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 currently considered to be an effective soil organic carbon (SOC) management to prevent land degradation by enhancing SOC stock. However, quantitative information on the impact of biochar application on carbon dioxide (CO2) flux and associated microbial responses is still scarce, especially in degraded tropical agroecosystems. Here, we 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; 8.2 Mg biochar-C ha-1 and 1.1 Mg FYM-C ha-1 yr-1)) on CO2 flux, SOC stock, microbial biomass C (MBC), and metabolic quotient (qCO2) in degraded tropical alkaline cropland of southern India, based on a 27-month field experiment. Cumulative CO2 flux over the experiment was 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 and SOC stock, though did not affect CO2 flux, MBC, and qCO2, indicating the limited response of microbes to increased soil moisture because of small amount of SOC. Combined application of biochar and FYM did not increase CO2 flux compared with FYM alone, due to little difference of microbial responses between the M and BM treatments. Additionally, SOC increment (8.9 Mg C ha-1) and the rate of C-input retention in soil (0.78) was most significant in the BM treatment. Hence, the combined application of biochar and FYM could be sustainable land management by efficient increase of SOC stock in the tropical degraded cropland.

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ABSTRACT:

Biochar application is currently considered to be an effective soil organic carbon (SOC) management to prevent land degradation by enhancing SOC stock. However, quantitative information on the impact of biochar application on carbon dioxide (CO_2) flux and associated microbial responses is still scarce, especially in degraded tropical agroecosystems. Here, we 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; 8.2 Mg biochar-C ha^{-1} and 1.1 Mg FYM-C ha^{-1}yr^{-1})) on CO_2 flux, SOC stock, microbial biomass C (MBC), and metabolic quotient (qCO_2) in degraded tropical alkaline cropland of southern India, based on a 27-month field experiment. Cumulative CO_2 flux over the experiment was 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 and SOC stock, though did not affect CO_2flux, MBC, and qCO_2, indicating the limited response of microbes to increased soil moisture because of small amount of SOC. Combined application of biochar and FYM did not increase CO_2 flux compared with FYM alone, due to little difference of microbial responses between the M and BM treatments. Additionally, SOC increment (8.9 Mg C ha^{-1}) and the rate of C-input retention in soil (0.78) was most significant in the BM treatment. Hence, the combined application of biochar and FYM could be sustainable land management by efficient increase of SOC stock in the tropical degraded cropland.

Keywords :

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

Main Text:

INTRODUCTION

Proper soil organic carbon (SOC) management is essential to prevent land degradation and mitigate climate change in the world (Lal, 2004; Minasny et al., 2017). Accurate evaluation of carbon dioxide (CO_2) flux is vital to develop effective SOC management strategies (Lehmann & Kleber, 2015). Changes in annual CO_2 flux could substantially alter the pool size of SOC (Moinet et al., 2016). Soils in dry tropical areas retain low SOC, and soil fertility is correspondingly low (Powlson et al., 2016) because of the small amount of fresh litter return to the soil 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 in degraded soils of tropical agroecosystems.

Biochar, made by biomass pyrolysis with low/no oxygen, has become globally popular to increase soil C stocks because of its high resistance to microbial decomposition (Lehmann et al., 2011; Al-Wabel et al., 2018). Recent research found that biochar application increased soil C decomposition by increasing soil water holding capacity (Jeffery et al., 2011) and/or soil microbial biomass C (MBC) (Thies & Rillig, 2012), 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, i.e., environmental factors containing soil moisture and temperature (Kim et al., 2015) and microbial factors such as MBC and metabolic quotient (Schmidt et al., 2011). Many studies have been conducted on the impact of biochar addition on soil respiration (Senbayram et al., 2019), soil 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. While these studies are important, they do not integrate all the biotic and abiotic factors impacting *in situ* CO_2 fluxes, such as moisture and temperature fluctuations. Zhou et al. (2017) reviewed the literature from 2001 to 2015 focusing on soil respiration and/or MBC with biochar addition to croplands, and they found that 26 studies investigated both soil respiration and MBC, nine of which were conducted in the field. Moreover, most studies of biochar addition were conducted in acidic soils because biochar addition can ameliorate soil acidity (Hernandez-Soriano et al., 2016). Therefore, there is limited research on the impact 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).

