Impact of biochar and manure application on in situ carbon dioxide flux, microbial activity, and carbon budget in degraded cropland soil of southern India

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Impact of biochar and manure application on in situ carbon dioxide flux, microbial activity, and carbon budget in degraded cropland soil of southern India

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Abstract

Biochar application is attracting attention to be an effective soil organic carbon (SOC) management to prevent land degradation, though quantitative information of its effect on carbon dioxide (CO_2) flux and associated microbial responses is still scarce, especially in degraded tropical agroecosystems. We conducted a 27-month field experiment with periodically measuring environmental factors, CO_2 efflux rate, microbial biomass C (MBC), and SOC stock, and evaluated the impact of land management (control (C), biochar (B; 8.2 Mg C ha^{-1}), farmyard manure (FYM) (M; 1.1 Mg C ha^{-1} yr^{-1}), and a mixture of both (BM) on CO_2 flux, microbial responses (MBC and qCO_2 as microbial activity) and C budget, in tropical alkaline cropland of southern India. Based on the relationship between the CO_2 efflux rate and environmental factors, cumulative CO_2 flux was estimated at 2.4, 2.7, 4.0, and 3.7 Mg C ha^{-1} in the C, B, M, and BM treatments, respectively. Biochar application increased soil moisture though did not affect CO_2 flux, causing a positive C budget (6.7 Mg C ha^{-1}), because of the limited response of microbes to increased soil moisture due to the small amount of SOC. Biochar and FYM combined application did not increase CO_2 flux compared with FYM alone, contributing to the largest SOC increment (8.9 Mg C ha^{-1}) with a positive C budget (9.1 Mg C ha^{-1}), due to little difference of microbial responses between the two treatments. Hence, biochar application combined with FYM could be an effective SOC management in the degraded cropland of southern India.

KEYWORDS

biochar, land degradation, microbial activity, SOC management, tropical alkaline soil

1 | INTRODUCTION

Soil organic carbon (SOC) is a central component in maintaining soil fertility and subsequent food security, and hence, soil C sequestration is vital to prevent land degradation worldwide (Lal, 2004a; Minasny et al., 2017). Since changes in soil CO_2 flux could substantially change the amount of SOC (Moinet et al., 2016), it is necessary to assess accurate CO_2 flux for carrying out appropriate SOC management to sustain SOC level and prevent land degradation (Lehmann & Kleber, 2015). Soils in dry tropical areas retain low SOC, and soil fertility is correspondingly low (Powlson et al., 2016) because of the small amount of plant residue input and fast decomposition of litter and SOC under tropical climate conditions. Therefore, it is critically important to estimate annual CO_2 flux to conduct sustainable SOC management for preventing land degradation in degraded soils of tropical agroecosystems.

Biochar, made by biomass pyrolysis with low/no oxygen, has attracted attention as its potential for preventing land degradation, because it increases soil C stocks due to its high resistance to soil microbial decomposition (Al-Wabel et al., 2018; Lehmann et al., 2011). Recent research found that biochar application increased soil C decomposition due to improved soil water holding capacity (WHC) (Lorenz & Lal, 2014) and/or soil microbial biomass C (MBC) (Thies & Rillig, 2009), while other studies found that it decreased soil C decomposition because of reduced soil microbial activity (Li et al., 2018), and/or the sorption of SOM to biochar (Zimmerman et al., 2011). To assess accurate CO_2 fluxes following biochar application, the controlling factors need to be evaluated, that is, environmental factors containing soil moisture and temperature (Kim et al., 2015) and microbial factors such as MBC and microbial activity (Schmidt et al., 2011). There are many studies conducted on the effect of biochar addition on microbial respiration (Senbayram et al., 2019), C sequestration (El-Naggar et al., 2018), and associated microbial responses (Gul et al., 2015), though these studies have mainly been conducted under controlled conditions, which do not integrate all the biotic and abiotic factors impacting in situ CO_2 fluxes. Moreover, most studies on biochar were conducted in acidic soils because biochar application can ameliorate soil acidity (Hernandez-Soriano et al., 2016). There is limited research on the effect of biochar application on in situ CO_2 flux and associated microbial responses in tropical alkaline soils, although they are globally distributed and are subject to the critical problem of land degradation such as low SOC accumulation (Tavakkoli et al., 2015). Hence, it is essential to assess the accurate CO_2 flux with related environmental and microbial responses, and to assess the impact of biochar application on C budget based on in situ CO_2 flux, for conducting proper SOC management in the tropical alkaline soils.

Tropical alkaline soils in India are mostly degraded and characterized by low soil C stock due to the long-term use of extractive cultivation and removal of crop residue, especially in croplands (Lal, 2004b). Srinivasarao et al. (2009) investigated soil C stocks at 21 locations under different land uses in India and found low soil C contents (<5 g kg^{-1}), which was less than the threshold level of SOC for crop production in the tropics (1.1%) (Aune & Lal, 1997). Indian farmers conventionally utilize the crop residues as livestock food, and then composted farmyard manure (FYM) from livestock excrement, which is applied to the soil (Srinivasarao et al., 2014). Many studies have reported that increased CO_2 flux and negative C budget with FYM application (Lai et al., 2017) resulted from larger microbial responses (Lian et al., 2016), by the fast decomposition of FYM with relatively low C:N ratio. Some previous studies suggested that larger C inputs are necessary to keep SOC level and prevent land degradation in degraded soils of India (Pathak et al., 2011; Seki et al., 2019). Despite such degradations in cropland soils of India, the availability of FYM have declined because of its utility for other domestic purposes such as fuel (Indoria et al., 2018), and hence, other management options, such as biochar application and/or combined application of biochar and OM, have been paid attention as effective management to enhance SOC stock. Hamer et al. (2004) revealed that combined biochar and organic substrate application stimulated biochar decomposition, resulting from increased MBC, in a 26-day incubation experiment in Germany. In contrast, Zavalloni et al. (2011) found that fresh OM decomposition was decreased with combined biochar and plant residue application because of physical protection by biochar, that is, substrate sorption to the biochar surface and pores, in an 84-day incubation experiment in Cambisols. These contradictory results make it difficult to evaluate whether the biochar and FYM combined application increase or decrease soil respiration and/or SOC stock in tropical alkaline soils, especially under field conditions.

