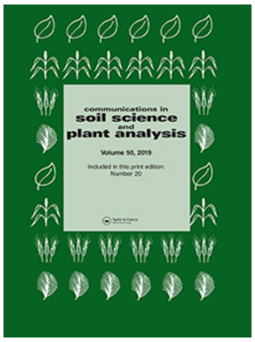

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Kinetics of Carbon Mineralization and Sequestration of Sole and/or Co-amended Biochar and Cattle Manure in a Sandy Soil

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ABSTRACT
The interactive effect of biochar, cattle manure and nitrogen (N) fertilizer on the dynamics of carbon (C) mineralization and stabilization was investigated in a sandy soil amended with three sole biochar (0, 20 or 40 t ha^{-1}) or manure (0, 13 or 26 t ha^{-1}) and four combined biochar-manure levels (20 or 40 t ha^{-1} biochar plus 13 or 26 t ha^{-1} manure) with or without N fertilizer (0 or 90 kg ha^{-1}) and CO_2-C evolution measured over 54-d incubation period. Biochar application, solely or combined with manure resulted in lower applied C mineralized (ACM), indicating C sequestration in the soils. Negative attributable effect (AE) of co-application of biochar and manure on C mineralization was observed relative to the sole treatments. Both ACM and AE were negatively correlated with C/N ratio and mineral N content of the soil-mixtures ($r \geq -0.573$; $p \leq 0.01$), indicating microbial N limitation. The double first-order exponential model described CO_2-C efflux very well and indicated that ≥94% of C applied was apportioned to stable C pools with slower mineralization rate constant and longer half-life. Cumulative C mineralized and modeled C pools were positively correlated with each other ($r \geq 0.853$; $p \leq 0.001$) and with readily oxidizable C of soil-amendment mixtures ($r \geq 0.861$; $p \leq 0.001$). The results suggested that co-application of biochar and manure can promote initial rapid mineralization to release plant nutrients but sequester larger amounts of applied C in refractive C pool, resulting in larger C sequestration in sandy soils.

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KEYWORDS
Biochar; C mineralization; C sequestration; cattle manure; kinetic models; sandy soils

Introduction

The development of soil management systems to mitigate climate change through stabilization of soil organic matter (SOM) and sequestration of carbon (C) is an important and critical component of soil fertility management for crop production in sandy soils (Lu et al. 2014; Novak et al. 2010; Rittl et al. 2015), especially in the semi-arid and sub-humid regions of the tropics. To replenish and/or maintain organic matter (OM) content in these soils, manure is extensively applied and incorporated into the surface 0–20 cm layer of the soil every growing season. However, due to its low stability, coupled with the high temperature and humid soil conditions peculiar to such areas, the manure is mineralized very fast. This results in slow buildup of SOM and contributes to significant increase in the concentration of CO_2 in the atmosphere, a major concern for global warming. In addition, the conventional inversion tillage practice in sandy soils invariably accelerates decomposition of added organic residues (Bauer et al. 2006), reducing not only its values as a soil amendment but also its capacity as a C sequestration strategy.

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Biochar is a C-rich material produced from thermal degradation of organic feedstocks under limited oxygen condition (Lehmann and Joseph 2009). Due to its recalcitrant nature, biochar has been suggested as an alternative C management strategy to sequester C and improve the fertility status of impoverished soils (Smider and Singh 2014). Furthermore, biochar contains high proportion of stable forms of aromatic rings, oxidized and easily degradable aliphatic C, and hence its application can ameliorate the rapid degradation of SOM in tropical soils (Sohi et al. 2010). The complex physico-chemical properties of biochar such as texture, particle size, surface area, and water holding capacity are determined by the feedstock it is made from, the temperature, heating rate and holding time during pyrolysis. The extent of aromatic structure formation in biochar increases with the pyrolysis temperature (Brewer et al. 2009), with low pyrolysis temperature yielding acidic biochar, while high pyrolysis temperature produces alkaline biochar (Zhang et al. 2013), higher C/N ratio (Novak, Cantrell, and Watts. 2013; Ronsse et al. 2013) and a lesser concentration of dissolved organic C (Budai et al. 2014; Rajapaksha et al. 2014). Higher pyrolysis temperatures also increase the surface area and sorption capacity of biochar (Tang et al. 2013), and micropore volume (Angin and Şensöz 2014).

Application of biochar has been shown to influence soil physical properties such as porosity, bulk density and improved soil structure, aggregation and water retention (Glaser, Lehmann, and Zech 2002; Laird et al. 2010; Sohi et al. 2010). In addition, amendment of soils with biochar has been shown to increase soil quality, nutrient cycling, C sequestration, and increased pH, potassium and available phosphorus in soils (Laird et al. 2010; Van de Voorde et al. 2014). Owing to its low content of major plant nutrients such as N, however, it is recommended to co-apply biochar with other organic amendments to improve its plant nutritive value and hence crop yields (Fernández et al. 2014). Indeed, co-application of biochar and other organic amendments have been reported to result in synergistic positive effect on soil organic carbon (SOC) content, nutrient levels in soils and plant uptake, leading to improved plant growth and yield (Agegnehu et al. 2015). Combined application of biochar and other organic amendments such as green and animal manures have also been shown to increase C mineralization compared to individual applications (Luo et al. 2011; Troy et al. 2013). However, these findings are not conclusive as other researchers have reported decreased mineralization of SOC following the combined application of biochar and other organic amendments (Rogovska et al. 2011).

When a mixture of organic materials of very different C pools are co-applied to the soil, a complex set of interactions can affect the kinetics of the C mineralization process, which can also change with the rate of application of these amendments. For example, a study in our laboratory indicated that sole application of biochar and manure resulted in up to 125% positive priming of SOC in a sandy soil (Dodor et al. 2018). However, co-application of the two amendments de-accelerated the rate of decomposition and stabilized C in the soils, subsequently leading up to 35% negative priming effect, albeit with a short-term mineral N limitation (Dodor et al. 2018). Owing to the fact that the effect of co-applied biochar and manure on C mineralization and stabilization depends on the interaction with soil properties, local edaphic conditions and cropping systems, it is of interest to conduct in-depth studies to elucidate the kinetics of C mineralization and stability of organic amendments as influenced by co-applied biochar, with or without N fertilizer application and the subsequent impact on plant nutrient release in this soil. Although this knowledge is essential for the proper management of sandy soils, it is still poorly understood.