Tropical alkaline soils in India are mostly degraded and characterized by low soil C stock due to the long-term use of excessive 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). Traditionally, most Indian farmers make farmyard manure (FYM) from livestock excreta and soil, which is applied to the soil to maintain soil C level and soil fertility (Srinivasarao et al., 2014). However, a decline in the availability of FYM because of its utility for other domestic purposes such as fuel, and replacement of manure with chemical fertilizers, have reduced SOC stocks over decades (Indoria et al., 2018). Therefore, alternative C management strategies such as biochar could enhance soil C stocks. 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, i.e., substrate sorption to the biochar surface and pores, in an 84-day incubation experiment using Cambisols. These contradictory results make it difficult to evaluate whether the combined application of biochar and FYM 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 qCO_2), and C budget in tropical alkaline degraded cropland soils 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 Experimental site

A field experiment was conducted from September 2017 to December 2019 (27 months total) in a farmer's field in Madurai, Tamil Nadu state, India (9deg43'22.37" N 77deg46'51.61" E; 175 m asl) (Seki et al., 2019). The mean annual air temperature was 24.7 degC and the annual rainfall was 820 mm (692–857 mm; 2017–2019). This area has 40–75% of annual rainfall during the rainy season (South-West monsoon: June–September, and North-East monsoon: 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 was classified into Typic Haplustepts (Soil Survey Staff, 2014). The value for the selective physicochemical properties of the surface layer (0–15 cm depth) in this site were: soil pH (1:5 water) of 8.5, SOC of 3.2 g kg^{-1}, inorganic carbon (IC) of 0.1 g kg^{-1}, clay content of 27.2%, cation exchange capacity of 25.1 cmol$_c$ kg^{-1}, and soil bulk density of 1.57 g cm^{-3}. Surface SOC stock (0–15 cm depth) was 8.3 Mg C ha^{-1}. TC was measured by a dry combustion method with a NC analyzer SUMIGRAPH NC TR-22 (Sumika Chemical Analysis Service, Japan). IC was measured following the method provided by Bundy and Bremner (1972). Briefly, the soil sample was treated with 1M HCl at room temperature for 24 h, and then unreacted HCl that was not released as CO_2 from carbonates was determined by titration with 1M NaOH to calculate the IC content. SOC was calculated as follows: SOC = total carbon (TC) – IC.

2.2 Experimental set-up

The experiment included the following four treatments with three replicates:

(1) Control plot (nothing applied to the soil); 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',

Each experimental plot (8 m x 5 m) was arranged in a randomized block design with a 1 m buffer zone.

Table S1 indicates the summary of three years of crop cultivation and land management. Sorghum (*Sorghum bicolor* (L.) Moench) was cultivated three times during the experimental period. Every year before cultivation, plowing (0–15 cm) was done using hand hoes. In the B and BM treatments, biochar was applied only in Sep 2017, while FYM was applied three times in Sep 2017, Aug 2018, and Aug 2019 (every year before sorghum cultivation) in the M and BM treatments. Both biochar and FYM were incorporated into the soil (0–15 cm depth) using hand hoes. Biochar applied in this experiment 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). *Prosopis 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. The application amount of biochar and FYM C was determined by measuring the dry weight, as well as the C content of biochar and FYM by a dry combustion method as mentioned above. Table 1 shows 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 Oct 2017 and harvested in Jan 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, i.e., sorghum roots, root biomass were collected from a soil volume of 30 cm (plant spacing) x 30 cm (plant spacing) x 15 cm (depth) for each plot by completely digging out the root system manually at the end of each cultivation period (in Jan 2018, Dec 2018, and Dec 2019). The root samples were washed and dried for more than two days at 70 degC, and the C content and its weight were measured as mentioned above.

During the non-cultivation period, i.e., from after harvesting to the next cultivation period (Feb–Jul in 2018, and Jan–Jul in 2019), weeding was conducted by hand every 2–3 months to maintain bare land in all treatment plots.

2.3 Environmental monitoring

The soil volumetric moisture content, air temperature, soil temperature and rainfall were measured by a data logger system (CR1000 data logger; Campbell Scientific, Inc., USA). The volumetric moisture content in the surface layer (0–15 cm depth) was recorded every 30 min in three replicates for each plot using time-domain reflectometer (TDR) probes (CS616; Campbell Scientific, Inc., USA). The moisture probes were installed near the polyvinyl chloride (PVC) columns (see below). Air temperature was recorded every 30 min, and soil temperature (5 cm depth) was recorded every 30 min in duplicate for each plot, using thermistor probes (Model 108; Campbell Scientific, Inc., USA). Rainfall was also recorded every 30 min using a TE525MM device (Campbell Scientific, Inc., USA). All sensors were connected to a data logger system (CR1000 data logger; Campbell Scientific, Inc., USA). 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 samples were collected 30 times throughout the experimental period, especially focusing on the crop growing season approximately every 2 weeks. For each sample, five composite soil samples (0–15 cm depth) were taken inside the plot (7 m x 4 m; avoiding the plot edge, and c.a. 1 m away from the CO_2 chambers mentioned below), and between plants (plant-to-plant distance was 30 cm) so as not to disturb plant roots. After transporting to the laboratory in a 4 degC cooler, soil samples were passed through a 4-mm sieve after removing stones and plant roots and stored at 4degC under field-moisture conditions until each measurement. SOC was measured at the start of the experiment (in Sep 2017) and at the end of the experiment (in Dec 2019). Soil moisture content was determined by the difference in soil weight before and 48 h after 105 degC drying. MBC was measured using the fumigation-extraction method (Vance et al., 1987), following the detailed described in Sugihara, et al., 2015. All data were expressed on a dry weight basis.