The objectives of this study were to evaluate the impact of land management (biochar and manure application) on in situ CO_2 fluxes, associated microbial responses (i.e., MBC and microbial activity as qCO_2), and C budget in tropical alkaline degraded cropland soil of southern India. We hypothesized that biochar and FYM combined application would stimulate microbial growth and activity, causing increased OC decomposition and high CO_2 flux in tropical alkaline cropland soil (Awad et al., 2013). To verify this hypothesis, we conducted a 27-month field experiment with three cropping periods and evaluated the CO_2 efflux rate with environmental factors, MBC, qCO_2, and SOC stock under different land management.

2 | MATERIALS AND METHODS

2.1 | Site description

A field experiment was carried out from September 2017 to December 2019 (27 months in total) in a farmer's field in Madurai, Tamil Nadu State, India (9°43′22.37″ N 77°46′51.61″ E; 175 m asl) (Seki et al., 2019). The mean annual air temperature was 24.7°C and the annual rainfall was 820 mm (692–857 mm; 2017–2019). The agriculture situation in this area mainly depends on rainfall amount during rainy season (June–September and October–December). Due to the low SOC content (Seki et al., 2019), the experimental field should be representative of the degraded cropland soils in this area (Lal, 2004b). Soil in the experimental site was classified as Typic Haplustepts (Soil Survey Staff, 2014). The soil of the surface layer has the following characteristics: pH (1:5 water) of 8.5, SOC of 3.2 g kg^{-1}, and clay content of 27.2% [details in Seki et al. (2019)]. SOC was calculated as follows: SOC = total carbon (TC) − inorganic carbon (IC). IC was measured using the method provided by Bundy and Bremner (1972). Briefly, the soil sample was treated with 1 M HCl at room temperature for 24 hr, and then unreacted HCl that was not released as CO_2 from carbonates was determined by titration with 1 M NaOH to calculate the IC content.

2.2 | Experimental set-up

In this study, the field experiment was carried out to assess the effect of biochar and FYM application on in situ CO_2 fluxes, associated microbial responses, and C budget. Biochar was selected to assess its potential effect for enhancing SOC stock to prevent land degradation, whereas FYM was selected to compare the impact of the traditional cultivation management with the combined management of biochar and FYM. Each experimental plot (5 × 8 m) was arranged into a randomized block design with a 1-m buffer zone. The experiment was set-up with the following four treatments with three replicates.

1. Control plot; hereafter referred to as 'C plot.'
2. Biochar plot (8.2 Mg C ha^{-1}) (applied only one time at the beginning of the experiment); hereafter referred to as 'B plot.'
3. FYM plot (1.1 Mg C ha^{-1} yr^{-1}) (applied every year, i.e., three times during the whole experiment); hereafter referred to as 'M plot.'
4. Biochar (8.2 Mg C ha^{-1}) and FYM (1.1 Mg C ha^{-1} yr^{-1}) plot (each applied in the same way as the B and M plots above); hereafter referred to as 'BM plot.'

Table S1 indicates the summary of 3 years of crop cultivation and land management. Sorghum (*Sorghum bicolor* (L.) Moench) was cultivated three times during the experimental period. Every year before cultivation, ploughing (0–15 cm) was done using hand hoes. In the B and BM treatments, biochar was applied only in September 2017, while FYM was applied three times in September 2017, August 2018, and August 2019 (every year before sorghum cultivation) in the M and BM treatments. Both biochar and FYM were incorporated into the surface layer (0–15 cm) using hand hoes. Biochar was produced from mesquite wood (*Prosopis juliflora*) and pyrolyzed with the heap method that local people traditionally use for making charcoal (Srinivasarao et al., 2013). *P. juliflora* has recently been utilized and/or eliminated in India to control its invasion because it is recognized as an invasive species that can cause reductions in water resources and farmlands (Wakie et al., 2016). The amount of FYM added was representative of the traditional amount applied in the experimental area, and FYM has been incorporated by local farmers every 1–3 years. Table 1 presents the chemical properties of biochar and FYM.

In all treatment plots, sorghum was planted according to rainfall in each season: in the first year, sorghum was planted in October 2017 and harvested in January 2018, while in the second and third years, sorghum was planted in August and harvested in December. Every year, sorghum was planted at the rate of 1.75 g m^{-2} (plant-to-plant distance was 30 cm). During each cultivation period, weeding was carried out with hand hoes every month after planting. After harvesting, aboveground biomass (leaf and stem) was removed outside the field, according to local farmers' traditional way for animal feed, while belowground biomass (root) was retained. To evaluate belowground C input, that is, sorghum roots, root biomass were collected from a soil volume of 30 cm (plant spacing) × 30 cm (plant spacing) × 15 cm (depth) for each plot by completely sampling the root system by hand at the end of each cultivation period (in January 2018, December 2018, and December 2019). The collected roots were washed and dried over 48 hr at 70°C, which then carbon contents were measured with a dry combustion method by an NC analyzer SUMIGRAPH NC TR-22 (Sumika Chemical Analysis Service).

