

Effects of biochar addition on CO₂ and CH₄ emissions from a cultivated sandy loam soil during freeze-thaw cycles

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ABSTRACT

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This study was conducted to examine the effects of biochar additions (0, 2 and 4%, w/w) on soil carbon dioxide (CO₂) and methane (CH₄) emissions during freeze-thaw cycles (FTC). The results showed that soil CO₂ emissions were stimulated by both FTC and biochar addition. However, the differences in soil CO₂ emissions between control (CK) and FTC treatments were not significant when biochar addition rate was 4%, indicating that high biochar addition rate may have stronger effect on stimulating soil CO₂ emissions than FTC. The increased soil dissolved organic carbon content, which attributed to the labile carbon in biochar, was the likely reason for the increased CO₂ emissions. The negative CH₄ emissions were promoted by biochar, especially under FTC conditions; possibly due to the structure of biochar soil aeration increased, which formed a favourable environment for methanotrophs. The results of this study indicate that biochar additions can increase soil CO₂ emissions and CH₄ uptakes during FTC, and such effects are different from those under CK conditions.

Keywords: agricultural soil; greenhouse gas mitigation; soil labile organic carbon; soil amendment; non-vegetation period

Carbon dioxide (CO₂) and methane (CH₄) are two major greenhouse gases (GHGs), which play important roles in the biogeochemical carbon (C) cycle as well as global warming (IPCC 2007). Agricultural soils are identified as major sources of GHGs (Smith et al. 2008). It is estimated that over 50 Gt of CO₂ were emitted from agricultural soils to the atmosphere through the mineralization of soil organic C (SOC) at the end of the 20th century (Paustian et al. 2000). Agriculture also accounts for 52% of global anthropogenic CH₄ emission (Smith et al. 2008). In the context

of global change, effective measures are strongly needed to mitigate CO₂ and CH₄ emissions from agricultural soils.

Biochar, which is a C-rich product derived from the slow pyrolysis of organic materials under oxygen (O₂) limited conditions, has drawn increasing attention for its potential to be used as an amendment to mitigate soil GHGs emissions (Liu et al. 2014). However, the observation periods of previous studies were mainly focused on vegetation periods (Castaldi et al. 2011, Zhang et al. 2012). In recent decades, enhanced soil GHGs emissions during

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freeze-thaw period were reported in both field and incubation investigations (Wolf et al. 2010, Wang et al. 2014, Wu et al. 2014). Some studies further demonstrated that freeze-thaw induced GHGs emissions were important parts of the annual GHGs budget (Liang et al. 2007, Wolf et al. 2010). In a short-term laboratory study, Kettunen and Saarnio (2013) found that soils amended with biochar decreased soil N_2O emissions by 61% during freeze-thaw cycles (FTC). However, to the best of our knowledge, there have been no studies on responses of soil CO_2 and CH_4 emissions to biochar addition during FTC.

Soil CO_2 and CH_4 are believed to be produced mostly from microbial processes, such as methanogenesis and biological oxidation of SOC (Smith et al. 2008). Previous studies demonstrated that soil microbial activities could be considerably influenced by FTC due to the important role of temperature on microbial metabolism. FTC can disrupt soil aggregates and some microbial cells, induce the release of aggregate-protected organic C and the decomposition of microbial cells (Yergeau and Kowalchuk 2008, Kim et al. 2012). These accumulated substrates can be utilized by microorganisms and then enhance microbial metabolism during thawing period (Kim et al. 2012). Soil dissolved organic C (DOC) and microbial biomass C (MBC) are easily accessible C sources for soil microorganisms (Wang et al. 2014, Yeboah et al. 2016). Previous studies proved that soil DOC and MBC contents were closely related to soil C emissions (Wang et al. 2014, Shaaban et al. 2016). However, under the joint effects of biochar and FTC, the relationships between soil C emissions and DOC/MBC contents are still poorly understood.

In this study, FTC was simulated in laboratory to: (1) examine the effects of biochar addition on soil CO_2 and CH_4 emissions, DOC and MBC contents during FTC; (2) estimate the relationships among soil C emissions, DOC and MBC contents under the joint effects of FTC and biochar.