To determine the soil bulk density, soil cores were also collected at the start of cultivation and at the end of cultivation every year, i.e., in Sep 2017, Jan 2018, Aug 2018, Dec 2018, Aug 2019, and Dec 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 was measured by a closed-chamber system (Seki et al., 2019) at a frequency of approximately every 2 week in the rainy season and every month in the dry season for a total of 40 times throughout the experimental period. Polyvinyl chloride (PVC) columns (diameter 13 cm, height 30 cm) were inserted randomly in each plot at the end of each September or August, i.e., after FYM application. We waited at least 1 week after the installation until measuring the CO_2 efflux rate, so as not to disturb the plots when installing columns. Columns were re-installed within a plot every year, as mentioned above. Since soil respiration is composed of microbial respiration and plant-root respiration, plant-root respiration was excluded by the trenching method (Shinjo et al., 2006), following the detailed in Seki et al (2019). Gases were sampled at 0 min and again 40 min after the top of the column was covered with a plastic sheet, and analyzed with an infrared CO_2 analyzer (ZFP9-AA11; Fuji Electric, Japan) equipped with a voltage capture detector (C-R8A; Shimadzu, Japan) and N_2 carrier gas (Shinjo et al., 2006). The CO_2 efflux rate was calculated based on the increase in CO_2 concentration in the column after 40 min. Two columns were installed in each plot, and we used the average data in each plot with three replicates (plots). The CO_2efflux rate was always measured between 08:00 and 11:00 am in the field.

To evaluate the microbial activity as qCO_2 (generally termed as a metabolic quotient) (Anderson & Domsch, 1985), we divided the measured CO_2 efflux rate by the MBC. In the calculation, both CO_2 efflux rate and MBC were expressed on an area basis (µg CO_2-C $m^{-2}h^{-1}$ and mg MBC m^{-2}, respectively).

2.6 Data analyses

All statistical analyses were conducted using SYSTAT 14.0 (SYSTAT Software, Richmond, CA, USA). Pearson's correlation coefficient was applied to evaluate the relationship between environmental factors and CO_2 efflux rate, MBC and qCO_2 in the C treatment. To evaluate the effect of treatment on soil moisture, CO_2 efflux rate, MBC and qCO_2 over the experimental period and also during each cultivation 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 assess the interaction effect of biochar application and FYM application during each cultivation period on CO_2 efflux rate, MBC and qCO_2, two-way RM-ANOVA was conducted. Surface SOC stock was calculated by multiplying soil C content by soil bulk density in each treatment plot. Tukey-Kramer test was used to determine the differences between treatments, in SOC stock in Sep 2017, SOC stock in Dec 2019 and SOC increment. Student's t-test was used to determine the differences between SOC stock in 2017 and Dec 2019 for each treatment. In all cases, $P < 0.05$ was considered significant.

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 soil temperature by multiple regression analysis, as shown in Sugihara et al. (2012), as follows:

$$\text{Cem} = aM^b \exp(-E / RT)$$

where Cem is the hourly CO_2 efflux rate (mol C ha^{-1} hr^{-1}), M is the volumetric soil moisture content (m^3m^{-3}; $0.12 < M < 0.27$), E is the activation energy (J mol^{-1}), R is the gas constant (8.31 J mol^{-1} K^{-1}), T is the absolute soil temperature (K), b is a coefficient related to the contribution of soil moisture, and a is a constant. 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, i.e., first-year (from Sep 2017 to Jul 2018; 11 months), second-year (from Aug 2018 to Jul 2019; 12 months), and third-year (from Aug 2019 to Dec 2019; 4 months).

3. RESULTS

3.1 Seasonal variations in environmental factors

Rainfall was generally occurred in the rainy season (i.e., June–December), although rainfall was unusually high during April and May 2018 (Fig. S1a). Cumulated rainfall during the first cultivation period (from Sep 2017 to Jan 2018) (218 mm) was less than half that of the second cultivation period (from Aug 2018 to Dec 2018) (531 mm) and the third cultivation period (from Aug 2019 to Dec 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 2), 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 that in the C treatment (0.12 m^3m^{-3}) throughout the experimental period, while FYM application did not affect the soil moisture (Fig. S1a).