In all treatment plots, during the non-cultivation period, that is, from after harvesting to the next cultivation period (February–July in 2018, and January–July in 2019), weeding was conducted by hand every 2–3 months to maintain bare land.

2.3 | Environmental monitoring

The soil volumetric moisture content (0–15 cm depth), soil temperature (5 cm depth), air temperature and rainfall were measured by a data logger (CR1000; Campbell Scientific, Inc.) with sensors as mentioned in Seki et al. (2019). For each plot, volumetric moisture content and soil temperature were recorded every 30 min with three replicates and duplicates, respectively. Air temperature and rainfall were also recorded every 30 min. The soil moisture sensors were calibrated in each treatment plot in each year by comparing measured field soil moisture (as mentioned below) and recorded soil moisture through sensors.

2.4 | Soil sampling and measurements

Soil was sampled, especially focusing on the crop growing season approximately every 2 weeks (30-times in total). For each sample, we collected five composite soil samples (0–15 cm) inside each plot (4 m × 7 m; c.a. 1 m away from the CO_2 chambers mentioned below and avoiding the plot edges) so as not to disturb plant roots. After

TABLE 1 Chemical properties of applied biochar and farmyard manure (FYM)

	pH (H_2O)	Total C (g kg^{-1})	Total N (g kg^{-1})	C:N ratio	DOC (mg kg^{-1})	DON (mg kg^{-1})
Biochar	8.0 ± 0.1a	515.5 ± 12.2a	10.6 ± 1.0a	48.4 ± 2.6a	116.2 ± 8.2b	8.2 ± 1.6b
FYM	7.8 ± 0.1a	119.9 ± 9.8b	12.4 ± 3.5a	9.6 ± 0.3b	1562.4 ± 157.4a	2416.1 ± 76.2a

Note: The pH (H_2O) was extracted by distilled water (1:20). Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were extracted by distilled water (1:10) (Luo et al., 2011). The values are average ± standard error ($n = 3$). Different small letters indicate significant differences between biochar and FYM ($p < 0.05$)

transporting to the laboratory in a 4°C cooler, soil samples were sieved (<4 mm) and stored at 4°C under field-moisture conditions until each measurement. SOC was measured at the beginning of the experiment (in September 2017) and at the end of the experiment (in December 2019). MBC was measured by the fumigation-extraction method (Vance et al., 1987), as mentioned in Sugihara et al. (2015).

To determine the soil bulk density, soil cores were also sampled at the start of cultivation and at the end of cultivation every year, that is, in September 2017, January 2018, August 2018, December 2018, August 2019, and December 2019, only in the C and B treatments. Five core samples were collected for each sample by inserting metal rings of 100 cm^3.

2.5 | Measurement of CO_2 efflux rate and microbial activity as qCO_2

The CO_2 efflux rate, that is, not plant-root respiration but microbial respiration (Shinjo et al., 2006), was measured with a closed-chamber method (detailed in Seki et al., 2019) at a frequency of approximately every 2 week in the rainy season and every month in the dry season (40 times in total). After FYM application each year, two polyvinyl chloride (PVC) columns (diameter 13 cm, height 30 cm) were inserted randomly in each plot, and the averaged data in each treatment with three replicates were used. Gases collected at 0 and 40 min were analyzed with an infrared CO_2 analyzer (ZFP9-AA11; Fuji Electric) equipped with a voltage capture detector (C-R8A; Shimadzu) and N_2 carrier gas (Shinjo et al., 2006).

To evaluate the microbial activity as qCO_2 (generally defined as a metabolic quotient) (Anderson & Domsch, 1985), the CO_2 efflux rate was divided by the MBC on an area basis (μg CO_2-C m^{-2} hr^{-1} and mg MBC m^{-2}, respectively).

2.6 | Data analysis

To evaluate the relationship between environmental factors and CO_2 efflux rate, MBC and qCO_2 in the C treatment, Pearson's correlation coefficient was applied. To evaluate the impact of treatment on soil moisture, CO_2 efflux rate, MBC and qCO_2 over the experimental period, repeated-measures analysis of variance (RM-ANOVA) was conducted, in which treatment and sampling time were treated as fixed effects and permitted to interact. When ANOVA indicated a significant difference for treatments, mean comparisons were performed with the Tukey–Kramer multiple comparison test. In addition, to test the interaction effect of biochar application and FYM application on CO_2 efflux rate, MBC and qCO_2 during each cultivation period, two-way RM-ANOVA was conducted. Surface SOC stock was calculated by multiplying soil C content by bulk density in each treatment plot. Tukey–Kramer test was done to determine the difference among treatments, in SOC stock in September 2017, SOC stock in December 2019, and SOC increment. The differences between SOC stock in September 2017 and December 2019 for each treatment were examined by Student's *t* test.

To estimate the annual CO_2 flux, we used an modified Arrhenius relationship between the measured CO_2 efflux rate and environmental factors such as soil moisture and temperature with multiple regression analysis as follows: Cem $= aM^b \exp(-E / RT)$ (see Sugihara et al., 2012). Because of the considerable annual variation in rainfall and disturbance by plowing and cultivation, we separated the period from the start of the cultivation and performed the above analysis for each year, that is, first-year (from September 2017 to July 2018; 11 months), second-year (from August 2018 to July 2019; 12 months), and third-year (from August 2019 to December 2019; 4 months). All of the statistical tests were conducted by SYSTAT 14.0 (SYSTAT Software).