MATERIAL AND METHODS

Soil sampling and biochar preparation. Soil samples were collected in a depth of 20 cm from a maize (*Zea mays* L.) field in the Ili River Valley (43°27'N, 82°54'E), northwest China. The soil is classified as Haplic Kastanozems (FAO) with a

sandy loam texture (4.2% clay, 23.2% silt and 72.6% sand). Soil samples were air-dried in the shade and sieved (≤ 2 mm) with removal of any visible plant material. Sieved soil samples were homogenized and then stored at 4°C until the incubation experiment.

Biochar used for this experiment was produced using bamboo subjected to pyrolysis at 500–600°C by the Seek Bio-Technology Company in Shanghai, China. The biochar was then ground up, passed through a 2 mm sieve and mixed thoroughly before experimental use.

Experimental design. A series of 250 mL Erlenmeyer flasks were prepared with 60.0 g (oven-dry basis) of soil samples. Biochar was then mixed well with soils at addition rates of 0% (BC0); 2% (BC2) and 4% (BC4) (w/w). Deionized water was added to the mixtures to maintain 60% of maximum water holding capacity (MWHC). All flasks were pre-incubated at 25°C in the dark condition for one week. After pre-incubation, flasks of each addition rate were randomly divided into three equal groups to experience three different FTC treatments: (1) treatment without FTC (CK); (2) treatment with small amplitude of FTC (SFT); (3) treatment with large amplitude of FTC (LFT). Therefore, there were nine treatments (three biochar addition rates \times three FTC amplitudes) in this experiment. For SFT, a single FTC consisted of freezing at -5°C for 24 h and thawing at 5°C for 24 h. By contrast, flasks of LFT were frozen at -10°C for 24 h and then thawed at 10°C for 24 h. Fifteen FTCs (30 days) were conducted in total to simulate the freeze-thaw period under field conditions. Flasks of CK were incubated at 5°C during the entire incubation. At the end of every two FTCs, deionized water was added into each flask to maintain constant soil moisture. Three flasks of each treatment were randomly selected for gas sampling after 1st, 3rd, 5th, 10th and 15th FTC. Soils of each flask were then destructively sampled for the measurements of soil DOC and MBC contents.

Chemical analysis. The pH and electrical conductivity (EC) of soil and biochar were measured in a volume ratio (H_2O) of 1:5 (w/v) using a pH meter (SevenEasy, Mettler-Toledo, Greifensee, Switzerland) and an EC meter (DDSJ-308A, Rex, Shanghai, China), respectively (Zhang et al. 2014). Soil total N (N_{tot}) was determined using an automatic azotometer (Kjeltec 8400, FOSS, Hillerød,

Denmark) according to the Kjeldahl method (Lu 1999). SOC was measured using the $\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$ oxidation method (Lu 1999). The C and N contents of biochar were measured using an elemental analyser (vario Micro cube, Elementar, Hanau, Germany) (Lan et al. 2017). The ammonium N ($\text{NH}_4^+\text{-N}$) and nitrate N ($\text{NO}_3^-\text{-N}$) of soil and biochar were measured using a continuous flow analyzer (AA3, SEAL Analytical, Norderstedt, Germany) (Yao et al. 2009). Soil texture was determined using a laser diffraction particle analyzer (Mastersizer 2000, Malvern, UK) (Gui et al. 2010). DOC of fresh soils and biochar were extracted with deionized water and 2 mol/L KCl (1:10, w/v), respectively, at 250 rpm for 30 min; the extracts were filtered (0.45 μm) after centrifuging at 8000 rpm for 10 min (Jones and Willett 2006). Biochar samples were recovered for further extraction using 2 mol/L hot (95°C) KCl (1:10, w/v) at 250 rpm for 16 h before centrifuging and filtering (0.45 μm). DOC contents of extracts were determined using a TOC analyzer (model 1030, OI Analytical, College Station, USA) and biochar DOC was obtained by adding up the DOC contents of both cold and hot KCl extracts (Lan et al. 2017). Soil MBC was measured using the CHCl_3 fumigation- K_2SO_4 extraction method (1:4, w/v). The extracts were analyzed at 280 nm using an UV spectrophotometer (Cary 60, Agilent Technologies, Santa Clara, USA) (Nunan et al. 1998). Selected physicochemical properties of soil and biochar are shown in Table 1.