The determination of quantitative information on the decomposition rates of co-applied biochar-manure mixtures in soils through modeling can provide important information about the processes influencing C mineralization and stability in soils. A variety of models have been used to determine the mineralization rates and kinetic parameters of organic amendments added to soils (Ajwa and Tabatabai 1994; Zimmerman, Gao, and Ahn 2011). Most of the C mineralization models involve the use of either the single or double-exponential first-order equations, depending on the heterogeneity of the organic material (Ajwa and Tabatabai 1994; Qayyum et al. 2012; Zhao, Coles, and Wu 2015; Zimmerman, Gao, and Ahn 2011).

In this study, we hypothesized that co-application of biochar and manure will change the C pools and induce slower C mineralization rates, resulting in increased C sequestration, but sole application of the amendments will decrease C sequestration in the soil. In addition, the implication of these interactions is likely to change with the application rates of the two amendments. Therefore, the objectives of the present study were to (i) investigate the influence of sole and/or co-applied rice husk biochar and cattle manure and their interaction with N fertilizer on C mineralization in a dry equatorial coastal savanna sandy soil, and (ii) determine the rate and kinetic parameters of the C mineralization process.

Materials and methods

Biochar, manure and soil

The biochar used was produced from rice husk (*Oryza sativa*) by a slow pyrolysis under limited oxygen conditions at target temperature of 500°C. The biochar was held in the furnace for 1.5 h once the target temperature was reached. After pyrolysis, the biochar samples were crushed and passed through 2-mm sieve to ensure homogeneous size fractions but were not exposed to any aging treatment before use. The cattle manure was sampled from small heaps on a farmer's field at Anloga, a semi-arid coastal savanna region located in the Keta District of the Volta Region in southeast Ghana (Longitude: 0° 53′50.21"E, Latitude: 5° 47′41.03" N). The manure did not undergo any stabilization treatment, except the natural biodegradation process during their storage in the heap. The manure was air-dried and crushed to pass through 2-mm sieve. Surface soil (0–20 cm depth) samples were collected from uncultivated fields in Anloga (Longitude: 0° 53′50.21"E, Latitude: 5° 47′41.03" N). After air-drying, the soil samples were passed through a 2-mm sieve and thoroughly homogenized. The mineral N fertilizer used was ammonium sulfate $(NH_4)_2SO_4$. The N fertilizer application rates were adapted to the common field application rates used by farmers in the region. The analytical procedure and physicochemical properties of the biochar, manure and soil have been presented by Dodor et al. (2018).

Experimental design

Three factors, namely biochar (0, 20 or 40 t ha^{-1}), manure (0, 13 or 26 t ha^{-1}) and N fertilizer addition (0 or 90 kg ha^{-1}) were tested in a full factorial experimental design. In all, there were 18 treatments which were factorially combined with three replications to give a total of 54 experimental units. The treatments were denoted with B (biochar), M (manure) and N (N fertilizer) followed by their respective sole and/or combined biochar-manure application rates. The amendments were thoroughly mixed with 500 g of soil samples and placed in 1.5 L wide mouth jars. Details of the procedure followed for the incubation experiment has been reported by Dodor et al. (2018).

The net CO_2-C efflux from biochar and/or manure amended soils were calculated using the following equation:

$$Net\ CO_2 - C = CO_2 - C_{(treatment)} - CO_2 - C_{(control)} \tag{1}$$

where CO_2-C $_{(treatment)}$ is the cumulative CO_2-C efflux from amended soils, and CO_2-C $_{(control)}$ is the CO_2-C evolved from soils without amendment. The amount of applied C mineralized (ACM; mg CO_2-C [g C applied]$^{-1}$) was calculated to evaluate the effect of adding different amounts of C to the soil in each treatment using the following equation:

$$ACM(\%) = \frac{[Net\ CO_2 - C(treatment]}{Orgnic\ C\ added} \times 100 \tag{2}$$

To evaluate the interactive influence of biochar, manure and N fertilizer on C mineralization, the net CO_2-C efflux from combined biochar and manure treatments were compared with the sum of the net CO_2-C evolved from the corresponding sole biochar and manure amended soils. The attributable effect (AE%) of co-application of biochar and manure on C mineralization, which is the percent increase or decrease in net CO_2-C evolution in the co-amended relative to the sole treatments, with or without N fertilizer, was calculated using the following equation:

$$AE(\%) = \frac{[Measured\ net\ CO_2 - C - Expected\ net\ CO_2 - C]}{Measured\ net\ CO_2 - C} \times 100 \tag{3}$$

where "measured" is the net CO_2-C efflux from combined biochar and manure amended soils and "expected" is the sum of the net CO_2-C evolved from the corresponding sole biochar and manure treatments. Positive AE value indicates increased C mineralization, and negative AE indicates the reverse.

The cumulative CO_2-C efflux data were fitted to the double-exponential first-order kinetic model representing a biphasic pattern of mineralization of the added C, using the following equation (Molina, Clapp, and Larson 1980):

$$C_m = C_l\left(1 - e^{k_l t}\right) + C_s\left(1 - e^{k_s t}\right) \tag{4}$$

where C_m is the amount of C mineralized at time t (day), C_l is the size of the labile mineralizable C pool, and C_s is the size of the stable mineralizable C pool, k_l and k_s are the corresponding mineralization rate constants (day^{-1}) for each C pool. The rate constants of the C_l and C_s pools were used to calculate their respective half-lives ($t_{½}$) using the following equation:

$$t_{1/2} = \frac{\ln(2)}{k} \tag{5}$$

At the end of the incubation period, readily oxidizable carbon (ROC) content was determined by shaking air-dried soil samples with 333 mM $KMnO_4$ for 1 h, followed by centrifugation and measuring of the absorbance of the filtrate at 565 nm (Blair, Lefroy, and Lisle 1995). Mineral N content of the soils after incubation were extracted with 2 M KCl and the amount of NH_4 and NO_3-N were determined by steam distillation (Mulvaney 1996).

Statistical analysis

The cumulative C mineralized (CCM), ACM and kinetic parameters were subjected to a three-way analysis of variance (ANOVA) to identify the primary factors influencing C mineralization. Tukey's LSD test was used to compare multiple means of variables when treatment effects were found at the 5% level of significance. Spearman correlation analysis was used to evaluate the relationship among CCM, ACM, AE, ROC, and C/N ratio of the soil mixtures. All statistical analysis were done using GenStat version 12 (VSN International); kinetic modeling of C mineralization was done using SigmaPlot 11.0, and figures were drawn using GraphPad Prims 7 for windows.