3 | RESULTS

3.1 | Seasonal variations in environmental factors

Rainfall was mostly occurred in the rainy season, although rainfall was unusually high during April and May 2018 (Figure S1a). Cumulated rainfall during the first cultivation period (from September 2017 to January 2018) (218 mm) was less than half that of the second cultivation period (from August 2018 to December 2018) (531 mm) and the third cultivation period (from August 2019 to December 2019) (606 mm). During the periods when rainfall events were concentrated, soil moisture kept high (c.a. 0.25 m^3 m^{-3}). According to the RM-ANOVA (Table S2), soil moisture was weakly related to the treatment (16.6%). Average soil moisture in the B treatment (0.15 m^3 m^{-3}) was significantly higher than the C treatment (0.12 m^3 m^{-3}) throughout the experimental period, while FYM application did not affect the soil moisture (Figure S1a).

Over the experimental period, air temperature showed a fluctuation from 19.2 to 29.9°C, and average air temperature was 24.7°C (Figure S1b). Average soil temperature was 33.9, 33.5, 33.4, and 33.8°C in the C, B, M, and BM treatments, respectively, and there were no significant differences among treatments.

3.2 | Seasonal variation in CO_2 efflux rate

The average CO_2 efflux rate of each cultivation period was 13.8, 16.4, 21.5, and 16.6 mg CO_2-C m^{-2} hr^{-1} (first-year), 15.8, 19.4, 27.3, and 23.0 mg CO_2-C m^{-2} hr^{-1} (second-year), and 20.1, 22.8, 35.9, and 34.8 mg CO_2-C m^{-2} hr^{-1} (third-year) in the C, B, M, and BM treatments, respectively (Figure 1). The CO_2 efflux rates were significantly impacted by treatments, time, and their interactions (Table S2). For all treatments, the average CO_2 efflux rate in the cultivation period of the first year tended to be smaller than the second and third years. Averaged CO_2 efflux rate during the non-cultivation period was 9.8, 12.5, 12.5, and 11.7 mg CO_2-C m^{-2} hr^{-1} (first-year), and 9.9, 10.5, 13.5, and 12.7 mg CO_2-C m^{-2} hr^{-1} (second-year), in the C, B, M, and BM treatments, respectively (Figure 1). The CO_2 efflux rates in all treatments were mostly high during the rainy season and low during