The concentrations of CO_2 and CH_4 were detected using a gas chromatograph (7890B, Agilent Technologies, Santa Clara, USA). The gas chromatograph was equipped with a thermal conductivity detector for CO_2 analysis and a flame ionization detector for CH_4 analysis. The CH_4 and CO_2 emissions were calculated according to the method of Lim and Choi (2014). Cumulative gas emissions during the whole incubation were directly computed from the measured emissions

and were estimated by linear interpolation for days when no measurements were available (Gao et al. 2013).

Statistical analysis. The effects of FTC amplitude and biochar addition rate on soil C emissions, DOC and MBC contents were tested using two-way ANOVA. Differences in cumulative gas emissions of the entire incubation among different FTC amplitudes or biochar addition rates were examined using one-way ANOVA with *LSD* (least significant difference) test. Data sets were tested for normality and heterogeneity before analyses. Pearson correlation test was employed to examine the relationships among soil C emissions, DOC and MBC contents. Differences and correlations were considered statistically significant if $P < 0.05$ and highly significant if $P < 0.001$. SPSS 16.0 (SPSS Inc., Chicago, USA) were used to perform statistical analysis.

RESULTS AND DISCUSSION

Effects of biochar addition on soil DOC and MBC contents during FTC. In general, soil DOC contents of BC2 and BC4 were significantly higher than those of BC0 for all temperature treatments (Figure 1, Table 2), indicating that soil DOC content can be increased by adding biochar. Lin et al. (2012) demonstrated that labile or leachable organic C could be generated during the production of biochar. These C can be adsorbed onto the surface of biochar and act as a source of soil DOC after being mixed into soils (Lin et al. 2012). In the present study, the high DOC content of biochar (695.1 mg/kg, Table 1) demonstrated that the DOC in biochar was the main reason for the increased soil DOC contents after adding biochar. Except for BC2 under CK condition, DOC contents of other treatments decreased by 8.4–43.3% after the whole incubation. Soil DOC utilized by soil

Table 1. Selected physicochemical properties of soil and biochar (mean \pm standard error, $n = 3$)

	C_{org}	C_{tot}	N_{tot}	DOC	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	pH	EC	MWHC
		(g/kg)			(mg/kg)			($\mu\text{S}/\text{cm}$)	(%)
Soil	11.0 \pm 0.2	–	1.2 \pm 0.1	285.6 \pm 2.1	4.0 \pm 0.10	21.5 \pm 0.1	8.0 \pm 0.04	315.3 \pm 2.1	39.5
Biochar	–	664.8 \pm 37.1	9.8 \pm 0.5	695.1 \pm 62.3	3.1 \pm 0.2	1.8 \pm 0.0	9.2 \pm 0.1	2393.3 \pm 57.7	–

– not detected. C_{org} – organic carbon; C_{tot} – total carbon; N_{tot} – total nitrogen; DOC – dissolved organic carbon; EC – electrical conductivity; MWHC – maximum water holding capacity