Air temperature showed a fluctuation from 19.2 °C to 29.9 °C, and average air temperature was 24.7 °C over the experimental period (Fig. S1b). Seasonal variations in soil temperature followed that of air temperature throughout the experimental period (Fig. S1b). Average soil temperature was 33.9 °C, 33.5 °C, 33.4 °C 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} h^{-1} (first-year), 15.8, 19.4, 27.3 and 23.0 mg CO_2-C $m^{-2}h^{-1}$ (second-year), and 20.1, 22.8, 35.9, and 34.8 mg CO_2-C m^{-2} h^{-1}(third-year) in the C, B, M and BM treatments, respectively (Fig. 1 and Table 3). The CO_2 efflux rates were significantly impacted by treatments, time, and their interactions (Table 2). For all treatments, the average CO_2 efflux rate in the cultivation period of the first year tended to be smaller than that of the second and third years. During the non-cultivation period, the average CO_2 efflux rate was 9.8, 12.5, 12.5, and 11.7 mg CO_2-C m^{-2} h^{-1}(first-year), and 9.9, 10.5, 13.5, and 12.7 mg CO_2-C m^{-2} h^{-1} (second-year), in the C, B, M and BM treatments, respectively (Fig. 1). The CO_2efflux rates in all treatments were generally high during the rainy season and low during the dry season. The CO_2 efflux rate in the C treatment was significantly and positively correlated with soil moisture throughout the experimental period (Fig. S2a).

During all cultivation periods, there were no significant differences in CO_2 efflux rate between the C and B treatments (Table 3); however, the CO_2 efflux rate in the B treatment tended to be higher than that in the C treatment. During all cultivation periods, the CO_2 efflux rate in the M treatment was significantly higher than that in the C treatment, while the CO_2 efflux rate in the BM treatment was significantly higher than that in 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 that in the M treatment (Fig. 1 magnified part). There was a significant interaction effect of biochar application and FYM application on CO_2 efflux rate only at the cultivation period of the first year (Table S2).

3.3 Microbial biomass and qCO_2 responses influenced by land management

According to the RM-ANOVA (Table 2), 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 (Fig. 2a-c and Table 3). 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 that in 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 (Fig. 2d-e). In the third year, qCO_2 fluctuated over the whole cultivation period (Fig. 2f). As for MBC, during all cultivation periods, there were no significant differences in qCO_2between the C and B treatments, while qCO_2 in the M and BM treatments were significantly higher than that in the C and B treatments in most cultivation periods (Table 3). 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 (Fig. 2d), qCO_2 in the BM treatment (9.3–19.1 µg CO_2-C mg MBC^{-1} h^{-1}) was 30% lower than that in the M treatment (15.1–29.4 µg CO_2-C mg MBC^{-1} h^{-1}), while qCO_2 in the M and BM treatments showed a similar fluctuation during the second and third cultivation periods. As with the CO_2 efflux rate, a significant interaction effect between biochar application and FYM application on $qCO_2$was shown during the cultivation period of the first year (Table S2). In the C treatment, the MBC was independent of soil moisture (data not shown), while qCO_2 was significantly correlated with soil moisture (Fig. S2b).

3.4 Estimation of annual CO_2 flux and C budget

In all treatments, the estimated annual CO_2 flux in the first year tended to be lower than that in the second year (Table 4). 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 (Fig. 3 and Table S3). Cumulative CO_2 flux in the M treatment was 1.6 Mg C ha^{-1} larger than that in the C treatment, while cumulative CO_2 flux in the B treatment was 0.3 Mg C ha^{-1} larger than that in 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.

Surface SOC stock in all treatment plots except for the C treatment significantly increased from Sep 2017 to Dec 2019 (Fig. 3 and Table S3). In the C treatment, SOC stock decreased from 7.9 Mg C ha^{-1} (in Sep 2017) to 7.0 Mg C ha^{-1}(in Dec 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} (0–15 cm), while SOC stock in the M treatment increased significantly by 2.0 Mg C ha^{-1} (0–15 cm). These variations in SOC stock led to SOC increments in the B and BM treatments that were significantly higher than those in 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 soils of southern India

The average CO_2 efflux rate in the C treatment was 15.2 mg CO_2-C m^{-2} h^{-1}, and this value was in line with our previous study conducted in the same field (20.5 mg CO_2-C m^{-2}h^{-1}; Seki et al., 2019). These values were relatively small when compared with those in the other studies in similar tropical ecosystems, such as 46.0 mg CO_2-C m^{-2}h^{-1} in cropland of Tanzania with 13.8 g C kg-^{1} of soil (Sugihara et al., 2012), and 63.1 mg CO_2-C m^{-2} h^{-1} in bare land of Brazil with 12.2 g C kg^{-1} of soil (La Scala et al., 2000). The low CO_2 efflux rate in this study might be explained by 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 12.2 to 13.8 g C kg-^{1} of soil.