Results

Carbon mineralization

The temporal pattern of C mineralization rates in the various treatments showed that maximum CO_2-C release rates were observed in all amended soils during the first 7-d of incubation followed by a slower rate throughout the remaining period (Figure 1). The C mineralization rates during the initial first 7-d of the incubation ranged from 16.3 mg kg^{-1} day^{-1} in the control to 41.4 mg kg^{-1} day^{-1} in the B40M26F90 treatment (Table 1), with the amount of C mineralized during this period (7-d) constituting 33 – 47% of the total C mineralized during the entire 56-d incubation period (Table 1). The initial C mineralization (ICM) rates were significantly ($P < .05$) higher in soils amended with manure, solely or combined with biochar compared with sole biochar treated or control soils (Table 1). The ICM values increased with increasing manure application rates in the sole manure and combined biochar-manure treated soils (Table 1). Except for the control and the lower sole biochar (B20M0) treatments,

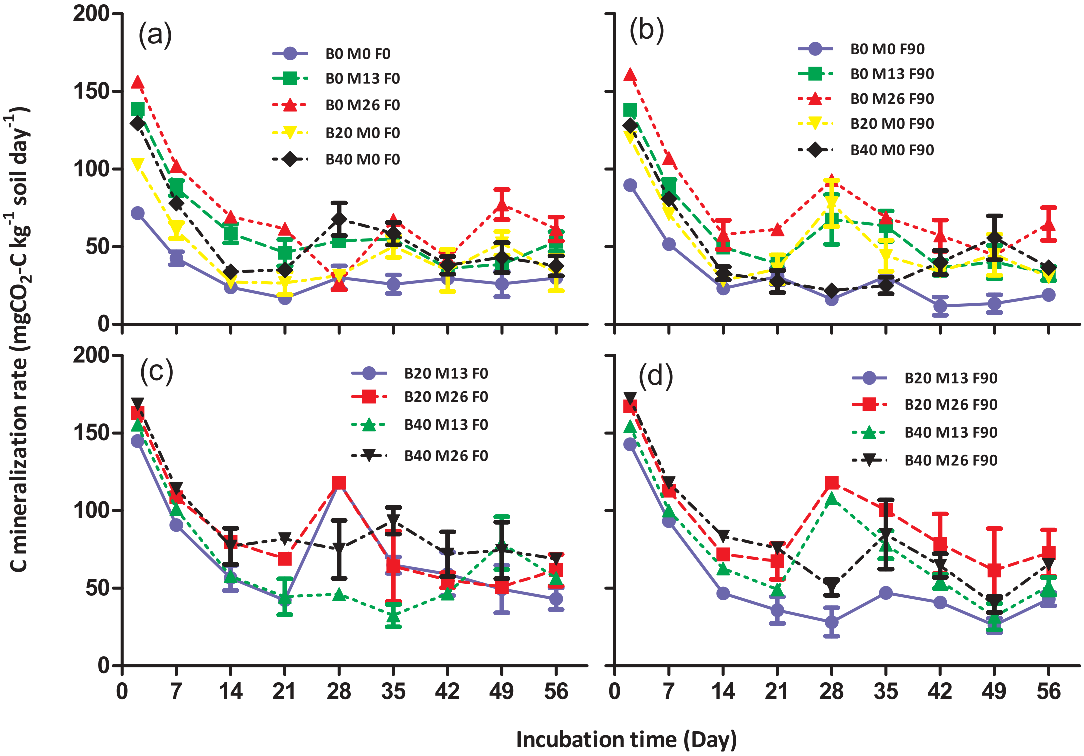

Figure 1. Carbon mineralization rates in soils amended with (a) sole biochar (B) or manure (M) without N fertilizer (F), (b) sole B or M with F, (c) combined B-M without F, and (d) combined B-M with F and incubated for 56 days (n = 3). B0, B20 and B40 are B rates at 0, 20 and 40 t ha-1, respectively. M0, M13 and M26 are M rates at 0, 13 and 26 t ha-1, respectively. F0 and F90 are F rates at 0 and 90 kg kg-1 soil, respectively.

Table 1. Cumulative CO_2-C flux values from soils amended with sole and/or combined manure and biochar on days 7 and 56 of incubation.

		Cumulative CO_2-C efflux (mg CO_2-C kg^{-1})		
		Incubation period (days)		
Treament[a]	N treatment	7	56	Initial C mineralization rate (mg CO_2-C kg^{-1} day^{-1})
B0M0	0	114	297	16.3
	90	142	287	20.2
B0M13	0	227	568	32.4
	90	226	555	32.3
B0M26	0	259	666	36.9
	90	268	716	38.3
B20M0	0	163	419	23.4
	90	191	487	27.3
B40M0	0	208	522	29.7
	90	209	449	29.9
B20M13	0	236	670	33.7
	90	236	504	33.7
B20M26	0	272	771	38.8
	90	280	851	40.0
B40M13	0	257	620	36.7
	90	254	690	36.4
B40M26	0	283	826	40.4
	90	290	754	41.4
LSD		11	80	

[a]B0, B20 and B40 are biochar rates at 0, 20 and 40 t ha^{-1}, respectively. M0, M13 and M26 are manure rates at 0, 13 and 26 t ha^{-1}, respectively.

N application did not influence ICM rates insole and/or combined biochar and manure amended soils (Table 1). The ICM rates were positively and significantly correlated with initial C applied (r = 0.633; $P < .01$) and C/N ratio of the soil-mixtures (r = 0.431; $P < .05$).

The cumulative amounts of C mineralized as CO_2 showed a curvilinear relationship with two major inflections in the exponential curves during the 56-d incubation period (data not shown). Sole application of biochar or manure, with or without N fertilizer, resulted in significant ($P < .05$) increase in the total amount of CO_2-C release relative to the unamended control soil, with total CO_2 -C efflux from the higher manure application rate being significantly ($P < .05$) higher than that from the biochar treated soils (Table 1). The total amount of CO_2-C evolved increased with increasing manure application rate (Table 1). Generally, the total amount of CO_2-C released from the amended soils, with or without N fertilizer, were similar (Table 1).

