48	45.27	41.28	33.84	209.76
T ₁₀ : Soil + PM @ 4 t/ha + Biochar @ 4 t/ha (1:1 ratio)	16.77	23.20	29.36	36.58	21.49	20.4	147.80
T ₁₁ : Soil + PM @ 4 t/ha + Biochar @ 8 t/ha (1:2 ratio)	17.63	25.60	32.15	35.23	22.68	18.3	151.59
SE(m) [±]	1.08	1.13	2.59	1.46	1.31	0.75	--
CD(5%)	NS	3.33	7.63	4.32	3.87	2.23	--

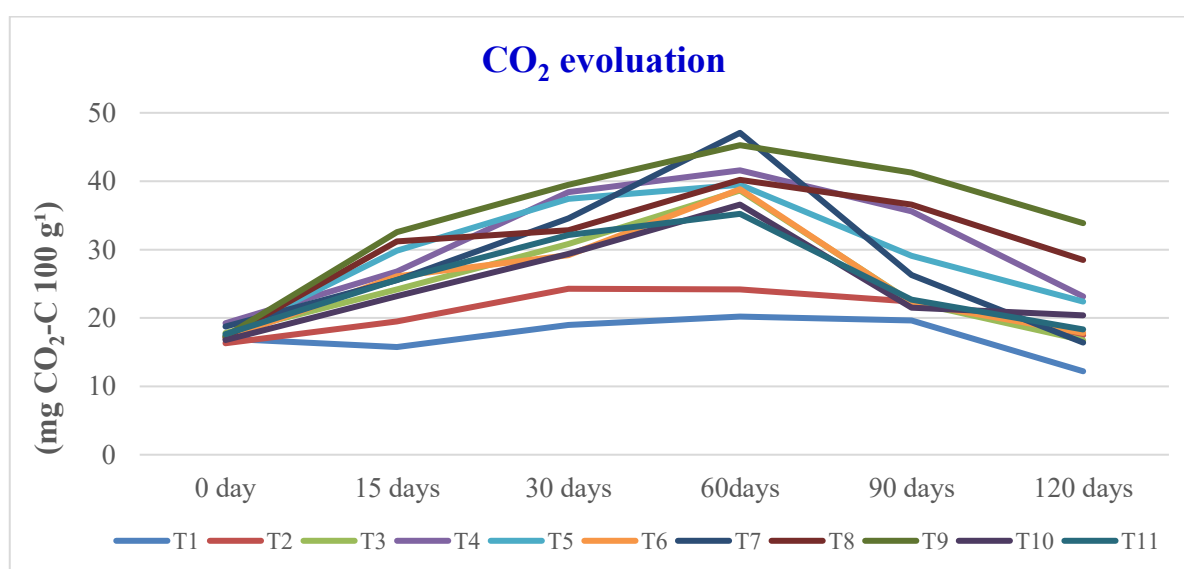


Fig. 1. Effect of wood biochar incubated with organic manures on periodical CO₂ evolution

The cumulative carbon mineralisation over 120 days of incubation was markedly influenced by the application of organic amendments and their combinations with wood biochar. The highest cumulative carbon mineralisation was recorded under soil + vermicompost @ 8 t/ha + biochar @ 16 t/ha (1:2 ratio) (209.76 mg CO₂-C 100 g⁻¹ soil), followed by vermicompost + biochar (1:1 ratio) (186.46 mg CO₂-C 100 g⁻¹ soil) and soil + vermicompost @ 8 t/ha (184.76 mg CO₂-C 100 g⁻¹ soil). The higher carbon mineralisation under soil + vermicompost @ 8 t/ha + biochar @ 16 t/ha (1:2 ratio) may be attributed to the availability of readily decomposable organic carbon from vermicompost together with the stimulatory effect of biochar on microbial activity. Among the manure-based treatments, soil + poultry manure @ 4 t/ha recorded 175.65 mg CO₂-C 100 g⁻¹ soil, followed by T7 (168.62), T11 (151.59), T6 (151.16), T3 (150.54), and T10 (147.80). The lowest cumulative carbon mineralisation was recorded under T1 (soil control) (103.74 mg CO₂-C 100 g⁻¹ soil), indicating comparatively lower microbial activity in the untreated soil. Overall, T9 (soil + vermicompost @ 8 t/ha + biochar @ 16 t/ha, 1:2 ratio) recorded the highest cumulative carbon mineralisation. A similar study reported that changes in CO₂ evolution during vermicomposting reflected the rapid decomposition of readily degradable organic matter and the release of carbon during microbial respiration (Dume *et al.*, 2021), while declining CO₂ evolution may indicate increasing stability of organic matter (Atiyeh *et al.*, 2000).