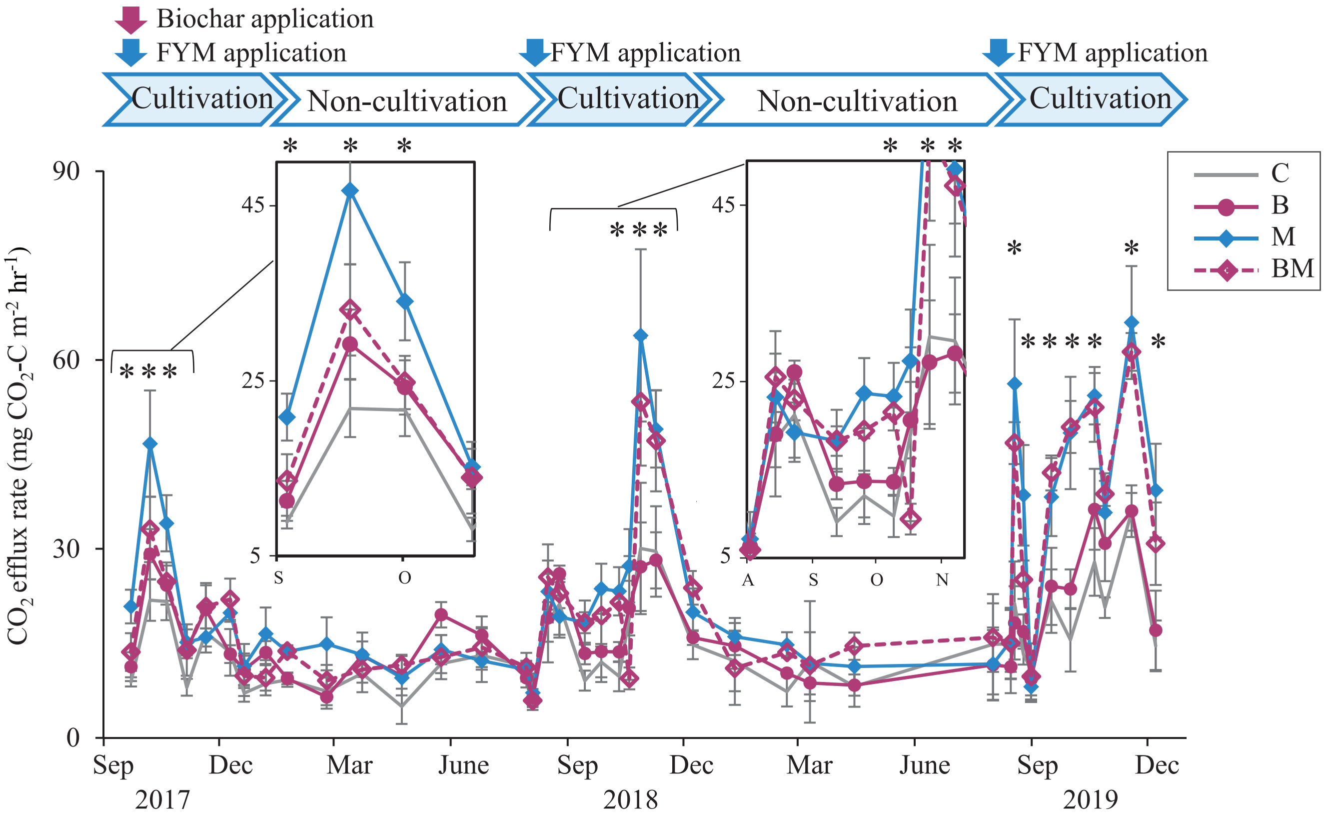

FIGURE 1 Seasonal variations in CO_2 efflux rate. C, control plot; B, biochar application plot, M; farmyard manure (FYM) plot, BM, biochar and FYM application plot. Bars present standard error. Stars represent significant differences among the treatments on each sampling date based on the Tukey–Kramer multiple comparison test (p < 0.05) [Color figure can be viewed at wileyonlinelibrary.com]

the dry season. The CO_2 efflux rate showed the positive correlation with soil moisture in the C treatment throughout the experimental period (Figure S2a).

During all cultivation periods, there were no significant differences in CO_2 efflux rate between the C and B treatments (Figure 1); however, the CO_2 efflux rate in the B treatment showed higher tendency than the C treatment. During all cultivation periods, the CO_2 efflux rate in the M treatment was significantly higher than the C treatment, while the CO_2 efflux rate in the BM treatment was significantly higher than the B treatment only at the cultivation period of the third year. There were no significant differences in CO_2 efflux rate between the M and BM treatments, except for the cultivation period of the first year. During this period only, the CO_2 efflux rate in the BM treatment was significantly lower than the M treatment (Figure 1, magnified part). Only at the cultivation period of the first year, the significant interaction effect of biochar application and FYM application on CO_2 efflux rate was shown (Table S3).

3.3 | Microbial biomass and qCO_2 responses influenced by land management

According to the RM-ANOVA (Table S2), MBC was explained well by treatment (83.9%). The average MBC of each cultivation period was 84.5, 94.8, 103.2, and 103.0 mg C kg^{-1} (first-year), 87.3, 93.0, 117.1, and 113.3 mg C kg^{-1} (second-year), and 79.4, 87.3, 115.5, and 119.1 mg C kg^{-1} (third-year) in the C, B, M, and BM treatments, respectively (Figure 2a–c). In all cultivation periods, there were no significant differences in MBC between the C and B treatments, while MBC in the M and BM treatments were significantly higher than the C and B treatments in most cultivation periods.

In the first year, qCO_2 tended to be high during the first half of the cultivation period, whereas it was high during the latter half of the cultivation period in the second year (Figure 2d,e). In the third year, qCO_2 fluctuated over the whole cultivation period (Figure 2f). As for MBC, during all cultivation periods, there were no significant differences in qCO_2 between the C and B treatments, while qCO_2 in the M and BM treatments were significantly higher than the C and B treatments in most cultivation periods. Only during the cultivation period of the first year, qCO_2 in the BM treatment was significantly lower than that in the M treatment. During the period when there was a significant difference in qCO_2 between the M and BM treatments (Figure 2d), qCO_2 in the BM treatment (9.3–19.1 μg CO_2-C mg MBC^{-1} hr^{-1}) was 30% lower than the M treatment (15.1–29.4 μg CO_2-C mg MBC^{-1} hr^{-1}), while qCO_2 in the M and BM treatments showed a similar fluctuation during the second and third cultivation periods.