Three-way ANOVA indicated that CCM was significantly ($P < .001$) influenced by biochar and manure applications, as well as their interactions (Table 2). Application of N fertilizer did not influence CCM, however, there was a significant interaction among the three factors. The CCM was positively and significantly correlated with the amount of C applied (r = 0.584; $P < .01$) and manure application rate at fixed biochar rates (r = 0.891; $P < .001$); however, the correlation with biochar application rate at fixed manure rate was not significant (r = 0.324; P= .1898). As expected, ICM and CCM were positively and significantly correlated (r = 0.946; $P < .001$).

Applied c mineralized (ACM) and attributable effect (AE)

The ACM in sole manure amended soils ranged from 18.6% to 27.4%, with values decreasing with increasing manure application rates (Figure 2). Sole application of biochar significantly ($P < .05$) decreased ACM values to 2.8–5.9%, with values at the two application rates being statistically ($P >$ .05) similar (Figure 2). The ACM values in the sole biochar treated soils are significantly ($P < .05$) lower than those in the manure amended soils, with or without N fertilizer application (Figure 2). The ACM values of soils treated with combined biochar and manure ranged from 4.7% to 6.8%, with lower values recorded at higher biochar at fixed manure application rates (Figure 2). The ACM values obtained in the combined biochar-manure treated soils were similar ($P > .05$) to those observed in the sole biochar treatments. Application of N fertilizer did not affect ACM values in both sole and combined biochar and manure amended soils (Figure 2).

The three-way ANOVA indicated that biochar and manure application significantly ($P < .001$) influenced ACM, and so were their interactive effects (Table 2). Application of N fertilizer did not influence ACM values, however, there was a significant interaction among the three factors. The ACM values were negatively and significantly correlated with biochar application rates (r = – 0.804; P= .005) but positively and significantly correlated with manure application rate (r = 0.640; P= .01). The ACM values were negatively and significantly correlated with the initial C applied (r = – 0.665; $P < .003$) and C/N ratio of the soil-mixtures (Figure 3; r = – 0.573; P= .013).

The net CO_2-C efflux from soils co-amended with biochar and manure (measured) were significantly ($P < .05$) lower than the sum of the net CO_2-C evolved from the corresponding sole

Table 2. Analysis of variance for cumulative C mineralized (CCM), applied C mineralized (ACM), sizes of the labile and stable C pools (C_l and C_s, respectively) estimated by the double-exponential model as affected by biochar (0, 20 and 40 t ha^{-1}), manure (0, 13 and 26 t ha^{-1}) and N fertilizer (0 and 90 kg^{-1} soil).

Source of variance	df	CCM	ACM	C_l	C_s
Biochar (B)	2	<0.001	<0.001	<0.001	<0.001
Manure (M)	2	<0.001	<0.001	<0.001	<0.001
Fertilizer (F)	1	0.583	0.140	<0.001	<0.001
B x M	4	0.006	<0.001	0.099	0.108
B x F	2	0.577	0.080	0.693	0.749
M x F	2	0.234	0.557	0.027	0.022
B x M x F	4	<0.001	<0.004	0.003	0.002

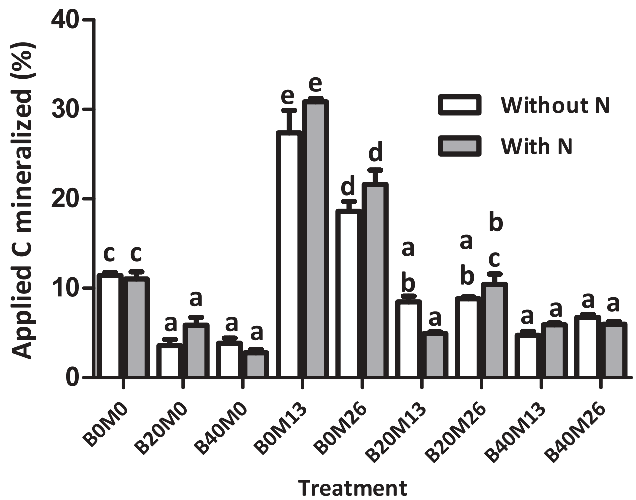

Figure 2. Applied C mineralized in soils amended with sole and/or combined biochar (B) or manure (M) with or without N fertilizer after 56 days incubation. Same letter(s) above the bars indicate no significant difference between treatments at $p = .05$. Vertical bars represent standard error of the means (n = 3). B0, B20 and B40 are B rates at 0, 20 and 40 t ha-1, respectively. M0, M13 and M26 are M rates at 0, 13 and 26 t ha-1, respectively. N fertilizer rates are 0 and 90 kg kg-1 soil.

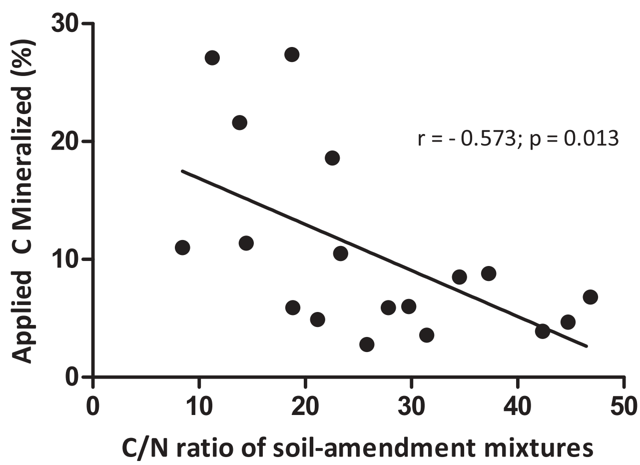

Figure 3. Relationship between percent of applied carbon mineralized (ACM) and C/N ratio of soil-amendment mixtures.

biochar and manure treated soils (expected), with or without N application. The calculated AE values ranged from −7 to −35%, with the most negative value recorded in the B40M13F90 treatment. Generally, AE values tended to be more negative in soils co-amended with biochar and manure that also received N fertilizer application.