In agreement with previous studies in dry tropical areas (Kim et al., 2015), the CO_2 efflux rate was positively correlated with soil moisture. Therefore, the low annual CO_2 flux in the first cultivation period 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 water holding capacity because of its high porosity (Jeffery et al., 2011), and this is consistent 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 water holding capacity (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 increased soil moisture because of the small amount of decomposable substrate in SOC poor soil of southern India (Sugihara et al., 2014), or (2) the increase in soil moisture was not enough to stimulate the microbial growth and/or activity. The biochar application significantly increased surface SOC stock, creating a positive C budget (Fig. 3 and Table S3), in agreement with many other studies which have reported C sequestration by biochar addition (Agegnehu et al., 2015; 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 significantly increased the CO_2 efflux rate (Table 3), 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_2flux with FYM application was associated with increased microbial responses, i.e., both increased MBC and qCO_2, in all cultivation periods (Table 3) (Lian et al., 2016). Additionally, FYM application significantly increased the surface SOC stock by 2.0 Mg C ha^{-1} over the experiment (Fig. 3 and Table S3). 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 (Kundu et al., 2001; Datta et al., 2018).

In the current study, combined application of biochar and FYM did not stimulate MBC and qCO_2, resulting in no clear difference in CO_2 flux between the M and BM treatments throughout most of the experimental period, in contrast to our hypothesis. Only for the first few months after both products' applications were the CO_2 efflux rate lower in the BM treatment than in 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 observed 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 (Zimmerman et al., 2011; Hernandez-Soriano et al., 2016). Based on 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 might also contribute to the inhibitory effect of biochar and FYM application on microbial activity. Further studies are required to elucidate the mechanism involved in the effect of the combined application on decreased microbial activity just after combined application, to develop effective C management in this area.

Finally, we found that the combined application of biochar and FYM increased SOC stock after 27 months, resulting in the largest SOC increment in the BM treatment (8.9 Mg C ha^{-1}; Table S3). 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. 0.78) was relatively higher than that in the B (ca. 0.74) and M (ca. 0.63) treatments, indicating that combined application of biochar and FYM would be more efficient to sequester C than an individual application of either amendment to soils (Jien et al., 2015). Hence, our results suggest that combined application of biochar and FYM would be an effective way to achieve sustainable SOC management for preventing land degradation both in terms of C output and C sequestration, in the tropical degraded cropland soils of southern India.

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Tables:

	pH (H_2O)	pH (H_2O)	pH (H_2O)	pH (H_2O)	pH (H_2O)	Total C	Total C	Total C	Total C	Total C
						g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
Biochar	8.0	(	0.1	)	a	515.5	(	12.2	)	a
FYM	7.8	(	0.1	)	a	119.9	(	9.8	)	b

Variables	Variables	df	%SS	F	p value
Soil moisture (m^3 m^{-3})	Soil moisture (m^3 m^{-3})				
	Treatment (C, B, M, BM)	3	16.6	2.8	0.04
	Time	39	81.5	13.6	< 0.001
	Treatment * Time	117	1.9	0.3	1.00
CO_2 efflux rate (mg CO_2-C m^{-2} h^{-1})	CO_2 efflux rate (mg CO_2-C m^{-2} h^{-1})				
	Treatment (C, B, M, BM)	3	61.7	42.5	< 0.001
	Time	39	35.3	24.3	< 0.001
	Treatment * Time	117	3.0	2.1	< 0.001
MBC (mg C kg^{-1})	MBC (mg C kg^{-1})				
	Treatment (C, B, M, BM)	3	83.9	72.7	< 0.001
	Time	28	15.0	13.0	< 0.001
	Treatment * Time	84	1.1	1.0	0.55
qCO_2 (µg CO_2-C mg MBC^{-1} h^{-1})	qCO_2 (µg CO_2-C mg MBC^{-1} h^{-1})				
	Treatment (C, B, M, BM)	3	30.4	30.4	< 0.001
	Time	28	66.4	66.5	< 0.001
	Treatment * Time	84	3.3	3.3	< 0.001

Treat-ment	CO_2 efflux rate (mg CO_2-C m^{-2} h^{-1})	CO_2 efflux rate (mg CO_2-C m^{-2} h^{-1})	CO_2 efflux rate (mg CO_2-C m^{-2}
	1st year	1st year	1st year
C	13.8	(	1.2
B	16.4	(	1.3
M	21.5	(	2.1
BM	16.6	(	1.4

Treatment	Year	CO_2 flux (Mg C ha^{-1})	R	R	n
C	Sep 2017–Jul 2018	0.75	0.47	+	14
	Aug 2018–Jul 2019	1.08	0.65	*	14
	Aug 2019–Dec 2019	0.62	0.53	+	11
B	Sep 2017–Jul 2018	0.94	0.60	*	14
	Aug 2018–Jul 2019	1.14	0.74	**	14
	Aug 2019–Dec 2019	0.70	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