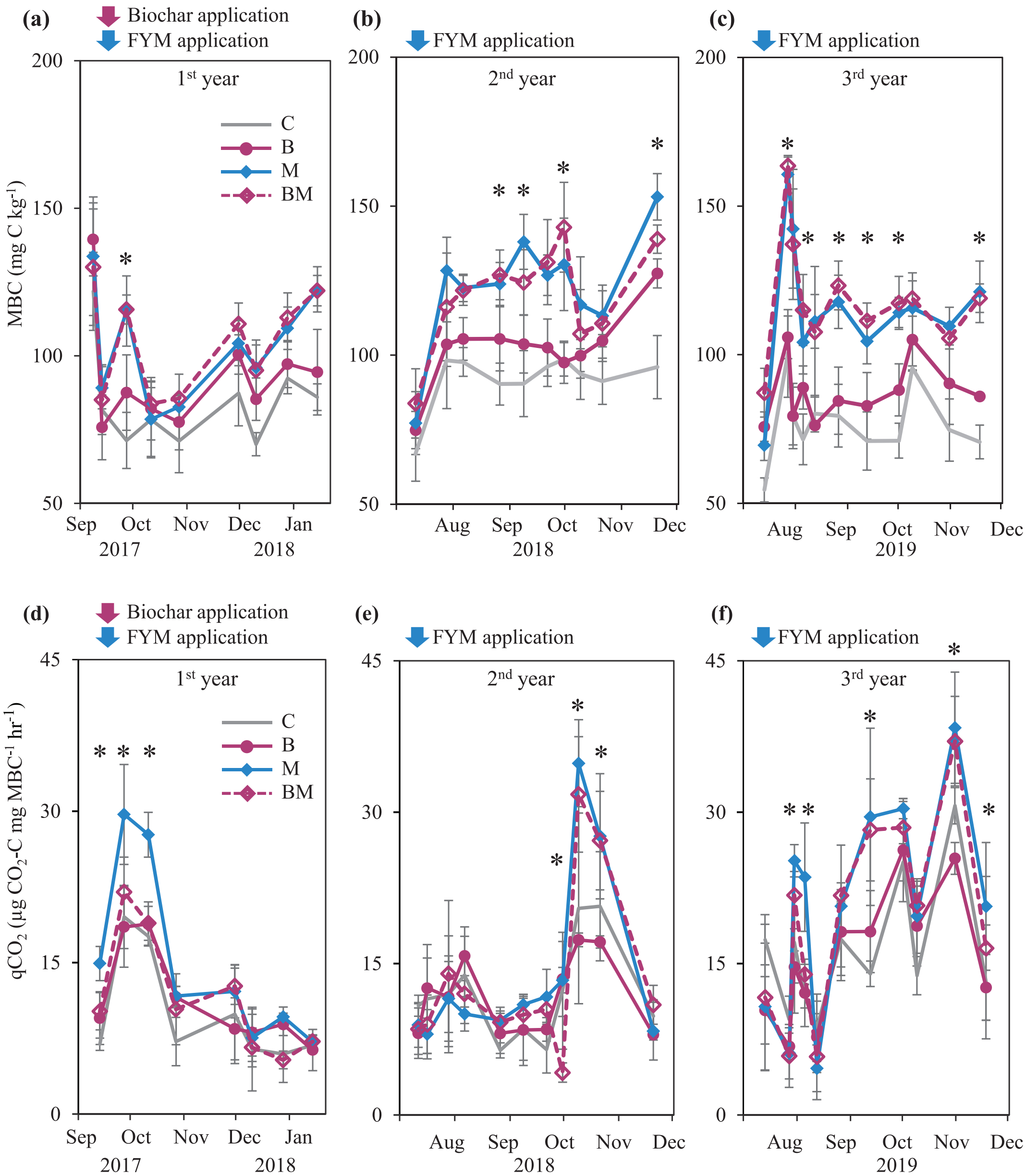

FIGURE 2 Seasonal variations in soil microbial biomass carbon (MBC) (a–c) and metabolic quotient (qCO_2) (d–f) in each cultivation period (a and d, 1st year; b and e, 2nd year; c and f, 3rd year). C, control plot; B, biochar application plot, M; farmyard manure (FYM) plot, BM, biochar and FYM application plot. Bars present standard error. Stars represent significant differences among the treatments on each sampling date based on the Tukey–Kramer multiple comparison test (p < 0.05) [Color figure can be viewed at wileyonlinelibrary.com]

As with the CO_2 efflux rate, a significant interaction effect between biochar application and FYM application on qCO_2 was shown during the first cultivation period (Table S3). The MBC in the C treatment was independent of soil moisture (data not shown), while qCO_2 was significantly correlated with soil moisture (Figure S2b).

3.4 | Estimation of annual CO_2 flux and C budget

Estimated annual CO_2 flux in the first year tended to be smaller than that in the second year, in all treatments (Table 2). Cumulative CO_2 flux as C output for the whole experimental period (27 months) was 2.4, 2.7, 4.0, and 3.7 Mg C ha^{-1} in the C, B, M, and BM treatments, respectively (Figure 3; Table S4). Cumulative CO_2 flux in the M treatment was 1.6 Mg C ha^{-1} larger than the C treatment, while cumulative CO_2 flux in the B treatment was 0.3 Mg C ha^{-1} larger than the C treatment. In addition, cumulative CO_2 flux in the BM treatment was 0.3 Mg C ha^{-1} lower than that in the M treatment. There were no clear differences in root biomass and aboveground biomass among treatments in all years, while aboveground biomass in the M and BM treatments tended to be larger than that in the C treatment by 44%–65%, depending on each cultivation year (data not shown).

TABLE 2 Estimated annual CO_2 flux in each land management

Treatment	Year	CO_2 flux ($Mg\ C\ ha^{-1}$)	R^a	n^b
C	Sep 2017—Jul 2018	0.75	0.47§	14
	Aug 2018—Jul 2019	1.08	0.65*	14
	Aug 2019—Dec 2019	0.61	0.53§	11
B	Sep 2017—Jul 2018	0.91	0.60*	14
	Aug 2018—Jul 2019	1.14	0.74**	14
	Aug 2019—Dec 2019	0.69	0.73*	11
M	Sep 2017—Jul 2018	1.16	0.71**	14
	Aug 2018—Jul 2019	1.58	0.70**	14
	Aug 2019—Dec 2019	1.29	0.83**	11
BM	Sep 2017—Jul 2018	1.00	0.59*	14
	Aug 2018—Jul 2019	1.62	0.75*	13
	Aug 2019—Dec 2019	1.12	0.91**	11

Note: To estimate annual CO_2 flux, we applied the modified Arrhenius equation by the stepwise regression analysis between the CO_2 efflux rate and environmental factors for each year, as mentioned in Section 2. In almost all treatment plots, CO_2 efflux rate was correlated with soil moisture (data not shown). $^*p < 0.05$, $^{**}p < 0.01$, $^{§}p < 0.10$

Abbreviations: C, control plot; B, biochar application plot, M; farmyard manure (FYM) plot, BM, biochar and FYM application plot.

[a] *R* means the correlation coefficient by the regression analysis for each treatment in each year.

[b] *n* means the number of measurements in CO_2 efflux rate in each treatment in each year.