Readily oxidizable C (ROC) and mineral N

Sole biochar and manure application resulted in significant ($P < .05$) increase in ROC content of the soils compared with the unamended control, with ROC of manure, treated soils being significantly higher ($P < .05$) than those of biochar-amended soils (Figure 4). The ROC contents of the soils were positively and significantly correlated with initial C applied (r = 0.635; $P = .005$), ICM rate (r = 0.568;

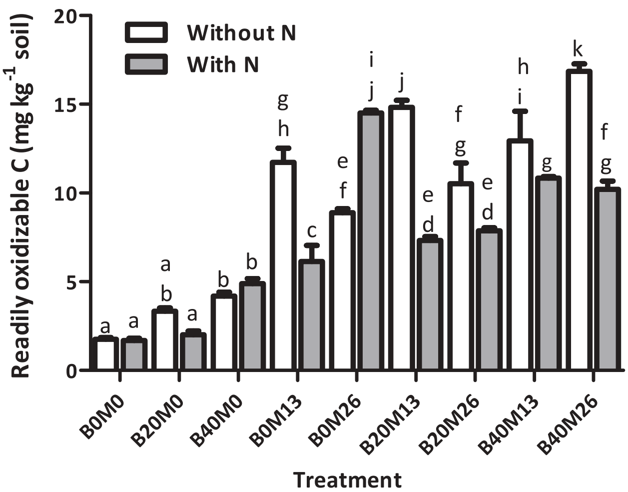

Figure 4. Effect of sole and/or combined biochar (B) or manure (M) with or without N fertilizer on readily oxidizable C content of the soils after 56-d incubation period. Same letter(s) above the bars indicate no significant difference between treatments at p = .05. Vertical bars represent standard error of the means (n = 3). B0, B20 and B40 are B rates at 0, 20 and 40 t ha-1, respectively. M0, M13 and M26 are M rates at 0, 13 and 26 t ha-1, respectively. N fertilizer rates are 0 and 90 kg kg-1 soil.

P= .013), CCM (Figure 5; r = 861; P < .001) and biochar application rate (r = 0.536; P < .023), but not with manure application rate (r = 0.414; P= .088).

The amount of mineral N ($NH_4 + NO_3$) extracted from the soils after 56-d incubation period ranged from 39.3 mg kg^{-1} soil in the B40M13F0 to 140 mg kg^{-1} soil in the B40M0F90 treatments (Figure 6). Generally, higher amounts of mineral N were produced in soils amended with sole biochar and manure, with or without N fertilizer application, relative to the control, resulting in positive net N mineralization in all sole-amended soils. Net N immobilization was observed mostly in soils co-amended with biochar and manure without N fertilizer application. The mineral N content of the soils were negatively and significantly correlated with AE values (Figure 7; r = – 0.781; P= .022). Three-way ANOVA indicated that the amount of mineral N produced was significantly influenced by biochar, manure and fertilizer, as well as their two and three-factor interactions.

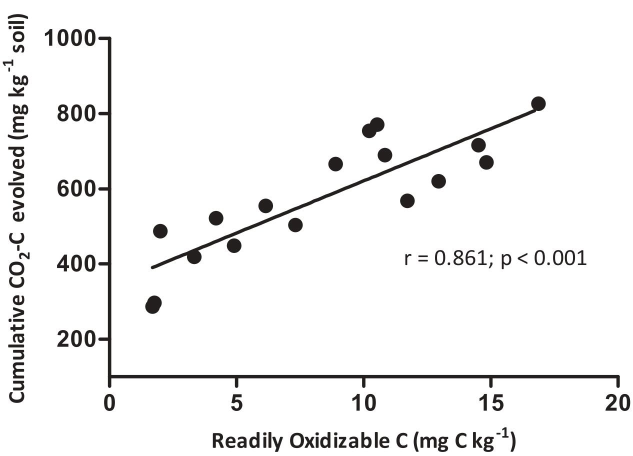

Figure 5. Relationship between total amount of CO2-C evolved and readily oxidizable C content of the soils after 56-d incubation period.

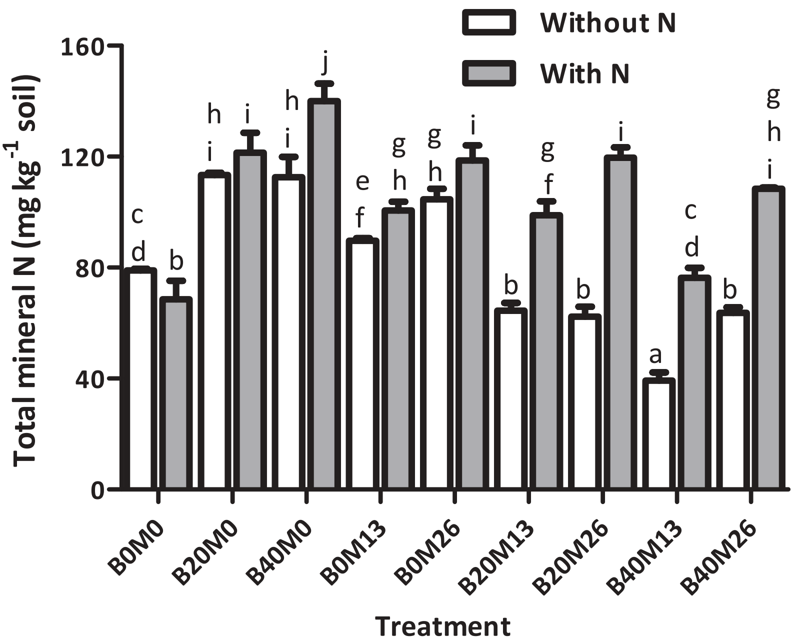

Figure 6. Effect of sole and/or combined biochar (B) or manure (M) with or without N fertilizer on mineral N content of the soils after 56-d incubation period. Same letter(s) above the bars indicate no significant difference between treatments at p = .05. Vertical bars represent standard error of the means (n = 3). B0, B20 and B40 are B rates at 0, 20 and 40 t ha-1, respectively. M0, M13 and M26 are M rates at 0, 13 and 26 t ha-1, respectively. N fertilizer rates are 0 and 90 kg kg-1 soil.

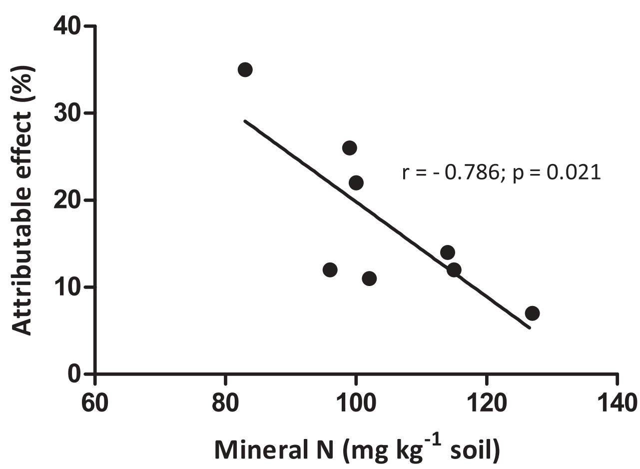

Figure 7. Relationship between attributable effect (AE) and mineral N content of the soils at the end of 56-d incubation period.