Treatment	Year	CO_2 flux (Mg C ha^{-1})	R	R	n
BM	Sep 2017–Jul 2018	1.00	0.59	*	14
	Aug 2018–Jul 2019	1.66	0.75	*	13
	Aug 2019–Dec 2019	1.12	0.91	**	11

Figures:

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Figure legends:

Supplementary Materials:

Treatment	Treatment	Treatment	C		B	
1st year (2017-2018)	Sorghum cultivation	16-Sep	Plowing	Plowing	Plowing	Plowing
		20-Sep	–		Biochar application (8.2)	
			–		–	
		6-Oct	Seeding	Seeding	Seeding	Seeding
		24-Jan	Harvesting	Harvesting	Harvesting	Harvestin
	Non-cultivation	Feb-Jul	Bare land	Bare land	Bare land	Bare lanc
2nd year (2018-2019)	Sorghum cultivation	1-Aug	Plowing	Plowing	Plowing	Plowing
		2-Aug	–		–	
		15-Aug	Seeding	Seeding	Seeding	Seeding
		1-Dec	Harvesting	Harvesting	Harvesting	Harvestin
	Non-cultivation	Jan-Jul	Bare land	Bare land	Bare land	Bare lanc
3rd year (2019)	Sorghum cultivation	9-Aug	Plowing	Plowing	Plowing	Plowing
		11-May	–		–	
		18-Aug	Seeding	Seeding	Seeding	Seeding
		2-Dec	Harvesting	Harvesting	Harvesting	Harvestin

	CO_2 efflux rate	CO_2 efflux rate	CO_2 efflux rate	CO_2 efflux rate	CO_2 efflux rate	CO_2 ef
	1st year	1st year		2nd year	2nd year	
	F value	F value		F value	F value	
Biochar application (B)	0.0			0.1		
FYM application (M)	19.0	**	**	27.6	**	**
B*M	8.2	**	**	1.2		

	CO_2 efflux rate	CO_2 efflux rate	CO_2 efflux rate	CO_2 efflux rate	CO_2 efflux rate	CO_2 ef
Sampling time (T)	22.5	**	**	19.5	**	**
B*T	0.8			0.6		
M*T	1.9			3.8	**	**
B*M*T	1.5			0.5		

Treatment	C input	C input	C input	C input	C output as cu
	application amount Biochar	application amount FYM		Cumulative root biomass	
	Mg C ha-1: 0-15 cm	Mg C ha-1: 0-15 cm	Mg C ha-1: 0-15 cm	Mg C ha-1: 0-15 cm	Mg C ha-1: 0-1
C	0	0		1.0	2.4
B	8.2	0		1.2	2.7
M	0	3.2		1.3	4.0
BM	8.2	3.2		1.3	3.7