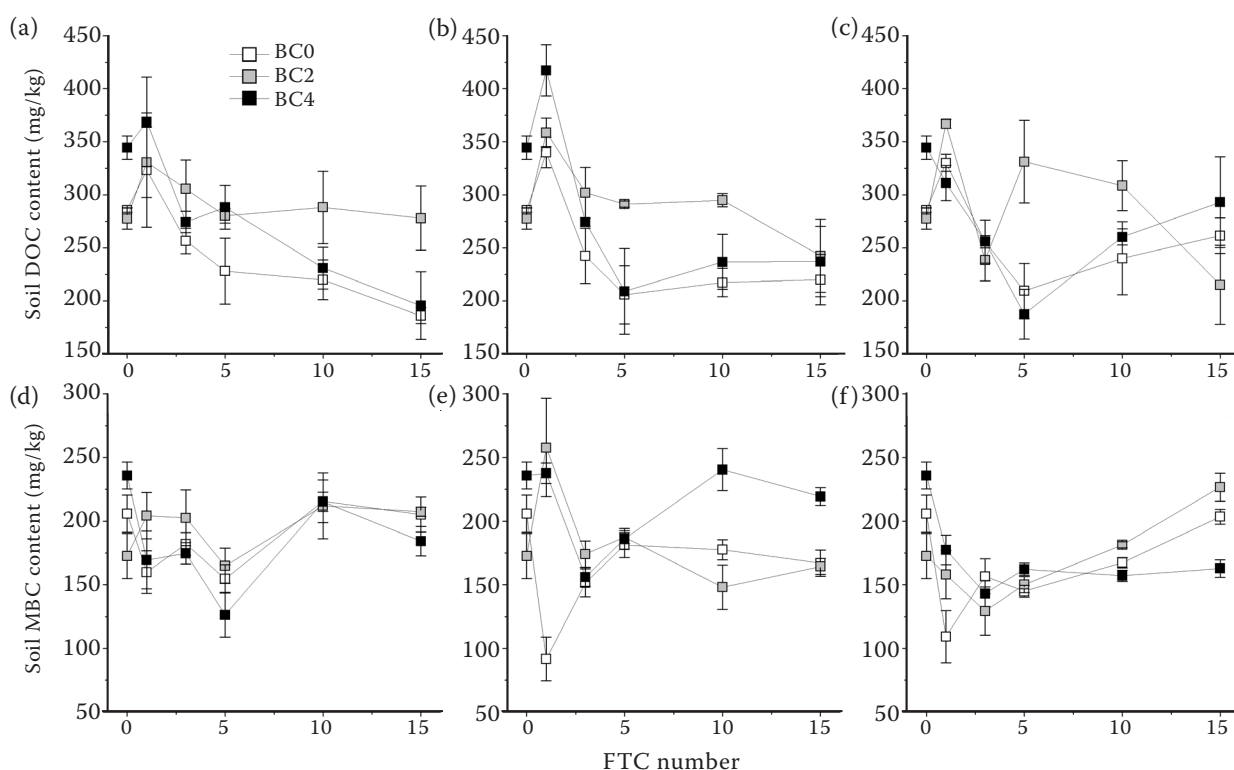


Figure 1. Responses of soil dissolved organic carbon (DOC) – (a) treatment without freeze-thaw cycles (FTC) (CK); (b) treatment with small amplitude of FTC (SFT); (c) treatment with large amplitude of FTC (LFT), and microbial biomass carbon (MBC) – (d) CK; (e) SFT; (f) LFT contents to biochar additions during the incubation. BC0 – 0%; BC2 – 2%; BC4 – 4% (w/w). Bars represent the standard error of the mean ($n = 3$)

microorganisms was the likely reason for such decreases because soil DOC is an easily accessible C source for soil microorganisms (Wang et al. 2014). However, the effect of FTC amplitude on soil DOC was not significant (Table 2), suggesting that the C in biochar may be quite stable and able to endure the FTC conditions.

As shown in Figure 1e,f, soil MBC contents of BC0 showed sharp decreases after the 1st FTC, and then gradually increased during the rest of

FTCs. The results were in agreement with observations of Wang et al. (2014). The sharp decreases might be attributed to some microbial cells that were damaged or destroyed by sudden changes in temperature (Yergeau and Kowalchuk 2008). Thereafter, soil microorganisms might be adapted to this environmental change. Under FTC conditions, MBC contents of soils with different biochar addition rates generally decreased in the following order: BC4 > BC2 > BC0. The results indicated that biochars were helpful in increasing soil microbial biomass during FTC. The reason was possibly that the macropores (> 200 nm) of biochars could serve as habitats for soil microorganisms such as bacteria, fungi, and protozoa (Gul et al. 2015), and protected them from being disturbed by the FTC process. The high EC of biochar might also influence soil microbial biomass because salinity is identified as an important factor that affects the growth of soil microorganisms (Wong et al. 2008), which should be paid more attention to in the future.