Kinetics of carbon mineralization

The kinetic parameters obtained by fitting the cumulative CO_2-C evolved from the treated soils to the double-exponential first-order model are shown in Table 3. The estimated size of the labile C pool (C_l) ranged from 82 mg kg^{-1} soil in the control soil to 260 mg kg^{-1} soil in the B40M26F90 treatment (Table 3). Generally, sole manure-amended soils recorded significantly (P < .05) larger C_l compared with the sole biochar amended soils, with or without N fertilizer application. Co-application of manure and biochar increased C_l pool compared to sole biochar treatments. The C_l

Table 3. Size, decomposition rates and half-lives of the labile and stable C pools (C_l, k_l, C_s and k_s, respectively) estimated by the double-exponential model in soils amended with biochar (B) at 0, 20 and 40 t ha^{-1}, manure (M) at 0, 13 and 26 t ha^{-1}, combined B and M (B at 20 or 40 t ha^{-1} plus M at 13 or 26 t ha^{-1}) with or without N fertilizer (F) at 0 and 90 kg ha^{-1} after 56 days of incubation.

Treatment	C_l (mg kg^{-1})	k_l (day^{-1})	Half-life (day)	C_s (mg kg^{-1})	k_s (day^{-1})	Half-life (day)	R^2	C_l/C_T (%)	C_s/C_T (%)	C_l/C_s (%)	k_l /k_s
Control											
B0M0F0	82	0.356	2.28	2518	1.5E-03	466	0.994	3.2	97	3.3	238
B0M0F90	145	0.240	3.05	2458	1.0E-03	752	0.995	5.6	94	5.9	231
Sole applied biochar or manure											
B0M13F0	197	0.272	2.30	3394	1.9E-03	360	0.998	5.5	95	5.8	141
B0M13F90	186	0.298	2.40	3405	2.0E-03	359	0.995	5.2	95	5.5	149
B0M26F0	212	0.310	2.22	4370	1.8E-03	389	0.996	4.6	95	4.8	173
B0M26F90	206	0.321	2.31	4375	2.1E-03	328	0.997	4.5	96	4.7	151
B20M0F0	112	0.392	1.97	5894	9.2E-04	762	0.990	1.9	98	1.9	427
B20M0F90	142	0.336	1.80	5863	1.1E-03	655	0.992	2.4	98	2.4	309
B40M0F0	155	0.343	2.11	8276	8.1E-04	882	0.995	1.8	98	1.9	425
B40M0F90	170	0.348	2.06	8261	5.7E-04	1239	0.988	2.0	98	2.1	612
Co-applied biochar and manure											
B20M13F0	170	0.322	2.26	6826	1.4E-03	508	0.992	2.4	98	2.5	234
B20M13F90	213	0.292	2.49	6783	7.5E-04	930	0.997	3.1	97	3.1	391
B20M26F0	237	0.241	2.79	7750	1.3E-03	552	0.995	3.0	97	3.1	190
B20M26F90	197	0.376	2.09	7793	1.6E-03	457	0.994	2.5	98	2.5	241
B40M13F0	211	0.324	2.36	9211	7.6E-04	934	0.994	2.2	98	2.3	427
B40M13F90	207	0.281	2.13	9215	9.8E-04	710	0.993	2.2	98	2.2	286
B40M26F0	212	0.331	2.43	10200	1.1E-03	643	0.999	2.0	98	2.1	303
B40M26F90	260	0.261	2.76	10152	8.7E-04	806	0.998	2.5	98	2.6	298
LSD	28	0.101	0.67	27	3.5E-04	213					

values expressed as a percent of the total organic C in the sole manure amended soils ranged from 4.5% to 5.5% compared to 1.8–2.4% for the sole biochar amended soils. The corresponding values for soils co-amended with biochar and manure ranged from 2.0% to 3.1% of the total organic C.

All treatments had larger proportion of their organic C apportioned to the stable C pool (C_s), with values ranging from 2.24 to 10.15 g C kg^{-1} soil; this is equivalent to ≥94% of the initial C applied. The C_l/C_s ratio ranged from 1.9% to 5.9%, with manure-amended soils recording higher values compared to sole and/or combined biochar and manure amended soils (Table 3). The size of both C_l and C_s pools increased with increasing application rates of the amendments (Table 3). Based on the double-exponential model, the amount of C sequestrated in the C_s pools in treatments containing biochar, solely or in combination with manure were 2.3–4.2 times higher than those of sole manure treatments or soil alone. The C_l pool was positively and significantly correlated with ICM (r = 0.921; $P < .001$), CCM (r = 0.785; $P < .001$) and ROC (r = 0.688; $p < .01$) (Table 4). As expected, the C_s pool was positively and significantly correlated with ICM (r = 0.623; $P < .01$) and CCM (r = 0.593; $P < .01$), but not with ROC (r = 0.432) (Table 3). Three-way ANOVA indicated that estimated C_l and C_s pools were influenced by biochar, manure, N fertilizer and their three factor interactions (Table 2).

Generally, the rate constant k_l for the C_l pool for the biochar and biochar-manure-amended soils were lower than those for the sole manure-amended soils (Table 3). Slower k_s values were recorded for the sole biochar treated soils, with k_l/k_s ratio ranging from 141 to 612. The half-lives of the C_l pools were narrow, with values ranging from 1.8 to 3.1 days, whereas those of the stable C_s pools were wide, ranging from 328 to 1239 days (Table 3). The half-lives of the C_s pool for all treatments containing biochar, either solely or in combination with manure were ≥1.4 folds longer than those of the sole manure amended soils.

Discussion

Cumulative c mineralization

The rapid increase in C mineralization immediately after incubation, especially during the first 7-d is consistent with the results of Ajwa and Tabatabai (1994) who reported that 45 – 47% of the organic

Table 4. Correlation coefficients among measured soil process and C pools estimated by the double-exponential model.