Based on the calculation of soil C budgets over the experiment (27 months), that is, C input as the summary of biochar and/or FYM application and root biomass—C output as cumulative CO_2 flux, C budget was negative in the C treatment (−1.4 $Mg\ C\ ha^{-1}$), while C budgets were positive in the B (6.7 $Mg\ C\ ha^{-1}$), M (0.6 $Mg\ C\ ha^{-1}$), and BM (9.1 $Mg\ C\ ha^{-1}$) treatments. Additionally, surface SOC stock in all treatment plots except for the C treatment significantly increased from September 2017 to December 2019 (Figure 3; Table S4). In the C treatment, SOC stock decreased from 7.9 $Mg\ C\ ha^{-1}$ (in September 2017) to 7.0 $Mg\ C\ ha^{-1}$ (in December 2019), although it was not significantly different. In the B and BM treatments, SOC stock increased significantly by 6.0–8.9 $Mg\ C\ ha^{-1}$, while SOC stock in the M treatment increased significantly by 2.0 $Mg\ C\ ha^{-1}$. These variations in SOC stock led to SOC increments in the B and BM treatments that were significantly larger than the C treatment. Additionally, BM treatment caused the largest SOC increment in this experiment.

4 | DISCUSSION

4.1 | CO_2 flux and its controlling factors in degraded cropland soil of southern India

The averaged CO_2 efflux rate in the C treatment was 15.2 mg CO_2-C $m^{-2}\ hr^{-1}$, which was in line with our previous study conducted in the same field (20.5 mg CO_2-C $m^{-2}\ hr^{-1}$; Seki et al., 2019). These values were small compared to other studies in similar tropical ecosystems, such as 46.0 mg CO_2-C $m^{-2}\ hr^{-1}$ in cropland of Tanzania with 13.8 g C kg − 1 of soil (Sugihara et al., 2012), and 23.6–266.1 mg CO_2-C $m^{-2}\ hr^{-1}$ in pasture of Kenya with 22.6 g C kg^{-1} of soil (Zhu et al., 2021). The low CO_2 efflux rate in this study might be because of the low C content of the degraded cropland soil in our study site (SOC; 3.2 g kg^{-1}), compared with those in the above studies that varied from 13.8 to 22.6 g C kg^{-1} of soil.

In agreement with previous studies in dry tropical areas (Kim et al., 2015), there are positive relationship between the CO_2 efflux rate and soil moisture. Therefore, the low annual CO_2 flux in the cultivation period of the first year was likely because of the low rainfall during this cultivation period of the first year.

4.2 | Impact of land management on CO_2 flux, C budget, and associated microbial responses

Biochar application did not affect CO_2 flux and microbial dynamics, although it increased the soil moisture throughout the experimental period. Increased soil moisture with biochar application indicates that biochar application improved the soil WHC because of its high porosity (Jeffery et al., 2011), which is in agreement with other studies with similar soil texture (Liu et al., 2016) and/or similar biochar application amount (Karhu et al., 2011). Previous research showed higher SOC or biochar decomposition with biochar application, caused by (1) improved soil WHC (Jeffery et al., 2011); (2) degradation of the easily decomposable fraction in biochar (Keith et al., 2011); and (3) increased MBC (Lehmann et al., 2011). In our study, like the CO_2 flux, MBC did not increase with biochar application. This is possibly because (1) soil microbes could not promptly respond to high soil moisture due to the limited amount of decomposable substrate in SOC poor soil of southern India (Sugihara et al., 2014), or (2) the soil moisture increments were not sufficient to stimulate the microbial growth and/or activity. The biochar application significantly increased