Effects of biochar addition on soil CO₂ and CH₄ emissions during FTC. Similar to soil DOC,

Table 2. Results of two-way ANOVA (P -values) testing the effects of freeze-thaw cycles (FTC) amplitude and biochar addition rate on soil CO₂ emission, CH₄ emission, dissolved organic carbon (DOC) content and microbial biomass carbon (MBC) content

Source	DOC	MBC	CO ₂	CH ₄
FTC amplitude	0.691	< 0.001	< 0.05	< 0.001
Biochar addition rate	< 0.05	0.352	< 0.001	< 0.05
FTC amplitude × biochar addition rate	0.936	< 0.05	< 0.05	< 0.001

Boldface values indicate which effects were significant

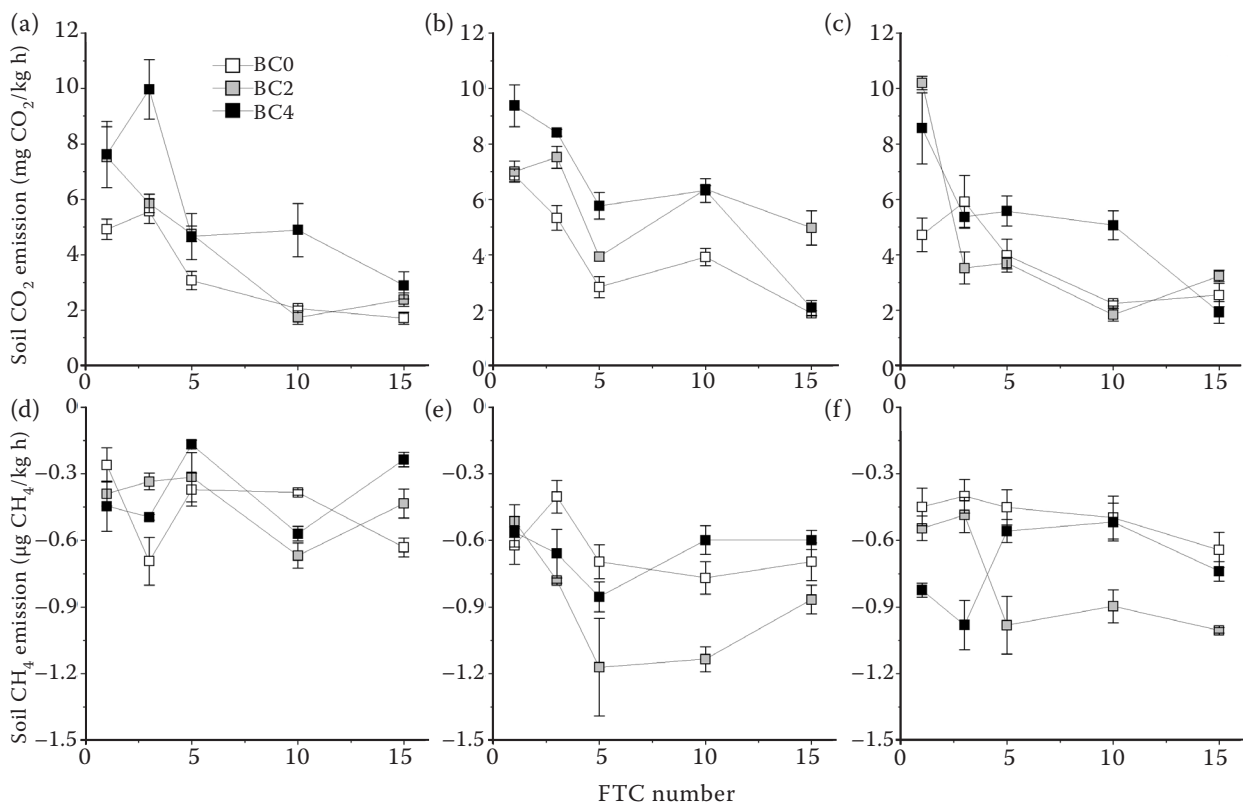


Figure 2. Responses of soil CO₂ – (a) treatment without freeze-thaw cycles (FTC) (CK); (b) treatment with small amplitude of FTC (SFT); (c) treatment with small amplitude of FTC (LFT), and CH₄ – (d) CK; (e) SFT; (f) LFT emissions to biochar additions during the incubation. BC0 – 0%; BC2 – 2%; BC4 – 4% (w/w). Bars represent the standard error of the mean ($n = 3$)

soil CO₂ emissions of most treatments gradually decreased with the increase of incubation time (Figure 2a–c). For BC0, cumulative CO₂ emissions from soils treated with FTC were significantly higher than those from soils under CK condition (Table 3), implying that soil CO₂ emissions can be increased by FTC. This was possibly due to some FTC-destroyed soil aggregates and microbial cells

through the transition phase of soil water and low temperature, which increased the availability of substrate and microbial respiration (Kim et al. 2012). Biochar additions increased cumulative CO₂ emissions by 6.8–50.9% (BC2) and 41.8–79.9% (BC4) compared to BC0 (Table 3), demonstrating that biochar additions may stimulate soil CO₂ emissions. This finding was in agreement with