Soil Process	C_l	C_s
ICM	0.921***	0.623**
CCM	0.785***	0.593**
ACM	0.171^{ns}	−0.673**
ROC	0.688**	0.432^{ns}
AE	0.297^{ns}	0.181^{ns}
C/N ratio	0.293^{ns}	0.808***
Mineral N	-0.355^{ns}	−0.456*

ICM, Initial C mineralization rate; CCM, Cumulative C mineralized; ACM, Applied C mineralized; AE, Attributable effect; ROC, Readily oxidizable C; Cl and Cs, labile and stable C pools, respectively; ns, not significant; *, ** and *** significant at p ≤ 0.05, 0.01 and 0.001, respectively.

C in cow manure was mineralized during the first week of a short-term incubation study. This increase in CO_2-C production is often attributed to changes in soil humidity and temperature following rewetting and availability of easily biodegradable organic C, which results in increased microbial activity and high CO_2 release rates (Borken and Matzner 2009; Quyang, Yu, and Zhang 2014; Ribeiro et al. 2010). Other researchers have also reported that rewetting of soils disrupts soil aggregates, releasing hitherto protected and unavailable organic C for rapid mineralization by microbial communities (Kieft, Soroker, and Firestone 1987; Lundquist, Jackson, and Scow 1999).

The significant increase in CO_2-C efflux in the biochar amended compared to the unamended soils indicates that the biochar contained some amount of readily mineralizable C that was assessible to the microbes, as has been reported by other workers (Ameloot et al. 2013; Qayyum et al. 2012). The results, however, contradict Grunwald, Kaiser, and Ludwig (2016) who reported comparable C mineralization rates in biochar amended compared to the control soil. The positive correlation between CCM and application rates of the amendments could be due to stimulation of microbial activity by the increased supply of readily mineralizable organic C and other limiting nutrients, such a P and N (Ribeiro et al. 2010).

Heterotrophic microorganisms responsible for organic C mineralization in soils utilize dissolved and readily available organic C as source of C, reducing equivalent and energy for cell biosynthesis and growth (Sylvia et al. 2005). The higher ROC content of the manure amended soils indicates that $KMnO_4$ indeed oxidized the easily mineralizable organic C components of the soil-mixtures, of which manure contained higher amounts compared to biochar. This higher amount of readily mineralizable C content of the soil-mixtures due to manure application resulted in significantly higher ICM and CCM rates in soils amended with manure, solely or combined with biochar compared to sole biochar treated soils. Furthermore, the increased ICM with increasing proportion of manure in the soil-mixtures at a fixed biochar rate is another strong indication that manure exerted considerable influence on C mineralization, especially at the initial stages of the incubation. The results further suggest that ROC content, as measured by susceptibility of SOC to oxidation by $KMnO_4$, can be used as an index of chemical reactivity, bioavailability and physical accessibility of organic C substrates to microorganisms in biochar-manure-amended sandy soils. Similarly, Dodor et al. (2018) reported a close association between water-extractable OC (WEOC) and net CO_2-C evolution in these soils and suggested the use of WEOC as an index of labile C content and an important source of C for microorganisms in soils.

Microbial mineralization of carbon is influenced by the proportion of labile organic C pool and N in a given amendment (Jien et al. 2017). Therefore, changes in composition due to biochar application led to differences in the nature and amount of readily mineralizable organic C and other nutrients, especially N in the soil-mixtures, resulting in the observed significantly higher

C mineralization rates in the manure amended compared to biochar-treated soils (Quyang, Yu, and Zhang 2014; Ribeiro et al. 2010).

Applied c mineralized (ACM) and attributable effect (AE)

The ACM for the sole biochar treatments, which is the total amount of CO_2 efflux expressed as a percentage of added C, were lower than that for the control. The significantly less C mineralization in the biochar compared to manure amended soils indicates that sole manure application resulted in more C mineralization whereas net C sequestration occurred in the sole biochar or biochar-manure-treated soils. Zimmerman, Gao, and Ahn (2011) reported similar finding in biochar amended compared to unamended control soils. Zhao, Coles, and Wu (2015) also reported lower ratios of mineralized to total C in biochar treated soils, indicating larger C sequestration in the biochar-soil mixture compared to the control soil without amendment.

The biochar mineralization rates reported in the present study are in the range reported by Baldock and Smernik (2002) for C mineralization in red pine wood biochar produced at pyrolysis temperature of 305°C. The mineralization rates are higher than those of Hamer et al. (2004) who reported C losses ranging from 0.3% to 0.8% in three biochars in a 60-day incubation study, and Kuzyakov et al. (2009) who measured mineralization rates between 1.8% and 2.1% for biochar incubated in a loess-soil during the first 2 months of incubation. The mineralization rates are, however, lower than those of Nguyen and Lehmann (2009) who reported 16% mineralization of corn-derived biochar after one-year incubation.

It is apparent that the negative AE values in the co-amended biochar-manure soils, which indicates negative C mineralization, was due to the low availability of readily mineralizable organic C in the mixtures because of biochar application. This assertion was supported by the significantly higher ICM and CCM in soils amended with manure, either sole or combined with biochar, which increased with increasing manure application rate at a fixed biochar rate. In addition, the positive correlation between ACM and manure application rate, coupled with the significant negative correlation between ACM and biochar application rates offer credence to this assertion. The results suggest that manure provided more readily available OC that stimulated microbial activity, leading to the slight but insignificant increase in CO_2-C efflux in the combined biochar-manure relative to the sole-amended soils. However, application of biochar might have stabilized the labile C in the mixture through sorption onto the organic coatings on the surface of biochar, by incorporation into its mineral fractions or absorption of the evolved CO_2-C onto the porous and large surface area of the biochar, as has been reported by other researchers (Hagemann, Joseph, and Schmidt et al. 2017; Jien et al. 2017; Keith and Singh 2011). The results further suggest that co-application of biochar and manure can provide the required nutrients for crop growth through mineralization while increasing the amount of C sequestrated in sandy soils.

Generally, interaction between C and N in amended soils determined the net direction of C mineralization process. Thus, the lack of influence of N application rates on ICM, coupled with the positive correlation between ICM and C/N ratio of the soil-mixtures strongly suggests that microbial populations were primarily limited by availability of C during the initial stages of incubation (Knapp, Elliott, and Campbell 1983; Reinertsen et al. 1984). The provision of copious amount of labile C from biochar and manure, therefore fueled higher ICM rates in the amended soils compared to the control and N only amended soils. However, as postulated by Liebig's Law of Minimum (Sylvia et al. 2005) and the stoichiometric balance between the two nutrients, after microbial requirement for C was met, N became the limiting nutrient as mineralization progressed.