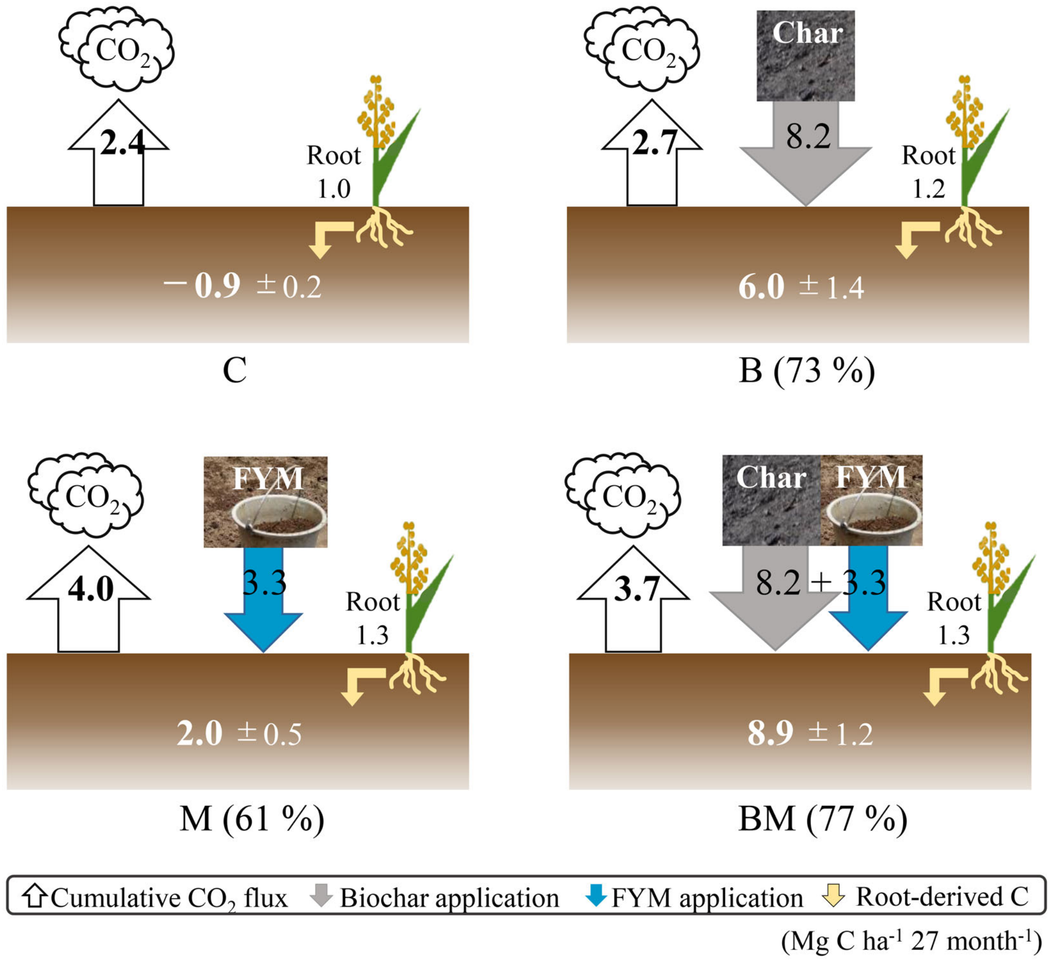

FIGURE 3 Summary of soil C budget (0–15 cm depth) in each treatment over the experiment. C, control plot; B, biochar application plot, M; farmyard manure (FYM) plot, BM, biochar and FYM application plot. Detailed calculations are shown in Table S4. Values inside each soil indicate the SOC increment ± standard error (S.E.). Values (%) next to each treatment name indicate the percentage of C-input retention in soil (SOC increment per C input as biochar and/or FYM; Kan et al., 2020) [Color figure can be viewed at wileyonlinelibrary.com]

surface SOC stock (6.0 Mg C ha^{-1}), creating a positive C budget (6.7 Mg C ha^{-1}) (Figure 3; Table S4), in line with other studies which mentioned C sequestration by biochar addition (El-Naggar et al., 2018). These results show that biochar application would be a sustainable and effective option to prevent or recover the soil degradation by increasing SOC stock in this area.

FYM application increased the CO_2 efflux rate, resulting in 1.6 Mg C ha^{-1} 27 $month^{-1}$ larger CO_2 flux in the M treatment than in the C treatment. Many studies have reported that manure application clearly increased soil respiration because of easily decomposable C addition (Lai et al., 2017). Larger CO_2 flux with FYM application was associated with increased microbial responses, that is, both increased MBC and qCO_2, in all cultivation periods (Lian et al., 2016). Additionally, FYM application significantly increased the surface SOC stock with a positive C budget (Figure 3; Table S4). These results suggest that 1.1 Mg C ha^{-1} FYM application every year would maintain and improve the SOC storage in this area. This is in agreement with our previous study (Seki et al., 2019) and other studies that estimated the necessary amount of C addition for sustaining SOC levels based on the fluctuations of soil C stock in India (Datta et al., 2018; Kundu et al., 2001).

In contrast to our hypothesis, biochar and FYM combined application did not stimulate MBC and qCO_2, resulting in no clear difference in CO_2 flux between the M and BM treatments during most of the experimental period. Only for the first few months after both products' applications were the CO_2 efflux rate lower in the BM treatment than the M treatment, resulting in 0.3 Mg C ha^{-1} smaller cumulative CO_2 flux in the BM treatment over the 27 months. Zavalloni et al. (2011) also found an inhibitory effect of biochar and plant residue application on residue decomposition. The difference in this period might have been caused by ca. 30% lower qCO_2 in the BM treatment than in the M treatment, although MBC did not change. Lehmann et al. (2011) speculated that the possible mechanism of low OM decomposition observed with biochar addition was because of changes in the enzyme activity and/or microbial community composition, while the physical protection provided by biochar could also be involved (Hernandez-Soriano et al., 2016; Zimmerman et al., 2011). Considering our calculation of the possible amount of absorbed DOC derived from applied FYM to biochar, in another equilibration experiment, ca. 1500 mg C kg^{-1} FYM could be absorbed on biochar, which was equivalent to only ca. 20 kg C ha^{-1} in this study (data not shown). This implies that sorption of FYM-derived DOC to biochar can only account for a limited part of the difference between the M and BM treatments in this study (Mukherjee & Zimmerman, 2013). Therefore, another factor could also induce the inhibitory effect of biochar and FYM application on microbial activity. Further studies are necessary to elucidate the mechanism regarding the effect of the combined application on decreased microbial activity just after combined application, for example, by evaluation of microbial community structure and diversity, to develop appropriate C management in this area.

Finally, biochar and FYM combined application increased SOC stock after 27 months, resulting in the largest SOC increment in the BM treatment (8.9 Mg C ha^{-1}) with positive C budget (9.1 Mg C ha^{-1}) (Table S4). The rate of C-input retention in soil (SOC increment per C input as biochar and/or FYM; Kan et al., 2020) in the BM treatment (ca. 0.77) was relatively higher than that in the B (ca. 0.73) and M (ca. 0.61) treatments, suggesting that the biochar and FYM combined application would be more effective to sequester C than an individual application of either amendment to soils (Jien et al., 2015). Hence, biochar application combined with FYM would be an effective way to achieve appropriate SOC management for preventing land degradation both in terms of C output and C sequestration, in the tropical cropland soils of southern India. Since there was no clear improvement of crop productivity in the B, M, and BM treatments (data not shown), due to inherently low soil fertility (Lu, 2020), further studies would be necessary to assess the sustainable land management in the long-term, to improve both SOC stock and subsequent crop productivity in this area.

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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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