Table 3. Cumulative CO₂ and CH₄ emissions of each treatment during the whole incubation (mean ± standard error)

Treatment	Cumulative CO ₂ emission (g C/kg)			Cumulative CH ₄ emission (mg C/kg)		
	CK	SFT	LFT	CK	SFT	LFT
BC0	2.19 ± 0.02 ^{Cb}	2.75 ± 0.12 ^{Ab}	2.51 ± 0.02 ^{Bb}	0.27 ± 0.01 ^{Ca}	0.48 ± 0.01 ^{Ab}	0.37 ± 0.03 ^{Bc}
BC2	2.72 ± 0.06 ^{Bb}	4.15 ± 0.09 ^{Aa}	2.68 ± 0.06 ^{Bb}	0.34 ± 0.02 ^{Ca}	0.70 ± 0.02 ^{Aa}	0.61 ± 0.02 ^{Ba}
BC4	3.94 ± 0.35 ^{Aa}	3.90 ± 0.14 ^{Aa}	3.64 ± 0.28 ^{Aa}	0.36 ± 0.04 ^{Ba}	0.48 ± 0.03 ^{Ab}	0.47 ± 0.01 ^{Ab}

Uppercase letters indicate significant differences ($P < 0.05$) among freeze-thaw cycles (FTC) amplitudes while under the same biochar addition rate; lowercase letters indicate significant differences ($P < 0.05$) among biochar addition rates while under the same FTC amplitude. CK – treatment without FTC; SFT – treatment with small amplitude of FTC; LFT – treatment with small amplitude of FTC; BC0 – 0%; BC2 – 2%; BC4 – 4% (w/w)

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Table 4. Correlation coefficients (*R*) among soil CO₂ emission, CH₄ emission, dissolved organic carbon (DOC) content and microbial biomass carbon (MBC) content (*n* = 135)

	DOC	MBC	CO ₂	CH ₄
DOC	1			
MBC	−0.027	1		
CO ₂	0.436**	−0.033	1	
CH ₄	0.066	0.194*	−0.008	1

Boldface values indicate correlations which are significant
P* < 0.05; *P* < 0.01

Zhang et al. (2012), who attributed such increases to enhanced soil labile C contents by adding biochar. In this study, BC2 and BC4 generally showed higher DOC contents than BC0. The results of Pearson correlation analysis showed that soil CO₂ emission was significantly correlated to soil DOC content (Table 4), partly supporting the previous assertions. However, cumulative CO₂ emissions of different temperature treatments did not differ significantly at BC4 (Table 4). This indicated that high biochar addition rate might have a stronger effect on stimulating soil CO₂ emissions than FTC, which might weaken the differences in soil CO₂ emission between CK and FTC.

Soil CH₄ emissions were negative for all treatments (Figure 2d–f), varying from −0.17 to −1.17 μg CH₄/(kg h) during the whole incubation period. Soil CH₄ uptakes were promoted by biochar, especially under FTC conditions (Table 3). Karhu et al. (2011) indicated that pores of biochar could increase aeration, porosity and surface area of soils, thus forming favourable environment for methanotrophs. Furthermore, low temperatures can reduce activities of some aerobic microorganisms, resulting in more O₂ in soils. The increased O₂ may favour methane oxidation while inhibits methanogenesis (Ding and Cai 2007). The positive correlation between soil MBC content and CH₄ uptake indicated that soil microorganism was an important factor that affected CH₄ emission (Table 4).

Although there are some limitations in this study (a short incubation period with single soil and biochar under laboratory conditions without plants), the results still indicate that the effects of biochar additions on soil C emissions may be

different between CK and FTC conditions. The effects of more types of biochar on GHGs emissions from different kinds of soil during FTC will be focused in further studies.

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