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`image4.emf` available at https://authorea.com/users/428303/articles/532189-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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`image5.emf` available at https://authorea.com/users/428303/articles/532189-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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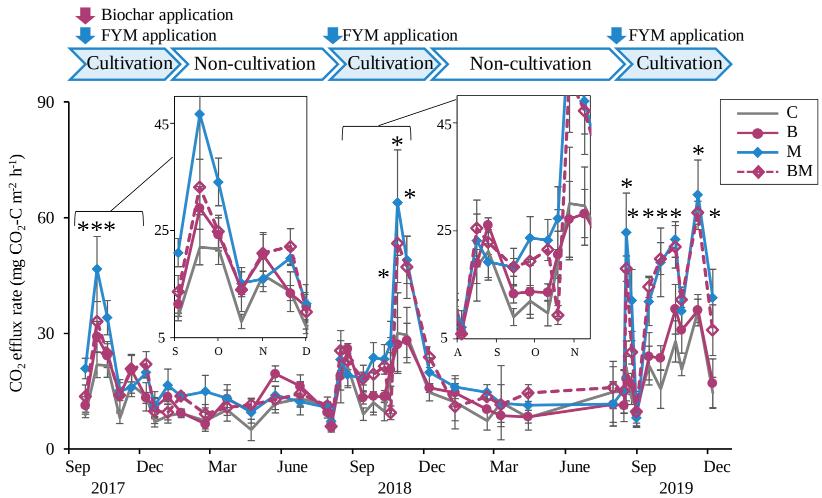
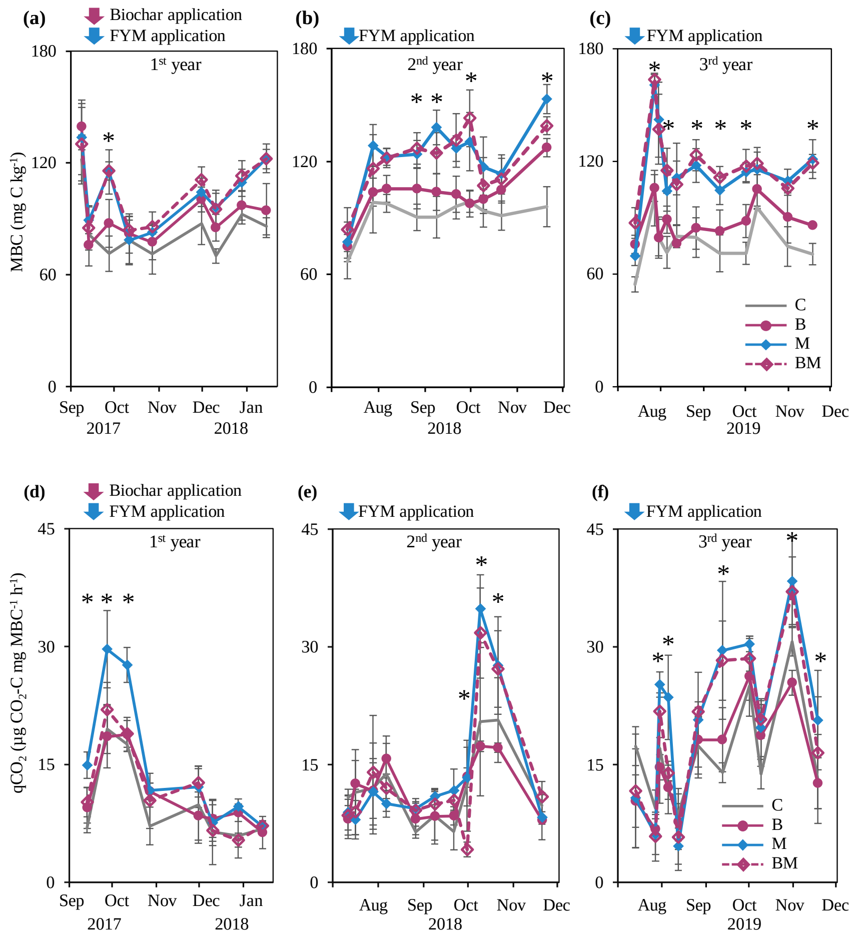
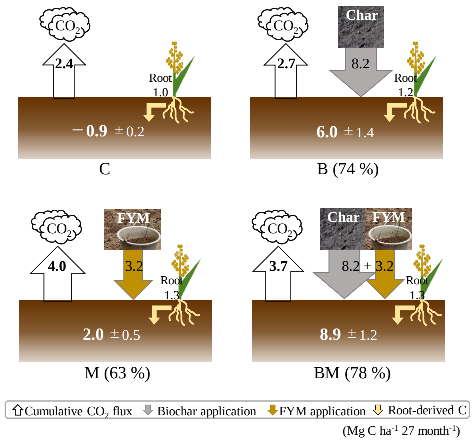
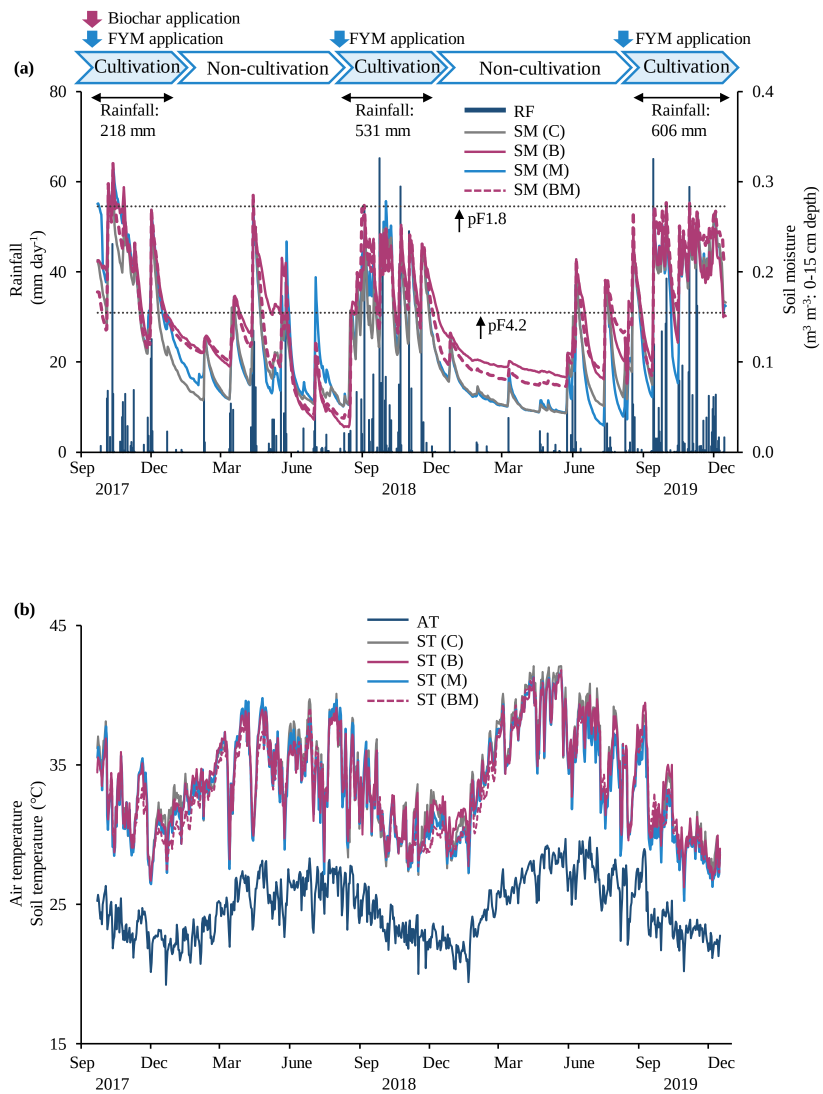