The purported shift in nutrient requirement as C mineralization continued was supported by the significant negative correlation between ACM and C/N ratio, indicating that the decreased ACM at the end of the incubation period was due to increased C/N ratio in the combined biochar-manure-amended soils without N fertilizer application, leading to mineral N limitation, culminating in reduced C mineralization. Furthermore, the negative correlation between AE and C/N ratio coupled with that between AE and mineral N content of the soils are also strong indications that microbial population were limited by N rather than C supply at the end of 56-d incubation period.

Although the results indicated that none of the parameters measured were influenced by N fertilizer application rates, they were significantly correlated with C/N ratio of the soil-mixtures. As noted above, because of the stoichiometric relationship between the amount of C and N that can be assimilated by microbes, it is conceivable that C/N ratio would be a better indicator of N requirement and/or limitation rather than N application rates. The results indicate that the use of N fertilizer application rate to evaluate the influence of N on microbial population and activity could lead to erroneous conclusions. Therefore, when predicting the potential effects of N fertilizer on C mineralization and sequestration, more attention should be paid to C/N ratio of the soil-mixtures, rather than N application rate.

Kinetics of C mineralization

The high R^2 values obtained in all treatments using the double-exponential model indicate that the model is a useful approximation of C mineralization in manure and biochar amended soils, and suggest that the C mineralization process was biphasic, with a smaller labile and larger stable C pools, as have been reported previously by other authors (Qayyum et al. 2012; Zimmerman, Gao, and Ahn 2011). This assertion was supported by the inflections in the exponential curves for CCM, indicating that C mineralization occurred in two major stages, an initial increase in microbial activity during which the easily degradable organic C were rapidly exhausted, and a second stage where microbes shifted to use the more recalcitrant materials until a new steady-state was established in the system (Ajwa and Tabatabai 1994; Ribeiro et al. 2010; Rivas et al. 2014).

The proportion of labile and recalcitrant organic C pools in an organic amendment determines its turnover rate and C sequestration potential when applied to soils. The low C_l/C_s ratio of the estimated C pools is in consonance with the results of Zimmerman, Gao, and Ahn (2011) who described the labile C pool as consisting of smaller sized volatile aliphatic compounds with low C and higher oxygen components that were readily mineralized, and a larger stable C pool made up of nonvolatile aromatic C and low oxygen compounds that were mineralized more slowly. Using a sequential C fractionation procedure, Bolan et al. (2012) reported that the proportion of labile C pool (resin and 0.1 M NaOH extractable) in biochar was very small compared to that for the stable C pool (1.0 M NaOH extractable). Bernal et al. (1998) reported that the labile C fraction of organic residues decomposed faster than the stable C fractions. The stronger positive correlation between ICM and the labile C pool, C_l compared with that for the recalcitrant C pool, C_s suggests that the easily mineralizable labile C pool exerted stronger influence on the C mineralization process, especially at the initial stages of the incubation.

Soil microorganisms are responsible for OC mineralization, therefore any useful kinetic parameter derived from empirical equations must correlate with experimentally measurable microbiochemical properties of the soil (Dodor 2002; Dodor et al. 2018). The significant correlation between the estimated indices of easily biodegradable organic C pools (C_l pool) and ICM, CCM and ROC affirmed our experimental results indicating that labile C was an important source of energy for the microorganisms, and that ROC can be used as a good proxy for estimating the size of the labile C content of manure and biochar amended soils. Similarly, Dodor et al. (2018) suggested the use of WEOC as a good estimate of labile and readily mineralizable C content of biochar amended soils. Saviozzi, Vanni, and Cardelli (2014) also reported a significant relationship between readily mineralizable C pools and the amount of C mineralized in urban soils during a 25 days incubation experiment. The close agreement between CCM from experimental results and the estimated C_l pools also suggest that the observed increase in C mineralization with increasing manure and biochar application rates were due to the increased supply of readily mineralizable C (Cross and Sohi 2011; Fernández et al. 2014).

The slower kinetic rate constants (k_l and k_s) of the labile and stable C pools in the biochar compared to manure amended soils, suggest that the chemical nature of mineralizable OC in the two amendments are different. The k_l and k_s values are also consistent with the experimental results showing higher CO_2-C evolution in the manure-amended soils, suggesting that biochar is relatively inert in the soils (Kuzyakov et al. 2009), which led to reduced CO_2-C efflux. The high k_l/k_s ratios indicate faster mineralization of the readily biodegradable C pool in the amendments leaving behind a larger and more refractive C_s pool with slower kinetic lost constant (k_s) and longer half-life (Zhao, Coles, and Wu 2015; Zimmerman, Gao, and Ahn 2011).

The shorter half-lives of the manure compared with biochar-treated soils are due to the varied nature and quality of C in the organic amendments (Flavel and Murphy 2009). The calculated shorter half-lives of the C_l pools and the longer half-life of the stable C_s pool suggest a rapid mineralization of the easily biodegradable organic C pools (C_l), leaving behind ≥94% of relatively stable C pool with reduced biodegradability, subsequently leading to greater C sequestration. The consistency between modeled kinetic parameters and experimental CO_2-C evolution suggest that co-application of biochar can promote an initial rapid mineralization of co-applied biochar and manure to release nutrients needed by plants but sequester larger amount of the applied amendment in the refractive Cs pool, resulting in larger amount of C sequestration and increased OC content of sandy soils.

Conclusions

This study evaluated the kinetics of C mineralization and sequestration in a sandy soil amended with sole and/or combined biochar and cattle manure, with or without N fertilizer application. Biochar application, solely or in combination with manure resulted in lower ACM values, indicating C sequestration in the soils. Negative attributable effect (AE) of co-application of biochar and manure on C mineralization was observed relative to the sole treatments. Both AE and ACM were negatively correlated with C/N ratio and mineral N content of the soil-amendment mixtures, indicating that microbial population were limited by N availability. The kinetic parameters obtained using the double-exponential model were consistent with experimental CO_2-C evolution and indicated that ≥94% of the initial C applied was apportioned to the stable C_s pool with slower rate constant and longer half-lives. The results suggest that co-application of biochar can promote initial faster mineralization to release plant nutrients but sequester larger amount of the applied amendment in the refractive C_s pool, resulting in larger amount of C sequestration in the soils. The best combination of the biochar and manure that can maximize C sequestration and sustain crop production in these soils require further investigation.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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