The cumulative pattern further indicates that amendment composition influenced the integrated respiratory response over 120 days. The comparatively high cumulative CO₂-C under the vermicompost + biochar 1:2 treatment may reflect the simultaneous presence of readily decomposable vermicompost carbon and biochar-mediated changes in substrate retention, nutrient availability, and microbial habitat. Comparable incubation work has shown that co-application effects are not necessarily additive: biochar and manure together can alter mineralisation relative to either material alone because sorption of labile compounds and nutrient immobilisation can change carbon accessibility (Dodor *et al.*, 2018). Recent field evidence from degraded soils likewise indicates that vermicompost preferentially supports more labile carbon fractions, whereas biochar contributes to more persistent carbon pools; their combination can therefore modify the balance between short-term turnover and longer-term stabilisation (Kenye *et al.*, 2026). The present cumulative CO₂-C values should consequently be interpreted as total mineralisation from the amended soil system rather than as a direct measure of net carbon sequestration. This distinction is important because greater absolute CO₂ evolution can occur simply when a treatment introduces more decomposable carbon, while a fraction of biochar- or manure-derived carbon may still remain stabilised in soil (Dey & Mavi, 2022). Source-partitioning approaches, such as isotopic labelling, would be required to quantify the separate contributions of native soil carbon, manure carbon, and biochar carbon to the measured CO₂-C.

4. Conclusions

Wood biochar co-applied with organic manures produced distinct temporal patterns of CO₂-C evolution during the 120-day incubation. Vermicompost alone recorded the highest CO₂ evolution at day 0, whereas vermicompost @ 8 t/ha + biochar @ 16 t/ha at a 1:2 ratio recorded the highest values at 15, 30, 90, and 120 days. At 60 days, FYM @ 12 t/ha + biochar @ 24 t/ha at a 1:2 ratio recorded the highest CO₂ evolution of 47.08 mg CO₂ 100 g⁻¹ soil. The vermicompost–biochar 1:2 treatment also produced the highest cumulative carbon mineralisation, reaching 209.76 mg CO₂-C 100 g⁻¹ soil compared with 103.74 mg CO₂-C 100 g⁻¹ soil in the untreated control. Overall, CO₂-C evolution increased towards 60 days and then declined or became comparatively stable. The results indicate that manure type, biochar proportion, and incubation duration influenced the respiratory response of the amended soil. Because the measured CO₂-C was not source-partitioned, the higher cumulative values should be interpreted as total mineralisation of the amended soil system rather than as direct evidence of biochar decomposition or net carbon sequestration.

5. Limitations

The study was a 120-day controlled laboratory incubation using one Inceptisol and three replications; therefore, the observed responses may not represent field conditions, other soil types, or longer-term amendment behaviour. CO₂-C measurements quantified total soil-system respiration but did not partition contributions from native soil organic carbon, manure carbon, and biochar carbon. Consequently, cumulative CO₂-C cannot distinguish direct amendment mineralisation from priming effects or quantify net carbon sequestration. Longer-term field studies and source-partitioning approaches, such as isotopic labelling, would strengthen interpretation of amendment-specific carbon dynamics.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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