Biochar application
FYM application
FYM application
FYM application
Cultivation
Non-cultivation
Cultivation
Non-cultivation
Cultivation
CO2 efflux rate (mg CO2-C m-2 h-1)
C
B
M
BM
Sep
Dec
Mar
June
Sep
Dec
Mar
June
Sep
Dec
2017
2018
2019

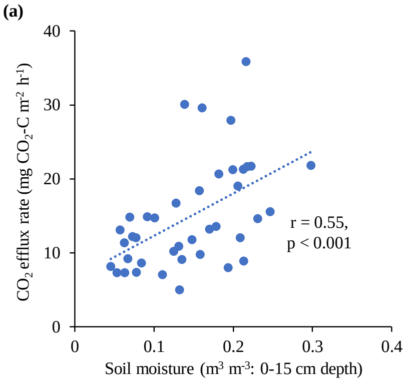


(a)
Biochar application
FYM application
1st year
MBC (mg C kg-1)
Sep
Oct
Nov
Dec
Jan
2017
2018
(b)
FYM application
2nd year
Aug
Sep
Oct
Nov
Dec
2018
(c)
FYM application
3rd year
C
B
M
BM
Aug
Sep
Oct
Nov
Dec
2019
(d)
Biochar application
FYM application
1st year
qCO2 (µg CO2-C mg MBC-1 h-1)
2017
2018
(e)
FYM application
2nd year
2018
(f)
FYM application
3rd year
2019


CO2
2.4
Root
1.0
− 0.9 ± 0.2
C
CO2
Char
2.7
8.2
Root
1.2
6.0 ± 1.4
B (74 %)
CO2
FYM
4.0
3.2
Root
1.3
2.0 ± 0.5
M (63 %)
CO2
Char
FYM
3.7
8.2 + 3.2
Root
1.3
8.9 ± 1.2
BM (78 %)
Cumulative CO2 flux
Biochar application
FYM application
Root-derived C
(Mg C ha-1 27 month-1)

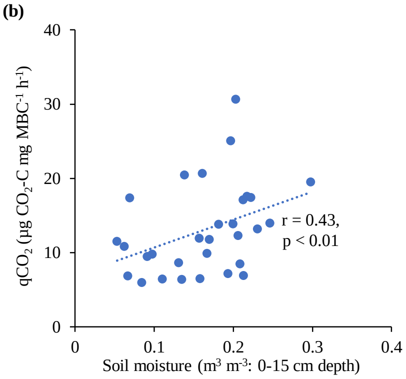

Biochar application
FYM application
FYM application
FYM application
(a)
Cultivation
Non-cultivation
Cultivation
Non-cultivation
Cultivation
Rainfall:
218 mm
Rainfall:
531 mm
Rainfall:
606 mm
RF
SM (C)
SM (B)
SM (M)
SM (BM)
pF1.8
pF4.2
Rainfall (mm day-1)
Soil moisture (m3 m-3: 0-15 cm depth)
80
60
40
20
0
0.4
0.3
0.2
0.1
0.0
Sep
Dec
Mar
June
Sep
Dec
Mar
June
Sep
Dec
2017
2018
2019
(b)
AT
ST (C)
ST (B)
ST (M)
ST (BM)
Air temperature
Soil temperature (°C)
45
35
25
15
Sep
Dec
Mar
June
Sep
Dec
Mar
June
Sep
Dec
2017
2018
2019

(a)



40
30
20
10
0
CO_2 efflux rate (mg CO_2-C m^{-2} h^{-1})
r = 0.55,
p < 0.001
0
0.1
0.2
0.3
0.4
Soil moisture (m^3 m^{-3}: 0-15 cm depth)

(b)



40
30
20
10
0
qCO_2 (µg CO_2-C mg MBC^{-1} h^{-1})
r = 0.43,
p < 0.01
0
0.1
0.2
0.3
0.4
Soil moisture (m^3 m^{-3}: 0-15 cm depth)